BRITISH STANDARD

BS EN ISO
17495:2003

Water quality — Determination of selected nitrophenols — Method by solid-phase extraction and gas chromatography with mass spectrometric detection

The European Standard EN ISO 17495:2003 has the status of a British Standard

ICS 13.060.50

Confirmed
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BSI
British Standards

National foreword

This British Standard is the official English language version of EN ISO 17495:2003. It is identical with ISO 17495:2001.

The UK participation in its preparation was entrusted by Technical Committee EH/3, Water quality, to Subcommittee EH/3/2, Physical, chemical and biochemical methods, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this subcommittee can be obtained on request to its secretary.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the *BSI Catalogue* under the section entitled "International Standards Correspondence Index", or by using the "Search" facility of the *BSI Electronic Catalogue* or of British Standards Online.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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English version

Water quality - Determination of selected nitrophenols - Method by solid-phase extraction and gas chromatography with mass spectrometric detection (ISO 17495:2001)

Qualité de l'eau - Dosage des nitrophénols sélectionnés - Méthode par extraction en phase solide avec détection par chromatographie en phase gazeuse et spectrométrie de masse (ISO 17495:2001)

Wasserbeschaffenheit - Bestimmung ausgewählter Nitrophenole - Verfahren mittels Festphasenanreicherung und Gaschromatographie mit massenspektrometrischer Detektion (ISO 17495:2001)

This European Standard was approved by CEN on 9 January 2003.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovak Republic, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
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Foreword

The text of ISO 17495:2001 has been prepared by Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 17495:2003 by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2003, and conflicting national standards shall be withdrawn at the latest by September 2003.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovak Republic, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of ISO 17495:2001 has been approved by CEN as EN ISO 17495:2003 without any modifications.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

EN ISO 17495:2003

INTERNATIONAL STANDARD

ISO 17495

First edition
2001-08-15

Water quality — Determination of selected nitrophenols — Method by solid-phase extraction and gas chromatography with mass spectrometric detection

Qualité de l'eau — Dosage des nitrophénols sélectionnés — Méthode par extraction en phase solide avec détection par chromatographie en phase gazeuse et spectrométrie de masse

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ISO 17495:2001(E)

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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 17495 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Annexes A to D of this International Standard are for information only.

Introduction

Several methods may be applied to determine nitrophenols in water. This International Standard describes a gas chromatographic/mass spectrometric determination after solid-phase extraction and derivatization with diazomethane. It should be investigated whether and to what extent particular problems will require the specification of additional marginal conditions.

Water quality — Determination of selected nitrophenols — Method by solid-phase extraction and gas chromatography with mass spectrometric detection

WARNING — Persons using this International Standard should be familar with normal laboratory practice. This International Standard does not purport to address all safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

1 Scope

This International Standard specifies a method for the determination of selected nitrophenols (see Table 1) in drinking, ground and surface water in mass concentrations > 0,5 µg/l[1)].

Table 1 — Nitrophenols to which this method is applicable

	CAS No.		CAS No.
2-Nitrophenol	88-75-5	2,4-Dinitrophenol	51-28-5
3-Nitrophenol	554-84-7	2,5-Dinitrophenol	329-71-5
4-Nitrophenol	100-02-7	2,6-Dinitrophenol	573-56-8
4-Methyl-2-nitrophenol	119-33-5	2,4-Dinitro-6-methylphenol	534-52-1
3-Methyl-4-nitrophenol	2581-34-2	2,6-Dimethyl-4-nitrophenol	2423-71-4
5-Methyl-2-nitrophenol	700-38-9	2,4-Dichloro-6-nitrophenol	609-89-2
3-Methyl-2-nitrophenol	4920-77-8	2,6-Dichloro-4-nitrophenol	618-80-4
CAS = Chemical Abstracts Service.			

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*.

1) See the results from the interlaboratory trial given in annex C.

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples*.

ISO 8466-1:1990, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*.

3 Principle

Solid-phase extraction of the nitrophenols is carried out from the acidified sample, followed by solvent elution, derivatization with diazomethane and determination by gas chromatography and mass spectrometry.

It is absolutely essential that the tests described in this International Standard be carried out by suitably qualified staff.

4 Interferences

4.1 Interferences during enrichment

In order to avoid losses, analyse the sample as soon as possible after sampling. If storage is unavoidable, store at 4 °C until sample pretreatment.

The commercially available adsorbance materials are often of varying quality. Considerable batch-to-batch variations in quality and selectivity of this material are possible.

The recovery may vary with the concentration, and shall therefore regularly be checked at different concentrations. Calibration and analysis shall be performed with material from one and the same batch only.

Suspended matter in the water sample (such as iron hydroxide, calcium carbonate) occurring on sampling, storage and sample preparation, as well as increased concentrations of microorganisms, may clog the packing. In this case the water sample may be filtered through a glass-fibre filter prior to the enrichment. A filtration step shall be mentioned in the test report.

