INTERNATIONAL STANDARD

ISO 10695

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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.

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 $\geqslant$ 0,05 µg/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	$C_8H_{14}ClN_5$	215,7	001912-24-9
Cyanazine	$C_9H_{13}ClN_6$	240,7	021725-46-2
Metazachlor	$C_{14}H_{16}ClN_3O$	277,8	067129-08-2
Parathion (ethyl)	$C_{10}H_{14}NO_5PS$	291,3	00056-38-2
Parathion (methyl)	$C_8H_{10}NO_5PS$	263,2	298-00-0
Pendimethalin	$C_{13}H_{19}N_3O_4$	281,3	040487-42-1
Propazine	$C_9H_{16}ClN_5$	229,7	000139-40-2
Sebuthylazine	$C_9H_{16}ClN_5$	229,7	007286-69-3
Simazine	$C_7H_{12}ClN_5$	201,7	000122-34-9
Terbuthylazine	$C_9H_{16}ClN_5$	229,7	005915-41-3
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	335,3	001582-09-8
Vinclozolin	$C_{12}H_9Cl_2NO_3$	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 °C ± 20 °C for 4 h ± 30 min, cool to about 200 °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(NaOH) = 1 mol/l.

b) **Hydrochloric acid**, aqueous solution, c(HCl) = 1 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 °C in the dark (they can be stored at around – 18 °C, but some septa closures may harden at such a temperature with possible losses of solvent).

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 °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 sensivity. 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 °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 °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 µl to 10 µl, 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.

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

4.2.6 Standard stock solutions.

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.

4.2.9 Inert gas, high purity, minimum 99,996 % (volume fraction), for drying and, if need be, for concentration by evaporation.

4.3 Apparatus

Equipment or parts of it which may come into contact with the sample or its extract shall be free from residues causing blanks. It is recommended to use glass, stainless steel or polytetrafluoroethylene (PTFE).

4.3.1 Gas chromatograph.

Identical to that used for liquid/liquid extraction. See 3.3.1.

4.3.2 Cartridges, made of glass or polypropylene, filled with RP-C18 material or other adsorbents.

The commercially available RP-C18 materials are often of varying quality. Considerable batch-to-batch differences in quality and selectivity of this material may occur. Therefore for each new batch of RP-C18 material, the recovery shall be checked according to 5.2.4. The recovery may also vary with the concentration. Calibration and analysis shall be performed with material from one and the same batch only.

4.3.3 Vacuum pump or pressure assembly.

4.3.4 Any suitable system of evaporation.

4.3.5 Microlitre syringes.

4.3.6 Glass-fibre filter.

4.3.7 Miscellaneous glassware.

Identical to that used for liquid/liquid extraction. See 3.3.6.

4.4 Sampling

Procedure identical to that used for liquid/liquid extraction. See 3.4.

Suspended matter in the water sample (such as iron hydroxide, calcium carbonate) occurring on sampling, storage and sample preparation can clog the packing. In this case, filter the water sample through a glass-fibre filter (4.3.6) prior to the enrichment.

4.5 Procedure (for RP-C18 material)

4.5.1 Conditioning

Wash the RP-C18 material (4.2.2) in the cartridge or glass column (4.3.2) with five times its volume of the solvent used in 4.5.2.

Then rewash with five times its volume of water (4.2.1) and use the moist carrier material for the enrichment. Do not let the cartridge/column run dry.

4.5.2 Enrichment

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

Run the water sample with a flowrate of < 1 000 ml/h over the carrier material, previously being conditioned according to 4.5.1. Regulate the flowrate by altering the vacuum or the over-pressure (4.3.3), respectively.

After the enrichment dry the cartridge with an inert gas (4.2.9) (30 min approximately 100 ml/min nitrogen, room temperature) or by freeze-drying.

Elute with at least 1 ml of solvent (4.2.4) per 500 mg of RP-C18 material (4.2.2), applied in small portions.

Put half of the solvent (4.2.4) on the column/cartridge and allow to equilibrate for about 15 min.

Then apply the rest of the solvent (4.2.4) and collect the eluate in a small graduated vial. Make up with the solvent (3.2.3) to a defined volume.

