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ISO 8165-2

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Water quality — Determination of selected monovalent phenols —

Part 2:
Method by derivatization and gas chromatography

Qualité de l'eau — Dosage des phénols monovalents sélectionnés —

Partie 2: Méthode par dérivatisation et chromatographie en phase gazeuse

Reference number
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International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@iso.ch

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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.

International Standard ISO 8165-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

ISO 8165 consists of the following parts, under the general title *Water quality — Determination of selected monovalent phenols*:

— *Part 1: Gas-chromatographic method after enrichment by extraction*

— *Part 2: Method by derivatization and gas chromatography*

Annex A of this part of ISO 8165 is for information only.

Water quality — Determination of selected monovalent phenols —

Part 2:
Method by derivatization and gas chromatography

1 Scope

This part of ISO 8165 specifies a method for the determination of phenols by gas chromatography, following pentafluorobenzoyl chloride (PFBC) derivatization. It may in particular be applied to the examination of drinking water, ground water and moderately contaminated surface water. With this method, lower limits of detection may be obtained compared with extraction procedures.

Since other reactive compounds such as amines and in some cases alcohols may also react, this method is not applicable in all cases to the examination of waste water. The applicability to the examination of waste water should be investigated for each individual case.

This method allows the determination of the phenols listed in Table 1 in a concentration range $\geqslant$ 0,1 µg/l. Other monovalent phenols may also be analysed using this method, but the applicability needs to be checked for each individual case.

Table 1 — Phenols to which this method is applicable

phenol	2-cyclopentyl-4-chlorophenol
2-methylphenol	4-chloro-2-benzylphenol
3-methylphenol	6-chloro-5-methyl-2-(1-methylethyl)phenol
4-methylphenol	2,3-dichlorophenol
2,4-dimethylphenol	2,4-dichlorophenol
4-ethylphenol	2,5-dichlorophenol
2,6-bis(1,1-dimethylethyl)-4-methylphenol	2,6-dichlorophenol
2-phenylphenol	2,4,6-trichlorophenol
2-benzylphenol	2,3,5-trichlorophenol
2-benzyl-4-methylphenol	2,4,5-trichlorophenol
2-chlorophenol	2,3,6-trichlorophenol
3-chlorophenol	2,3,4,5-tetrachlorophenol
4-chlorophenol	2,3,4,6-tetrachlorophenol
4-chloro-2-methylphenol	2,3,5,6-tetrachlorophenol
4-chloro-3-methylphenol	pentachlorophenol
6-chloro-3-methylphenol	
2,4-dichloro-3,5-dimethylphenol	
2-chloro-4-*t*-butylphenol	

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 8165. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 8165 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 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

The phenols contained in the unfiltered water sample are extractively derivatized by means of hexane and pentafluorobenzoyl chloride. The completion of the extractive derivatization is verified by the addition of the control solution (5.14). The gas chromatographic measurement uses two capillary columns of different polarity (simultaneous splitting) and detection with electron-capture detectors (ECD).

4 Interferences

Surfactants, emulsifiers or higher concentrations of polar solvents may affect the extractive derivatization step.

Suspended particles in the water may also interfere and reduce the recovery. A second liquid phase in the water sample (e.g. mineral oil compounds, highly volatile halogenated hydrocarbons, emulsified fats and waxes) disturbs sampling, sample preparation and the enrichment. In such cases the examination is restricted to the aqueous phase, and the portion of the non-aqueous phase is reported separately.

If problems are encountered in the use of the gas chromatographic system, reference should be made to the user's manual provided by the instrument manufacturer.

It is absolutely essential that the test described in this part of ISO 8165 be carried out by suitably qualified staff.

5 Reagents

5.1 General requirements

The content of monovalent phenols in water and reagents used shall be negligibly low, compared with the expected concentration levels. The water shall be suitable for trace analysis, its blank being determined in accordance with 8.1 and 8.2. If necessary, the water shall be purified by distillation at pH value > 12.

5.2 Sodium hydroxide solution, c(NaOH) = 1 mol/l.

5.3 Sodium sulfite, Na_2SO_3.

5.4 Sodium hydrogencarbonate solution, $c(NaHCO_3)$ = 1 mol/l.

5.5 Sulfuric acid

Carefully add 1 volume of sulfuric acid, $\rho(H_2SO_4)$ = 1,84 g/ml, to three volume portions of water.

5.6 Hexane, C_6H_{14}, highest purity grade.

5.7 Decane, $C_{10}H_{22}$.

5.8 Pentafluorobenzoyl chloride (PFBC), C_7OClF_5 (verify applicability, since batch-to-batch variations are likely to occur).

The purity of the PFBC is checked by running a blank chromatogram after an overall procedure blank sample preparation. If interfering peaks make a calculation impossible, the PFBC batch shall be rejected.