Possible losses due to heating and volume reduction of the eluate may be reduced by the addition of a keeper (for example iso-octane)

4.2 Interferences in the gas chromatograph

The operating conditions shall be set in accordance with the manufacturer's instructions. These settings shall be checked at regular intervals.

General interferences, caused by the injection system or insufficient separation, can be eliminated with the help of special laboratory experience and the instrument manuals.

Excess diazomethane may cause instrument failure or damage, due to its high reactivity. Therefore care should be taken to remove excess diazomethane as far as possible by reducing the volume of the solution.

The use of alcohol as solvent should be avoided because any alcohol may destroy the deactivation layer (poly-siloxane) of the glass liner, leading to column load and an overload of the detector. In this case, the quantification of the analyte and the reproducibility of the result are no longer possible.

The stability of the analyte system should be checked (for example by application of a measuring standard).

5 Reagents

Reagents "for residual analysis" shall be used. Impurities of the reagents and of the water contributing to the blank shall be negligibly low. The blank shall be checked before use, especially prior to the use of a new batch.

5.1 Water, double-distilled or of comparable purity.

5.2 Operating gases for the gas chromatograph/mass spectrometer, in accordance with the manufacturer's instructions.

The operating gases shall be of high purity.

5.3 Nitrogen, high purity, minimum 99,996 % (volume fraction), for drying and eventually for concentration by evaporation.

5.4 Hydrochloric acid, c(HCl) = 2 mol/l.

5.5 Diethyl ether, $C_4H_{10}O$, stabilized only with ethanol.

5.6 Potassium hydroxide, KOH, aqueous solution, $\rho = 0{,}6$.

5.7 Ethanol, C_2H_5OH.

5.8 *N*-methyl-*N*-nitroso-4-toluenesulfonamide, (Diazald) $C_8H_{10}N_2O_3S$.

5.9 Acetic acid, CH_3COOH , aqueous solution, 10 % volume fraction (used to destroy diazomethane).

5.10 Solvents

5.10.1 Acetone, C_3H_6O.

5.10.2 Methanol, CH_3OH.

5.10.3 Ethyl acetate, $C_4H_8O_2$.

5.11 Methylated phenols

Reference substances as methylated substances according to Table 1, with defined concentrations for the preparation of calibration solutions for gas chromatography (8.3)

5.11.1 Standard stock solution of methylated single substances

Weigh 50 mg of each of the reference substances into a 100-ml measuring flask, dissolve in ethyl acetate (5.10.3) or acetone (5.10.1) and bring to volume with ethyl acetate or acetone.

Store the solution in a refrigerator (about 4 °C). The shelf-life is limited (about 3 months) and the concentration shall be checked before use.

5.11.2 Intermediate standard solution of methylated single substances

Pipette into 100-ml measuring flasks 1 ml of the solution of the single substance (5.11.1), and bring to volume with ethyl acetate or acetone.

Store the solution in a refrigerator (about 4 °C). The shelf-life is limited (about 3 months) and the concentration shall be checked before use.

5.11.3 Calibration solutions for multipoint calibration (methylated phenols).

Prepare the calibration solutions by adequate dilution of the stock solutions (5.11.2) with ethyl acetate (5.10.3).

Store the solution in a refrigerator (about 4 °C). Their shelf-life is limited (about 3 months) and their concentration shall be checked regularly.

5.12 Reference substances

Reference substances (see Table 1) with defined concentrations for preparation of calibration solutions, for determination of the recovery (9.5) and for calibration over the overall procedure (9.3).

5.12.1 Standard stock solutions of non-methylated phenols

Weigh 50 mg of each of the reference substances in a 100-ml measuring flask, dissolve with acetone (5.10.1) or ethyl acetate (5.10.3) and bring to volume with ethyl acetate (5.10.3).

Store the solutions in a refrigerator (about 4 °C). Their shelf-life is limited (about 3 months) and their concentration shall be checked before use.

5.12.2 Intermediate standard solutions of non-methylated phenols

Pipette into 100-ml measuring flasks 1 ml of the solution of the single substances (5.12.1), and bring to volume with ethyl acetate (5.10.3)

Store the solutions in a refrigerator. Their shelf-life is limited (about 3 months) and their concentration shall be checked before use.

5.12.3 Calibration solutions for multipoint calibration (non-methylated phenols)

Prepare the calibration solutions by adequate dilution of the intermediate standard solutions (5.12.2) with solvent.

Store the solutions in a refrigerator. Their shelf-life is limited (about 3 months) and their concentration shall be checked regularly.

5.13 Diazomethane solution

WARNING — Diazomethane is explosive, extremely toxic and severely irritating, causing pulmonary oedema when inhaled in high concentrations. Long-term, low-level exposure may lead to sensitization, resulting in asthma-like symptoms. Diazald and diazomethane should be regarded as toxic, carcinogenic and mutagenic. Handle with care.