Larger volumes of solvent (4.2.4) may be required for some types of commercial apparatus to ensure the complete recovery of the extract.

Higher enrichment factors may be achieved by concentrating the eluate using a suitable system of evaporation (4.3.4). Evaporate at a temperature less than 40 °C, just to dryness. Make up with the solvent (3.2.3) to a defined volume.

Use an enriched aliquot for the gas chromatography.

The solvent used shall be the same for the extract and the calibration solutions.

4.5.3 Gas chromatography

Procedure identical to that used for liquid/liquid extraction. See 3.5.5.

4.5.4 Blank determination

Procedure identical to that used for liquid/liquid extraction. See 3.5.6.

5 Calibration

5.1 General

One of two possible calibration methods shall be used for the gas chromatograph, whether liquid/liquid extraction or liquid/solid extraction is used:

— calibration using external standards (see 5.2),

— calibration using an internal standard (see 5.3).

5.2 Calibration using external standards

5.2.1 General

For practical reasons, use mixed working standards.

Use the same injection volume for calibration and for measurement of the sample solutions.

For each compound, a separate calibration function and graph, consisting of at least five points from five different concentrations, shall be established.

The strategy of calibration is based on the following sequential steps:

a) Step 1: initial calibration

Determine the recovery in the following way:

1) direct injection of solvent working standard solutions (5.2.2),

2) injection of spiked aqueous standard solutions extracts (5.2.3).

The data obtained from 1) are compared with those from 2) in order to calculate the recovery of each determinand (5.2.4).

b) Step 2: recalibration

Carry out recalibration using two solvent working standard solutions (about 20 % and 80 % of the working range, respectively) with each batch of samples to analyse (5.2.5).

NOTE It is permitted to use the extracts of the spiked aqueous standard solutions to recalibrate if these extracts are stable (less than one week for organic nitrogen and phosphorus compounds).

c) Step 3: evaluation of data

1) if batch recalibration is carried out with solvent working standard solutions (5.2.2), the results obtained shall be corrected by mean recovery, using calculation (6.2.1),

2) if batch recalibration is carried out with extracts of spiked aqueous standard solutions, the results obtained take directly into account the correction for recovery (6.2.2),

3) if the calibration of the overall procedure (5.2.3) is carried out for each batch of samples to analyse, the results obtained take directly into account the correction for recovery (6.2.2).

Table 2 gives an explanation of the subscripts used in equations and the following text.

Table 2 — Explanation of the subscripts used in the symbols

Index	Meaning
i	Identity of the determinand
e	Calibration step
g	Overall procedure
I	Identity of internal standard
j	Consecutive number of pairs of values

5.2.2 Calibration with the solvent working standard solutions (not using the overall procedure)

Inject volumes of 1 µl to 10 µl of the working standard solutions (3.2.8) into the gas chromatograph.

Measure the gas chromatograph signals for each substance (peak heights or peak areas or area integration units respectively).

For a graphic presentation of the calibration curve, plot the respective measured values y_{ie} on the ordinate against the respective mass concentrations ρ_{ie} of the substance i on the abscissa.

The series of measured values thus obtained shall be used to establish the linear regression function as follows:

$$y_{ie} = m_i \rho_{ie} + b_i \quad (1)$$

where

y_{ie} is the (dependent variable) measured response of the substance i, depending on ρ_{ie}; the unit depends on the evaluation, for example, area value;

ρ_{ie} is the (independent variable) mass concentration of the substance i (external standard) in the working standard solution, in micrograms per litre;

m_i is the slope of the calibration curve of the substance i, the unit depends on the evaluation, for example, area value × (litres per microcrogram);

b_i is the intercept of the calibration curve on the ordinate, the unit depends on the evaluation, for example, area value. As a rule, the intercept is very small.

The working range shall be defined as the linear part of the curve.

5.2.3 Calibration of the overall procedure (spiked water)

To calibrate the entire procedure, prepare aqueous solutions of the compounds to be determined in an individual concentration range within the linear dynamic range of the detector, as follows.

a) Preparation of the spiked aqueous standard solutions

Prepare at least five aqueous standard solutions covering the range 0,02 µg/l to 0,5 µg/l by adding different volumes of the intermediate standard solutions (3.2.7) to water (3.2.1).