5.9 Sodium sulfate, anhydrous, Na_2SO_4.

5.10 Methanol, CH_3OH, or **acetone**, C_3H_6O; highest purity grade.

5.11 Phenol stock solutions

As an example, weigh at least 30 mg, to the nearest 0,1 mg, of each of the phenolic compounds in a 100 ml graduated flask and dissolve in methanol or acetone (5.10). For simultaneous determinations, several types of phenol may be dissolved in the appropriate volume of methanol. Keep the solutions cool (about 4 °C) and in dark glass bottles.

The solutions are stable for about four weeks.

5.12 Phenol standard solutions

Pipette 1 ml of the stock solution (5.11) into a 100 ml volumetric flask and make up to volume with methanol. The solution contains 3,0 mg/l of phenol. Prepare further standard solutions in accordance with the working range selected.

5.13 Stock solutions for the internal control solution

As an example, dissolve 0,1 g of 2,4-dibromophenol or 2,5-dibromophenol in 100 ml of methanol (5.10).

5.14 Standard solutions for the control solution

As an example, dilute 1 ml of the stock solution (5.13) with methanol (5.10) to 100 ml.

6 Apparatus

6.1 Flat-bottomed brown glass bottles, with conical shoulder, of capacity 250 ml and 1 000 ml.

Bottles should be rigorously cleaned.

6.2 Separating funnels, of capacity 100 ml and 250 ml, with stopcocks made of polytetrafluoroethene.

6.3 Graduated flasks, of capacity 5 ml, 10 ml and 100 ml.

6.4 Graduated round bottom flask, of capacity 50 ml, with a tapered tip in the base.

6.5 Graduated cylinder, of capacity 250 ml.

6.6 Injection syringes, of capacity 100 µl, 250 µl and 500 µl.

6.7 Gas chromatograph, with a glass insert and electron-capture detector (ECD), gas supply in accordance with the respective manufacturer's instructions.

7 Sampling and sample preparation

Sampling shall be carried out in accordance with ISO 5667-1, ISO 5667-2 and ISO 5667-3.

Place into brown, 250 ml to 1 000 ml bottles (6.1), 2 ml of sulfuric acid (5.5) per 1 000 ml of sample volume and fill the bottles completely with the water sample.

The pH value shall be < 2 (to be measured); if necessary add more acid.

Store the bottles in a refrigerator at about 4 °C until analysis.

If oxidizing agents are suspected, especially in presence of free chlorine, add approximately 1 g of sodium sulfite (5.3) per litre of sample.

Carry out the enrichment within preferably 48 h (see 8.2), but within 1 week.

8 Procedure

8.1 Clean-up procedure

Place 80 ml of the water sample into a 250 ml separating funnel.

Add sodium hydroxide solution (5.2) until pH 11 is reached (approximately 20 ml are required).

Add 10 µl of the control solution (5.13) and 20 ml of hexane (5.6).

Shake vigorously for 2 min and, after the phases have separated, run the aqueous solution into a 100 ml separating funnel. Discard the organic phase.

8.2 Extractive derivatization

Carry out the extractive derivatization immediately after the preliminary sample clean-up, by the following procedure.

Along with each series of analytical samples, an examination of the blank and the calibration shall be carried out in exactly the same way (overall procedure).

Reproducibility and repeatability shall be checked regularly.

Add 20 ml of sodium hydrogencarbonate solution (5.4), 20 ml of hexane (5.6), and then 20 µl of pentafluorobenzoyl chloride (5.8) to the hexane phase in the separating funnel.

Immediately shake vigorously for precisely (5 ± 0,1) min.

Allow 10 min for phase separation and centrifuge, if necessary.

Discard the aqueous solution.

Add 50 ml of sodium hydroxide solution (5.2) to the organic phase and shake for 1 min.

Allow the phases to separate and filter the organic phase through anhydrous sodium sulfate (5.9) into a test tube and analyse the extract by gas chromatography.

NOTE The extract may be concentrated, if necessary: transfer the extract into a round-bottomed flask (6.4), add 0,5 ml of decane (5.7) as keeper (to prevent losses of low-boiling phenol components) and evaporate at 40 °C to a final volume of 1 ml.

9 Gas chromatography

9.1 General requirements

The suitability of the capillary columns used shall be investigated with the examination of derivatized phenols. Non-polar and medium-polar separation capillaries are suitable, provided baseline-separated peaks, free from tailing, will be obtained. An electron-capture detector (ECD) may generally be used. To ensure qualitative and quantitative results, the compounds shall be analysed on two capillary columns of significantly differing polarity. It is advantageous to connect both columns to one injector for simultaneous sample application. However, with this technique, misinterpretation caused by peak overlapping cannot completely be ruled out. In this event, two quantitative results will be obtained, with the lower value probably being more accurate.

GC/MS may be used to confirm the identity of the phenol derivatives.

NOTE Suggested pairs of capillary columns are given in annex A.