Diazomethane may be prepared in a distillation apparatus or in a commercially available equipment, preferably in a fume cupboard. An example of a preparation method is as follows.

In a 250 ml reaction flask, add 8 ml of the KOH solution (5.6) and 10 ml of ethanol (5.7).

Dissolve 5,0 g of Diazald (5.8) in 45 ml of diethyl ether (5.5) in a filter funnel.

Cautiously warm the reaction flask to about 60 °C (water bath) and, within 20 min, dropwise add the solution from the filter funnel.

Collect the diazomethane being formed during this process and the diethyl ether in the cooled trap (cooling with ice/NaCl).

After this reaction, add a further 10 ml of diethyl ether through the filter funnel and distil the remaining diazomethane.

Stopper the trap and store at about – 18 °C for not longer than 1 month.

Glassware used in the preparation of diazomethane or which has come into contact with Diazald should be cleaned with a 10 % aqueous solution of acetic acid (5.9). It is advisable to clean the apparatus after rinsing the filter funnel and the reaction flask by distillation of about 50 ml of ethanol (5.7).

5.14 Internal standards

As internal standards, use two of the following phenols:

2,4-Dibromophenol, $C_6H_4OBr_2$, CAS No. 615-58-7

2,6-Dibromophenol, $C_6H_4OBr_2$, CAS No. 608-33-3

2,3,6-Trichlorophenol, $C_6H_3OCl_3$, CAS No. 933-75-5

2,4,6-Tribromophenol, $C_6H_3OBr_3$, CAS No. 118-79-6

Deuterated or ^{13}C-labelled substances are suitable as well.

NOTE The internal standards are used for the control of the analytical procedure. The choice of the substances depends on the expected phenols to be determined.

Prepare a mixed standard solution of the two components in a concentration which gives peak heights in the upper part of the linear range.

Usually, a concentration of 10 μg/ml is suitable; check the concentration prior to use.

6 Apparatus

Equipment or parts of it which may come into contact with the water sample or its extract should be free from residues causing significant blanks. It is recommended to use vessels made of glass or stainless steel.

6.1 Flat-bottomed flasks, preferably brown glass, 1 000 ml and 2 000 ml, with glass stoppers.

6.2 Cartridges, made of polypropene or glass, filled with solid-phase material, e.g. styrene/divinylbenzene polymer (see annex B).

6.3 Vacuum or pressure assembly for the enrichment step.

6.4 Measuring flasks, or graduated flasks with inert stopper, for the eluates.

In the case of autosampling, vials shall be made from glass and the septum covered with polytetrafluoroethene (PTFE).

6.5 Apparatus for preparing diazomethane, comprising:

— double-necked round-bottomed flask, 250 ml;

— filter funnel, 100 ml;

— distillation column, e.g. Vigreux column;

— distillation head;

— condenser, e.g. Liebig condenser;

— flask for absorption of diazomethane;

— security flask;

or a commercial distillation apparatus, preferably with fire-polished connections.

NOTE A closed system allows for a safer preparation of diazomethane.

6.6 Capillary gas chromatograph, equipped with a mass-spectrometric detector operated in the electron impact mode, gas supply according to the manufacturer.

The mass spectrometer should be capable of operating across the mass range of interest and incorporate a data system capable of quantifying ions using selected m/z values.

6.7 Injector, equipped for application with or without splitting, through-septum or on-column technique, or with temperature programming.

6.8 Capillary columns for gas chromatography (see examples in Table A.1).

6.9 Glass-fibre filters, made of borosilicate glass, fibre diameter e.g. 0,75 µm to 1,5 µm, with inorganic binding material.

6.10 Injection syringes, nominal capacity 5 µl or 10 µl.

7 Sampling

Collect samples in accordance with ISO 5667-1, 5667-2 and 5667-3.

Use for sampling carefully cleaned, preferably brown flat-bottomed glass flasks, of capacity 250 ml or 1 000 ml. Add 2 ml of hydrochloric acid (5.4) and fill to the brim with the sample.

In the case of possible presence of oxidizing agents, especially in presence of chlorine, add 1 g of sodium sulfite (Na_2SO_3) per 1 000 ml of sample.

The pH shall be < 2, if not, add more hydrochloric acid (5.4).

Prior to analysis, store the samples in the refrigerator. Storage time shall not exceed 3 days.

8 Procedure

8.1 Solid-phase enrichment

8.1.1 Pretreatment

As a rule, cartridges of 3 ml (sorbent mass 0,2 g) are suitable (see annex B for examples of suitable sorbents).

Condition the cartridges as follows:

a) Add 3 ml of ethyl acetate (5.10.3) , wait for 5 min, subsequently let it run dry.

b) Add 3 ml of methanol (5.10.2), and do not let it run dry.

c) Replace the methanol by 10 ml of water (5.1), acidified to pH 2. Do not let the cartridge run dry.

NOTE The size of the cartridges (volume, sorbent) depends on the mass concentration of phenols and the content of other organic substances present.