For blank measurements, add to one bottle of water (3.2.1), the same quantity of solvent used for the preparation of the aqueous standard solutions.

Use quantities such that the volume of solvent added is as small as possible (0,1 ml to 1 ml solvent per litre of water), in order to minimize any effect on the partition equilibrium.

Prepare the spiked aqueous standard solutions normally on the day of use.

b) Preparation of the calibration curve

Extract these aqueous solutions and concentrate as given in 3.5.3 and 3.5.4.

Inject volumes of 1 µl to 10 µl of the extracts of the blank and at least of five spiked aqueous standard solutions with concentrations ρ_{ieg} in ascending order, into the gas chromatograph.

Measure the peak values y_{ieg} of these solutions.

Calculate a regression function for each substance using the pairs of values y_{ieg} and ρ_{ieg}:

$$y_{ieg} = m_{ig} \cdot \rho_{ieg} + b_{ig} \qquad (2)$$

where

y_{ieg} is the (dependent variable) measured response of the substance i during calibration, depending on ρ_{ieg}; the unit depends on the evaluation, for example, area value;

ρ_{ieg} is the (independent variable) mass concentration of the substance i in the spiked aqueous standard solution, in micrograms per litre;

m_{ig} is the slope of the calibration curve of the substance i, often referred to as f_i; the unit depends on the evaluation, for example, area value × (litres per microgram);

b_{ig} is the axis intercept of the calibration curve on the ordinate; the unit depends on the evaluation, for example, area value.

Plot the reference functions in a graph, with the ordinate as the substance specific measured signals y_{ieg} and the abscissa as the mass concentrations ρ_{ieg} of the substance i in the spiked aqueous standard solution.

The working range shall be defined as the linear part of this curve.

5.2.4 Determination of the recovery

Determine, by means of the calibration procedure according to 5.2.2 and 5.2.3, the substance-specific mean recovery A_i, for the substance i [see equation (3)].

$$A_i = \frac{m_{ig}/m_i}{F_V} = \frac{m_{ig} \cdot V_E \cdot f}{m_i \cdot V_p} \quad (3)$$

where

A_i is the specific mean recovery for the substance i, dimensionless;

m_i see equation (1);

m_{ig} see equation (2);

F_V is the ratio of the volume of extraction solvent and sample.

This factor F_V shall be calculated taking into account sample volume, extraction solvent volume, dilution factors (if applicable). The following equation applies:

$$F_V = \frac{V_E \cdot f}{V_p} \quad (4)$$

where

V_E is the extraction solvent volume, in millilitres;

V_p is the sample volume, in millilitres.

NOTE Equation (3) is valid if b_i and b_{ig} are relatively small and if calibration according to equations (1) and (2) refers to the same range of concentration (in the extract and in the solvent standard solution), for example, comparable values y_{ie} and y_{ieg}.

A constant recovery is an essential prerequisite for good precision and accuracy of the analytical result. Variations of these values indicate problems in some stages of the analysis.

The recovery depends on the distribution coefficient and is characteristic for each substance and the working conditions. Consider a recovery greater than 60 % as “a good recovery”.

5.2.5 Recalibration with each batch

For routine recalibration of the method, it is essential to work within the previously established linear range (5.2.2 or 5.2.3). This shall be updated regularly, especially when contaminated samples are analysed, as these may affect the detector and hence the linear range.

The minimum requirement for batch recalibration shall be injections of two working standard solutions (3.2.8) or two spiked aqueous standard solutions (5.2.3). The concentration of the first solution shall be about 20 % of the selected linear working range, the concentration of the second solution about 80 %.

Calculate a regression function.

Compare this line to the previous established calibration curve (5.2.2 or 5.2.3). If the values are within the range of the confidence limits of the previous established calibration curve (5.2.2 or 5.2.3), use this new line as the calibration line for evaluation.

If not, check the system and establish a complete new calibration curve.

5.3 Calibration using an internal standard

The procedure of the internal standard described in this paragraph is restricted to the addition of a suitable internal standard to all extracts and working standard solutions (this procedure is also known as using an injection standard). The retention time of the internal standard shall be in an area of the chromatogram free from the determinands and interfering peaks.