9.2 Measurement of the blank

Prior to the examination, and if necessary, during analysis, blank determinations with water (5.1) shall be performed. The blanks shall be run through the complete analytical procedure, starting with the sampling and including all steps, down to the gas chromatographic evaluation. The blank results shall be negligibly low. If not, the cause for this shall be found by a step-to-step examination of the procedure. The blanks shall then be minimized by adequate procedures. Subtraction of the blank is not recommended if the standard deviation of the blanks exceeds the standard deviation of the overall procedure. In this case the blank shall be lowered.

9.3 Identification of the individual compounds

Individual compounds in the sample are identified by comparing the retention times of the respective peaks in the chromatograms with those of the substance peaks, measured under identical conditions, in the chromatogram of a reference solution.

NOTE If a mass spectrometer is used as detector, the individual compounds may be identified by interpretation or comparison of the mass spectra.

10 Calibration

Calibration covering the overall procedure shall be carried out with external standard (including the extraction and derivatization steps, according to 8.2).

The working range is adapted to the expected concentration range (see clauses 8 and 9) and is chosen to obtain a linear calibration function.

The calibration function established for a given substance is valid only for the calibration range applied. In addition, it depends on the operating conditions of the chromatographic system and on the quality of the derivatization agent. Consequently, the calibration shall regularly be checked.

The designation of the indices used in calculation of the calibration function are listed in Table 2.

Table 2 — Meaning of the subscripts

Subscript	Meaning
i	Identity of the substance
e	Measured value in the calibration
j	Consecutive figure for pairs of values
g	Reference to the overall procedure

As an example:

Use 1 ml of standard solution (5.12) and make up to 100 ml with water (5.1) [ρ(phenol) = 30 μg/l].

Prepare further standard solutions covering the working range chosen.

The total content of methanol in the standard solution shall not exceed 1 %.

A calibration function for each substance to be determined shall be established from at least five points.

It is advisable to examine all phenols in question in one working cycle. The retention times of the respective phenol derivates shall be known.

Derivate and extract the solution as described in clause 8, and analyse. Maintain the same conditions for calibration and measurements.

For the calibration of the overall procedure, add, at least six times, 1 ml of stock solution (5.11) to each 100 ml portion of water (5.1).

Carry out the extractive derivatization as described in 8.2 and analyse.

Establish the calibration function graphically by plotting the measured values y_{iej} (peak areas, peak heights or integration units, respectively) for each substance i on the ordinate, and the allocated mass concentrations ρ_{iej} on the abscissa.

In case of a satisfactory linearity (in accordance with ISO 8466-1), estimate the function of the regression line, using equation (1):

$$y_{ieg} = m_{ig}\rho_{ieg} + b_{ig} \quad (1)$$

where

y_{ieg} is the measured value of substance i obtained from the calibration, depending on ρ_{ieg}, its unit depends on the evaluation, e.g. area value;

ρ_{ieg} is the mass concentration of substance i (external standard in the reference solution), in micrograms per litre;

m_{ig} is the slope of the calibration line of substance i (corresponding to the substance-specific response factor), e.g. in area value × litres/microgram;

b_{ig} is the ordinate intercept of the calibration line.

11 Calculation of results

Calculation of the individual results on calibrating with external standard covering the overall procedure.

The mass concentration ρ_{ig} of substance i is calculated using equation (2):

$$\rho_{ig} = \frac{y_{ig} - b_{ig}}{m_{ig}} \quad (2)$$

where

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

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

m_{ig}, b_{ig} see equation (1), substance i.

12 Expression of results

The mass concentrations of the individual phenols shall be expressed in micrograms per litre, with at most two significant figures.

EXAMPLES

Phenol	17 µg/l.
Pentachlorophenol	0,5 µg/l.

13 Test report

The report shall refer to this part of ISO 8165 and contain the following information:

a) complete identity of the water sample;

b) expression of results in accordance with clause 12;

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

Annex A
(informative)

Capillary column pairs

Table A.1 — Capillary column pairs that may be used

Pairs of capillaries (for example)	**Temperature programme** (suggested)
OV 1701 / SE 54	65 °C; 3 °C/min; 220 °C
SE 30 / SE 54 DB 1 / DB 1701	140 °C; 10 min isoth.; 5 °C/min; 270 °C; 10 min
DB 5 / DX 2	70 °C; 1 min isoth.; 8 °C/min; 220 °C
Column length: 25 m to 40 m Inner diameter: 0,2 mm to 0,3 mm Film thickness: 0,2 µm to 0,3 µm	
NOTE Column length, inner diameter and film thickness shall be the same in both columns in order to achieve a split ratio of 1:1.	

Bibliography

[1] RENBERG, L. Gas chromatographic determination of phenolic compounds in water, as their pentafluorobenzoyl derivatives; *Chemosphere*, **10** (1981), pp. 767-773.

[2] Her Majesty's Stationery Office. *The Determination of Microgram and Submicrogram Amounts of Individual Phenols in River and Potable Waters*, London, 1988.

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