8.1.2 Enrichment

Start the enrichment immediately after conditioning.

Make sure that no air bubbles are trapped in the sorbent bed when changing from rinsing solution to sample.

Let a defined volume of the sample run through the column with a flowrate of 3 ml/min to 5 ml/min, make sure that the flowrate remains constant.

Rinse the sorbent with 10 ml of water (5.1), acidified to pH 2, and subsequently dry with nitrogen (5.3) at a flowrate of about 7 ml/s for at least 10 min.

NOTE The drying procedure may last up to 1 h. The end of the procedure is normally recognized by a brightening of the sorbent.

8.1.3 Elution

Elute with 2 ml of ethyl acetate (5.10.3), add the solvent in small portions at intervals of about 1 min.

Concentrate the eluate to about 0,5 ml using nitrogen (5.3) at room temperature.

NOTE As a rule and in case of dry sorbent, only 1 ml of ethyl acetate will allow complete elution. Larger volumes of solvents, or combinations of solvents, may be used if it is expected that the sorbent cannot be dried within a reasonable time. In these cases it may be advantageous to carry out a pre-elution with a small amount of methanol. This should be stated in the test report.

8.2 Derivatization with diazomethane

Add a sufficient volume of diazomethane solution (about 200 µl to 500 µl) (5.13), stopper the flask and allow the reaction to proceed in the dark.

After the reaction is complete (about 30 min; solution stays yellow), evaporate the solution with nitrogen (5.3) to a volume not smaller than 200 µl.

NOTE The addition of a keeper (e.g. about 0,05 ml of iso-octane) may be helpful to avoid losses during evaporation.

In the case of calibration with an external standard, bring the solution to volume with ethyl acetate (5.10.3).

8.3 Gas chromatography

Optimize the instrument parameters as described in the operator's manual.

Capillary columns with almost non-polar or non-polar stationary phases based on methyl silicones are especially suitable (see Table A.1). Depending on the case, a semi-polar or polar column may be helpful.

Use a mass spectrometric detector.

Record mass spectra in the full-scan mode for a relevant mass range within 35 m/z and 260 m/z, with the upper limit at least 10 m/z above the highest molecular mass of interest. The electron energy is set at approximately + 70 eV. If for the sake of sensitivity only selected ions are detected, register at least three diagnostic ions, preferably of the highest m/z values.

Annex D provides examples of some typical spectra.

8.4 Blank monitoring

Check the proper condition of instruments and reagents by blank monitoring at regular intervals.

For the blank measurements, prepare and analyse 1 000 ml of water (5.1) in the same way as the sample.

In case of blanks reading higher than the limit of detection, systematic investigations shall be carried out to detect and consequently eliminate the source of contamination.

8.5 Identification of individual compounds (see Table 2)

The target compound is present (is identified) in the sample analysed if:

— the relative or the absolute retention time measured in the sample does not differ by more than ± 1 % (or a maximum of ± 6 s) from the relative or the absolute retention time determined in the last measured external standard solution

and

Table 2 — Ethers and typical quantification masses (EI, 70 eV)

Substance (derivative)	Formula	CAS No.	Ion for quantification, *m/z*	
			First choice	Second choice
2,4-dibromoanisole	$C_7H_6Br_2O$	21702-84-1	266	264, 268
2,6-dibromoanisole	$C_7H_6Br_2O$	38603-09-7	266	264, 268
2,4,6-tribromoanisole	$C_7H_5Br_3O$	607-99-8	344	346, 329, 331
2,3,6-trichloroanisole	$C_7H_5Cl_3O$	50375-10-5	210	212, 195, 197
2-nitroanisole	$C_7H_7NO_3$	91-23-6	153	106, 92
3-nitroanisole	$C_7H_7NO_3$	555-03-3	153	92, 107
4-nitroanisole	$C_7H_7NO_3$	100-17-4	153	92, 123
4-methyl-2-nitroanisole	$C_8H_9NO_3$	119-10-8	167	120, 137
3-methyl-4-nitroanisole	$C_8H_9NO_3$	5367-32-8	167	150
3-methyl-2-nitroanisole	$C_8H_9NO_3$	5345-42-6	167	150
5-methyl-2-nitroanisole	$C_8H_9NO_3$	38512-82-2	167	150
2,6-dimethyl-4-nitroanisole	$C_9H_{11}NO_3$	14804-39-8	181	151
2,4-dichloro-6-nitroanisole	$C_7H_5Cl_2NO_3$	37138-82-2	221	223, 191, 193
2,6-dichloro-4-nitroanisole	$C_7H_5Cl_2NO_3$	17742-69-7	221	223, 191, 193
2,4-dinitroanisole	$C_7H_6N_2O_5$	119-27-7	198	168, 151
2,5-dinitroanisole	$C_7H_6N_2O_5$	3962-77-4	198	168
2,6-dinitroanisole	$C_7H_6N_2O_5$	3535-67-9	198	151, 198
2,4-dinitro-6-methylanisole	$C_8H_8N_2O_5$	29027-13-2	212	182, 165

— the relative intensities of all selected diagnostic ions measured in the sample do not deviate by more than $\pm (0{,}1 \times I + 10)$ % from the relative intensities determined in the external standard solution, where I is the relative intensity of the diagnostic ion in the external standard solution).