Follow a procedure similar to the calibration using external standards (5.2), except that each extract and working standard solutions are spiked with the same amount of internal standard.

Keep the same solvent composition and internal standard concentration for the working standard solutions and the extracts.

Evaluate the gas chromatographic signals for each substance against concentration.

Plot the rational values y_{ieg}/y_{leg} (peak areas, peaks heights or integration units) for each substance i on the ordinate and the associated rational mass concentration ρ_{iej}/ρ_{lej} on the abscissa.

Establish the linear regression function using the pairs of values y_{ieg}/y_{leg} and ρ_{iej}/ρ_{lej} of the measured series in the following equation:

$$\frac{y_{ie}}{y_{le}} = m_{il} \cdot \frac{\rho_{ie}}{\rho_{le}} + b_{il} \qquad (5)$$

where

y_{ie} is the (dependent variable) measured response of the substance i in the calibration, depending on ρ_{ie}, the unit depends on the evaluation, for example, area value;

y_{le} is the measured response of the internal standard l in the calibration, depending on ρ_{le}, the unit depends on the evaluation, for example, area value;

ρ_{ie} is the (independent variable) mass concentration of the substance i in the calibration solution, in micrograms per litre;

ρ_{le} is the (independent variable) mass concentration of the internal standard l, in micrograms per litre;

m_{il} is the slope of the calibration curve from Y_{ie}/Y_{le} as a function of the mass concentration ratio ρ_{ie}/ρ_{le}, often called the response factor;

b_{il} is the axis intercept of the calibration curve on the ordinate.

6 Identification and calculation

6.1 Identification of individual compounds

Compare the gas chromatogram obtained to those of the standards (to be run before a batch of samples) consistent with the chosen quantification method.

— If in the chromatogram of the sample extract, run on a particular capillary column, no peak appears at the substance specific retention time, consider the compound as not being detected.

— If a peak appears at a particular substance-specific retention time, the presence of the compound requested is possible. The identity of this compound shall be confirmed.

Repeat the complete comparison procedure, using capillary columns with stationary phases of different polarity.

Normally, the reliability of the identification increases with increasing difference in the polarities of the columns applied. If the comparative study with two capillary columns with stationary phases of different polarity reveals the presence of peaks at the expected substance-specific retention times, consider the identity of the substance as highly probable.

If confirmation is performed by mass spectrometry and if specific ions are present in the correct ratios for at least three to five specific ions at the substance-specific retention time, then consider the identity of the substance as highly probable (see annex C).

6.2 Calculation

6.2.1 Calculation using the (re)calibration according to 5.2.2

Calculate the mass concentration ρ_{ic} of the substance i using equation (6) after solving equation (1) for the mass concentration ρ_{ic}.

$$\rho_{ic} = \frac{y_i - b_i}{m_i \cdot A_i} \qquad (6)$$

where

ρ_{ic} is the mass concentration of the substance i in the water sample (corrected by mean recovery), in micrograms per litre;

y_i is the measured value of the substance i in the extract of the water sample (on the condition of the same procedure being applied as with the calibration and the sample measurement); the unit depends on the evaluation, for example, area value;

m_i is the slope of the calibration curve (5.2.2 or 5.2.5) of the substance i; the unit depends on the evaluation, for example, area value × (litres per microgram);

b_i is the axis intercept of the reference line on the ordinate; the unit depends on the evaluation, for example, area value;

A_i is the specific mean recovery for the substance i.

6.2.2 Calculation using the calibration according to 5.2.3

Calculate the mass concentration ρ_{ig} of the substance i in the water sample using equation (7) after solving equation (2) for the mass concentration ρ_{ig}:

$$\rho_{ig} = \frac{y_{ig} - b_{ig}}{m_{ig}} \quad (7)$$

where

ρ_{ig} is the mass concentration of the substance i in the water sample (corrected by recovery), in micrograms per litre;

y_{ig} is the measured value of the substance i in the extract of the water sample (on the condition of the same procedure being applied as with the calibration and the sample measurement); the unit depends on the evaluation, for example, area value;

m_{ig} is the slope of the calibration curve (5.2.3 or 5.2.5) of the substance i; the unit depends on the evaluation, for example, area value × (litres per microgram);

b_{ig} is the axis intercept of the reference line on the ordinate; the unit depends on the evaluation, for example, area value.