EXAMPLE Three selected diagnostic ions have the following relative intensities: 100 %, 50 % and 15 %.

The maximum allowed deviation in the sample is:

$\pm (0{,}1 \times 100 + 10)$ % = ± 20 %; relative intensity in the sample shall lie within 80 % and 120 %;

$\pm (0{,}1 \times 50 + 10)$ % = ± 15 %; relative intensity in the sample shall lie between 35 % and 65 %;

$\pm (0{,}1 \times 15 + 10)$ % = ± 11,5 %; relative intensity in the sample shall lie between 3,5 % and 26,5 %.

Only if identification of the target compound has been performed in the manner described above is the compound regarded as identified.

9 Calibration

9.1 General requirements

For practical reasons, the calibration is based on multicomponent solutions.

Make sure to achieve a linear dependence of signal to concentration.

Determine the linear working range using at least five measuring points of different concentration (in accordance with ISO 8466-1).

The calibration function for a substance is valid only for the measured concentration range. Additionally, the calibration function depends on the condition of the gas chromatograph and shall be checked regularly. For routine analysis, a check of the calibration function by measurement of two points is sufficient.

There are three different ways to set up the calibration function, the working range is then adjusted to the given demands (5.11 and 5.12):

— calibration of the GC-MS step (calibration with external standard, not covering the total procedure), (9.2);

— calibration of the GC-MS step with external standard (including the extraction and derivatization step), (9.3);

— calibration of the total procedure with internal standard (including the extraction step), (9.4).

The two first steps serve to estimate the recovery. For routine analysis, only calibration with internal standards should be applied.

Table 3 gives an explanation of the subscripts used in the equations and in the following text.

Table 3 — Definition of subscripts

Subscript	Meaning
i	Identity of the substance
e	Calibration step
I	Identity of internal standard
g	Overall procedure
j	Consecutive figure for pairs of values

9.2 Calibration of the GC-MS step (calibration with external standard, not covering the total procedure)

For each analyte, establish a calibration function from at least five points; it is practicable to include in one step all phenols mentioned in Table 1.

The knowledge of the retention times of the respective single substances is a prerequisite. These are evaluated with the aid of solutions of the single substances (5.11).

Establish the calibration function by injecting the calibration solution (5.11.3 for the methylated compounds).

The injection volume in the calibration step and in the measurement shall be the same.

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:

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

where

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

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

m_i is the slope of the calibration function of substance i, the unit depends on the evaluation, e.g. area value $\times$ litres per microgram;

b_i is the ordinate intercept of the calibration curve. The unit depends on the evaluation, e.g. area value.

9.3 Calibration with external standard of the overall procedure

To calibrate the entire procedure, pipette 1 ml of each calibration solution (5.12.3) into e.g. 1 000 ml of water (5.1).

Treat and analyse the solution as given in clause 8.

In accordance with 9.2, set up a calibration curve from the values y_{ieg} and ρ_{ieg}.

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

where

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

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

m_{ig} is the slope of the calibration curve of substance i, the unit depends on the evaluation, e.g. area value $\times$ litres per microgram;

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

9.4 Calibration using internal standards

When using internal standards, the determination of the concentration is independent from possible errors made during injection. Apart from this, errors caused by sample losses during distinct steps of sample pretreatment or the difficult adjustment to a (low) sample volume can be avoided. Additionally, the concentration determination is independent from matrix effects in the sample, provided the recoveries of the substance in question and the internal standard are about the same.

Choose as internal standard a substance with physicochemical properties similar to the substance to be determined (enrichment behaviour, retention time, derivatization ability). The internal standard shall not be present in the sample itself. The choice of a substance may be difficult and depends on the problem to be resolved; in any case, the suitability shall be checked. ^{13}C-labelled substances or nucleus-deuterated substances are especially suitable.

Add the internal standard l in a known amount to the water sample prior to analysis. The mass concentration ρ_{l} shall be the same for calibration and sample measurement.

For calibration over the total procedure, add to each of 1 000 ml of water (5.1) 1 ml of calibration solution (5.12.3) and the internal standard ρ_{l}, always in the same concentration.

Pretreat and analyse the samples as given in clause 8.

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

Plot the rational values $y_{\mathrm{iegj}}/y_{\mathrm{legj}}$ (peak areas, peaks heights or integration units) for each substance i on the ordinate and the associated rational mass concentration $\rho_{\mathrm{iegj}}/\rho_{\mathrm{legj}}$ on the abscissa.

Establish the linear regression function using the pairs of values $y_{\mathrm{iej}}/y_{\mathrm{lej}}$ and $\rho_{\mathrm{iej}}/\rho_{\mathrm{lej}}$ of the measured series in the following equation:

$$\frac{y_{\mathrm{ige}}}{y_{\mathrm{lge}}} = m_{\mathrm{igl}} \frac{\rho_{\mathrm{ieg}}}{\rho_{\mathrm{leg}}} + b_{\mathrm{igl}} \qquad (3)$$

where

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

y_{lge} is the measured response of the internal standard i, e.g. in the calibration, depending on ρ_{ie}; the unit depends on the evaluation, e.g. area value;

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

ρ_{leg} is the (independent variable) mass concentration of the internal standard, e.g. i, in micrograms per litre;

m_{igl} is the slope of the calibration curve from $y_{\mathrm{ie}}/y_{\mathrm{le}}$ as a function of the mass concentration ratio $\rho_{\mathrm{ie}}/\rho_{\mathrm{le}}$, often called the response factor;

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

9.5 Determination of procedural recovery values

Reliable recovery data are obtained from analysis of water and fortified control water samples (5.1) at different concentration levels, equidistantly spread over the working range. From these individual results a mean specific recovery $\bar{A}_{\mathrm{i}}$ is calculated.

Add to e.g. 1 000 ml of water (5.1) 1,0 ml each of the respective reference solutions (5.12.3) and proceed in the same way as described for a test sample.

a) Using the calibration function in 9.2, calculate the single mass concentrations $\rho_{i\ fnd}$ for each concentration level n and for each substance i;

b) calculate the single recovery $A_{i,n}$ according to equation (4).

$$A_{i,n} = \frac{\rho_{i,fnd}}{\rho_{i,nnom}} \cdot f \qquad (4)$$

where

$A_{i,n}$ is the recovery of substance i on the concentration level n_i;

$\rho_{i,\ fnd}$ is the recovered mass concentration of substance i on the concentration level n, determined according to equation (1), in micrograms per litre ;

$\rho_{i,\ nnom}$ is the original mass concentration of substance i on the concentration level n, in micrograms per litre;

f is the conversion factor; here $f = 100$.

Calculate with these single results the mean recovery $\bar{A}_i$ according to equation (5)

$$\overline{A_i} = \frac{\sum_{n=1}^{N} A_{i,n}}{N} \qquad (5)$$

where

n is the number of a single value $\bar{A}_{i,n}$;

$A_{i,n}$ is the recovery of substance i on the concentration level n_i;

$\bar{A}_i$ is the mean recovery of substance i, in percent;

N is the number of individual measurement values A_i.

With the ratio of mass to phase material as stated in clause 8, high recoveries are usually achieved. Low or unstable recoveries indicate matrix effects or difficulties during extraction.

10 Calculation

10.1 Calculation of single results after calibration with external standard, not covering the total procedure

Calculate the mass concentration ρ_i of the substance i according to equation (6).

$$\rho_i = \frac{(y_i - b_i)V_o}{m_i \cdot V_p \cdot \bar{A}_i} \cdot f \qquad (6)$$

where

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

y_i is the measured value of the substance i in the sample, e.g. area value;

m_i, b_i see equation (1);

V_o is the volume of the measured extract, in millilitres;

$\bar{A}_i$ see equation (5);

f conversion factor; here $f = 100$;

V_p is the sample volume, in millilitres.

10.2 Calculation of single results after calibration with external standard over the total procedure

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

$$\rho_{ig} = \frac{y_i - b_{ig}}{m_{ig}} \tag{7}$$

where

ρ_{ig} is the mass concentration of the substance i in the water sample, in micrograms per litre;

y_i is the measured value of the substance i in the extract of the water sample, e.g. area value;

m_{ig}, b_{ig} see equation (2).

10.3 Evaluation with the internal standard

Calculate the mass concentration ρ_{gi} of the substance using equation (8) after solving equations (3), (4) and (5).

$$\rho_{gi} = \frac{\frac{y_{gi}}{y_{gI}} - b_{igI}}{m_{igI}} \cdot \rho_{Ig} \tag{8}$$

where

y_{gi} is the measured value of the substance i in the water sample; e.g. area value;

y_{gI} is the measured value of the internal standard I in the water sample; the unit depends on the evaluation, e.g. area value;

ρ_{gi} is the mass concentration of the substance i in the water sample, in micrograms per litre;

ρ_{gI} is the mass concentration of the internal standard I, in micrograms per litre;

b_{igI} see equation (3);

m_{igI} see equation (3).

11 Expression of results

The mass concentration of the individual nitrophenol is reported in terms of micrograms per litre (μg/l), to two significant figures. In the case of mass concentration below 0,5 μg/l, only one significant figure is reported.

EXAMPLES

2-nitrophenol	1,8 μg/l
2,4-dinitrophenol	16 μg/l

12 Test report

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

a) identity of the sample;

b) expression of the results, in accordance with clause 11;

c) filtration, if applicable;

d) measuring conditions. It shall be reported whether the substances were identified by the full-spectra mode or only by identification of certain masses.

e) any deviations from this procedure and all circumstances which may have affected the results.