6.2.3 Calculation using internal standard according to 5.3

Calculate the mass concentration ρ_i of the substance i in the water sample using equation (8) after solving equations (3), (4) and (5).

$$\rho_i = \frac{\left[(y_i/y_l) - b_{il}\right] \cdot \rho_l}{m_{il}} \cdot \frac{F_V}{A_i} \quad (8)$$

where

y_i is the measured value of the substance i in the water sample; the unit depends on the evaluation, for example, area value;

y_l is the measured value of the internal standard I in the water sample; the unit depends on the evaluation, for example, area value;

ρ_i is the mass concentration of the substance i in the water sample, in micrograms per litre;

ρ_l is the mass concentration of the internal standard I, in micrograms per litre;

b_{il} see equation (5);

m_{il} see equation (5);

A_i is the specific mean recovery for the substance i;

F_V is the ratio of the final extraction solvent volume to sample volume.

6.3 Summary of results

When the procedure described is applied, gas chromatography provides one individual result for each column used. Derive the quantitative final result from these two individual results using a) or b) as follows.

a) Take the arithmetic mean, provided the differences between the individual results are less than 10 % relative to the lower result.

b) Choose the smallest value, in the case of larger differences, provided that this value is not caused by leakage in the gas chromatographic system. Larger differences may be the result of peak overlap. Such a result shall be reported as a measured value, obtained from a single separation only.

7 Expression of results

The mass concentrations of the organic nitrogen and phosphorus compounds shall be reported in micrograms per litre; at mass concentrations > 0,02 µg/l, expressed to two significant figures.

Precision data regarding liquid/solid extraction is given in annex D.

8 Test report

The test report shall refer to this International Standard and shall contain the following detailed information:

a) identity of the water sample;

b) method used (liquid/liquid or liquid/solid, by reference to the relevant clause of this International Standard);

c) sample preparation, if necessary (pH adjustment, extent of delay between sampling and extraction or between sampling and elution, filtration);

d) procedures used for extraction, concentration and separation (for liquid/liquid extraction), or solvents used for conditioning, elution, calibration solutions and procedure used for separation (for liquid/solid extraction), by reference to the relevant subclauses of this International Standard;

e) evaluation function used, in accordance with 6.2;

f) expression of results in accordance with clause 7;

g) any deviation from this method and any circumstances which might have affected the results.

Annex A
(informative)

Detection limits of certain organic nitrogen and phosphorus compounds

Detection limits and recovery rates of some organic nitrogen and phosphorus compounds in waste waters according to the liquid/liquid extraction method (clause 3) are given in Table A.1.

These values are given for information, as they depend on local practice, types of waste water, and equipment.

Table A.1 — Detection limits (liquid/liquid extraction method)

Compound	Detection limits µg/l	Recovery %
Atrazine	0,5	91
Fenpropimorph	1,0	94
Dimethoate	0,1	83
Vinclozolin	1,0	89
Metolachlor	0,5	83
Isochloridazon	0,5	100
Metazachlor	0,5	98
Simazine	0,5	90

Detection limits of some organic nitrogen and phosphorus compounds in drinking water, ground water and surface water according to the liquid/solid extraction method (clause 4) are given in Table A.2.

Table A.2 — Detection limits (liquid/solid extraction method)

Compound	Detection limits µg/l
Simazine	0,012
Atrazine	0,015
Dimethoate	0,024
Metolachlor	0,060
Metazachlor	0,060
Isochloridazon	0,060
Fenpropimorph	0,059
Vinclozolin	0,061

Annex B
(informative)

Examples of gas chromatograms

B.1 Conditions of chromatograph to obtain Figure B.1 chromatogram

DB 1701 column, 30 m length, 0,32 mm internal diameter; 1 µm film thickness

Injection volume: 1,5 µl

Injector: split 1:1, 250 °C

Detector: N/P, 255 °C

Carrier gas: He, ca. 2 ml/min

Temperature programme: 80 °C for 0 min, then 25 °C/min to 205 °C, hold for 10 min, then 20 °C/min to 240 °C, hold for 7 min, then 20 °C/min to 255 °C, hold for 14 min.