Annex A
(informative)

Examples of suitable capillary columns

Table A.1

Non-polar phase [a]	DB-1, HP-1, DB-5, HP-5, RTX-5, PTE-5, DBXLB
Medium polar phase [a]	OV 17, OV 1701, RTX-200
Polar phase [a]	DB-Waw, Durawax, DX 2
Column length	25 m to 30 m
Inner diameter	0,2 mm to 0,35 mm
Film thickness	< 0,3 µm
Temperature programme	from 60 °C (1 min) at 8 °C/min to 280 °C (5 min)
[a] These are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.	

Modern instruments are automatically adjusted to an optimal condition. As a general rule, the following marginal conditions may be set:

— instruments regulated by pressure: 2 bar of He pre-pressure;

— instruments regulated by flow: 1 ml/min of He.

Annex B
(informative)

Examples of sorbents suitable for solid-phase extraction of nitrophenols

Sorbent	Product name (supplier) [a]
Styrene/divinylbenzene copolymer	Lichrolut EN (Merck) Chromabond HR-P (Macherey & Nagel) SDB 1 (Mallinckrodt Baker)
Hydroxylated styrene/divinylbenzene copolymer	Isolute ENV + (International Sorbent Technology)
N-vinylpyrrolidone/divinylbenzene copolymer	Oasis HLB (Waters)

[a] These are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

Annex C
(informative)

Precision data

An interlaboratory trial on spiked surface water samples, carried out in Germany in autumn 1998, provided the results given in Table C.1.

Table C.1 — Precision data

Sample	Substance	L	N	NAP	$\bar{\bar{x}}$	x_{soll}	RR	s_R	CV_R	s_r	CV_r
				%	µg/l	µg/l	%	µg/l	%	µg/l	%
	2-Nitrophenol	6	21	4,6	1,36	1,00	136,3	0,387	28,4	0,165	12,1
	4-Methyl-2-nitrophenol	6	21	4,6	1,28	1,00	128,0	0,416	32,5	0,148	11,6
1	2,6-Dimethyl-4-nitrophenol	6	21	4,6	0,59	0,75	78,9	0,149	25,1	0,059	9,9
	2,4-Dichloro-6-nitrophenol	6	21	4,6	0,77	0,50	154,2	0,165	21,5	0,084	11,0
	2,5-Dinitrophenol	6	22	0,0	0,83	0,75	110,6	0,221	26,6	0,123	14,9
	2,4-Dinitro-6-methylphenol	6	22	0,0	0,53	0,50	106,3	0,199	37,4	0,077	14,5
	2-Nitrophenol	6	16	23,8	5,04	5,00	100,8	0,340	6,7	0,371	7,4
	4-Methyl-2-nitrophenol	6	21	0,0	5,11	5,00	102,2	0,508	9,9	0,438	8,6
2	2,6-Dimethyl-4-nitrophenol	6	21	0,0	4,11	3,75	109,7	1,029	25,0	0,403	9,8
	2,4-Dichloro-6-nitrophenol	6	20	4,8	7,99	7,50	106,5	0,775	9,7	0,709	8,9
	2,5-Dinitrophenol	6	20	4,8	6,46	6,25	103,3	1,952	30,2	1,330	20,6
	2,4-Dinitro-6-methylphenol	6	21	0,0	4,12	3,75	109,9	1,057	25,6	0,569	13,8

L is the number of laboratories after elimination of outliers;

N is the number of results after elimination of outliers;

NAP are the outliers, in percent;

x_{soll} is the true value, in micrograms per litre;

$\bar{\bar{x}}$ is the total mean, in micrograms per litre;

RR is the recovery rate, in percent;

s_R is the reproducibility standard deviation, in micrograms per litre;

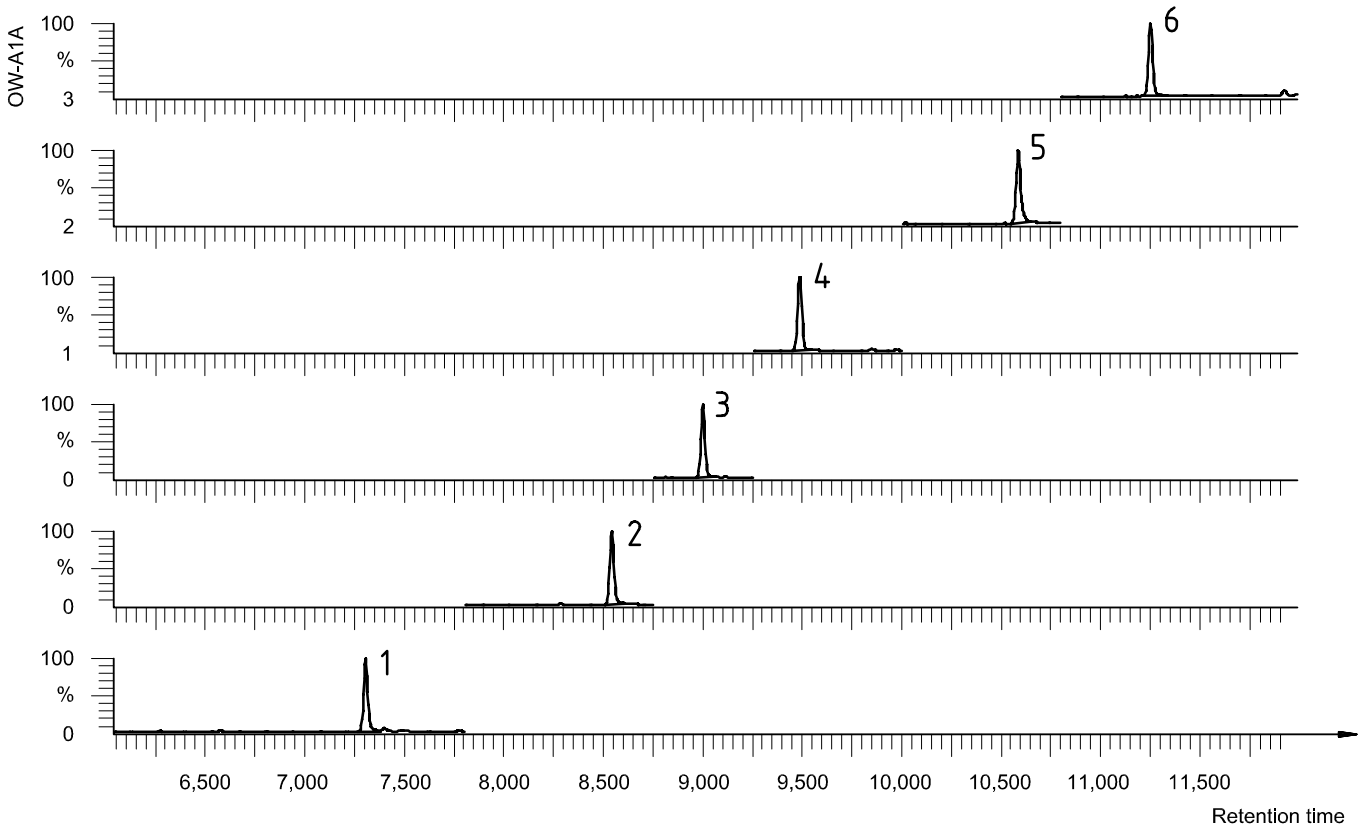
CV_R is the reproducibility variation coefficient, in percent;

s_r is the repeatability standard deviation, in micrograms per litre;

CV_r is the repeatability variation coefficient, in percent.

Annex D
(informative)

Example of typical spectra



Key

1 2-Nitrophenol, SIR of 3 Channels EI + TIC 2,87 10^8

2 4-Methyl-2-nitrophenol, SIR of 3 Channels EI + TIC 2,42 10^8

3 2,6-Dimethyl-4-nitrophenol, SIR of 3 Channels EI + TIC 1,52 10^8

4 2,4-Dichloro-6-nitrophenol, SIR of 3 Channels EI + TIC 1,94 10^8

5 2,5-Dinitrophenol, SIR of 2 Channels EI + TIC 4,84 10^7

6 2,4-Dinitro-6-methylphenol, SIR of 3 Channels EI + TIC 6,96 10^7

Figure D.1 — Surface water, spiked with six nitrophenols, solid-phase enrichment with Lichrolut EN

Sample:	Surface water, spiked with 0,1 mg of substance in 1 000 ml of water; enrichment with Lichrolut EN (200 mg); elution with 2 ml of ethyl acetate
Measurement:	GC/MS in MID mode
Column:	30 m HP-1ms × 0,25 mm × 0,25 μm
Temperature:	From 60 °C (1 min) at 8 °C/min to 280 °C (5 min)
Carrier gas:	He = 1,0 ml/min; flow-regulated

Bibliography

[1] Glastrup, J., Diazomethane preparation for gas chromatograpic analysis; *J. Chromatog. A*, **827** (1998), pp. 133-136.

Annex ZA
(normative)

Normative references to international publications with their relevant European publications

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

NOTE Where an International Publication has been modified by common modifications, indicated by (mod.), the relevant EN/HD applies.

Publication	Year	Title	EN	Year
ISO 5667-1	1980	Water quality - Sampling - Part 1: Guidance on the design of sampling programmes	EN 25667-1	1993
ISO 5667-2	1991	Water quality - Sampling - Part 2: Guidance on sampling techniques	EN 25667-2	1993
ISO 5667-3	1994	Water quality - Sampling - Part 3: Guidance on the preservation and handling of samples	EN ISO 5667-3	1995

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