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Water quality — Determination of tributyltin (TBT) in whole water samples — Method using solid phase extraction (SPE) with SPE disks and gas chromatography with triple quadrupole mass spectrometry

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Water quality - Determination of tributyltin (TBT) in whole water samples - Method using solid phase extraction (SPE) with SPE disks and gas chromatography with triple quadrupole mass spectrometry

Qualité de l'eau - Dosage du tributylétain (TBT) dans la totalité des échantillons d'eau - Méthode par extraction sur phase solide (SPE) avec disques SPE et chromatographie en phase gazeuse avec spectrométrie de masse triple quadrupôle

Wasserbeschaffenheit - Bestimmung von Tributylzinn (TBT) in Gesamtwasserproben - Verfahren mittels Festphasenextraktion (SPE) mit SPE-Disks und Gaschromatographie mit Triple-Quadrupole Massenspektrometrie

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Foreword

This document (CEN/TS 16692:2015) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

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This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of the Water Framework Directive (WFD, 2000/60/EC), and the Directive on Environmental Quality Standards (Directive 2008/105/EC).

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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

IMPORTANT — It is absolutely essential that tests conducted according to this Technical Specification be carried out by suitably trained staff.

Introduction

Tributyltin (TBT) is a priority substance listed in Annex X of the EU Water Framework Directive (WFD, Directive 2000/60/EC) for which Environmental Quality Standards (EQS) have been set at EU level for inland as well as other surface waters to protect the aquatic environment against chemical pollution (Directive 2008/105/EC). With the exception of metals, the EQSs are expressed as total concentrations in the whole water sample. Furthermore, analytical methods used in WFD monitoring need to meet certain requirements as regards the minimum limit of quantification and the maximum tolerable measurement uncertainty (Directive 2009/90/EC). So far, there is no standardized method available for the determination of TBT in whole water samples fulfilling those requirements. Hence, the European Commission mandated CEN to develop or improve standards in support of the implementation of the monitoring requirements of WFD.

Directive 2008/105/EC has been amended by Directive 2013/39/EU, however this standard has been developed for the analysis of TBT as listed in Annex A of Directive 2008/105/EC.

The annual average environmental quality standard (AA-EQS) value for TBT is 0,0002 µg/l and is defined for the concentration in the whole water sample, including suspended particulate matter (SPM) present in the sample. As compounds like TBT, sorb strongly to environmental solids, the fraction bound to particles may be substantial. Therefore it is important to be able to handle whole water samples within the analytical process. Identification and quantification of TBT at trace level concentrations often require both high sensitive chromatographic equipment and effective enrichment steps.

1 Scope

This Technical Specification specifies a method for the determination of tributyltin cation (TBT) in whole water samples. It is applicable to the analysis of TBT in surface water, which may contain suspended particulate matter (SPM) up to 500 mg/l (whole water samples), groundwater, drinking water and seawater. The working range is 0,04 ng/l to 20 ng/l. The LOQ will be mainly determined by the blank value obtained during validation of this method.

NOTE 1 The method has been successfully applied to seawater samples during method development, but sea water samples were not included in the interlaboratory comparison.

NOTE 2 In this document TBT is synonymous for tributyltin cation.

NOTE 3 Near the lower limit of the working range the measurement uncertainties may be higher.

2 Normative references

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

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696)*

EN ISO 5667-3, *Water quality - Sampling - Part 3: Preservation and handling of water samples (ISO 5667-3)*

3 Principle

TBT in the whole water sample is derivatized while stirring thoroughly. The derivatized target analyte is extracted using solid phase extraction with SPE disks and subsequently concentrated by evaporation. An aliquot of the extract is brought on the gas chromatograph with programmed temperature vaporizing - large volume injection (PTV-LVI) and identified and quantified with a triple quadrupole mass spectrometric detection in single GC-MS reaction mode.

Water samples of 1 l are spiked with internal standards and brought to pH 4 to pH 5 with a sodium acetate buffer. Subsequently, TBT is ethylated by adding sodium tetra ethylborate ($NaEt_4B$) to the water samples, while rigorously stirring. Solid phase disk extraction is used for extracting ethylated TBT with mainly hexane as a solvent. The extract is then brought to pH 12 with sodium hydroxide [1]. The final extract is then concentrated to 300 µl. A volume of 20 µl is injected into the gas chromatograph using a PTV-LV injection technique followed by GC-MS/MS in single GC-MS reaction mode for separation and detection.

A deuterium-labelled TBT substance is used as internal standard for quantification of TBT. A TBT-spiking solution is used for the determination of procedural recovery values. A tri-alkylated spiking solution is used for checking the efficiency of the alkylation process and is added to each sample. Tetra-alkylated reference solutions are used for the calibration.

4 Interferences

The reagents can contain impurities of organotin compounds, including the derivatization reagent. It is absolutely essential to verify contamination before use by analysis of blanks. Glassware can become contaminated. Heat all used glassware to 450 °C, or clean the glassware with a 10 % (volume fraction) nitric acid solution before use, by decontamination overnight. Make sure the GC-system is not contaminated. Regularly refresh the wash solvents to ensure that no contamination occurs via the injection needle. Verify the GC-system before analysis by analysis of blank solvent.

Commercially available solid-phase extraction disks (SPE-disks) differ frequently in quality. Variations in the selectivity of the materials also frequently occur from batch to batch, thus possibly causing significant

deviations in extraction yield. This does not basically impair their suitability, apart from a resulting higher detection limit of individual substances. To ensure that the measuring results show high trueness and precision, use materials of one batch for both measurement and calibration. Avoid major fluctuations in the extraction times and elution procedures within one sample sequence when analysing the samples. SPE - disks may be contaminated with mono-, di- or tributyltin cation compounds. Verify the contamination of the disks before use. Pre-cleaning can be achieved by pre-extracting the disks with a derivatization agent. To thoroughly clean magnetic stirrers, they can be shaken in a methanol-hexane solution (9:1, v/v) with 1 ml of 2 % derivatization agent. Decontaminate overnight and rinse three times with deionized water.

5 Reagents

The reagents shall be free from impurities possibly interfering with the GC-MS analysis.

Use solvents and reagents of sufficient purity, i.e. with negligibly low impurities compared with the concentration of analytes to be determined. As reagents use, as far as available “residual grade” or better in order to obtain low blanks. Verify by blank determinations and, if necessary, apply additional cleaning steps.

5.1 Water, Grade 1 according to EN ISO 3696.

5.2 Operating gases for the gas chromatography mass spectrometry, of high purity and in accordance with manufacturer’s specifications.

5.3 Nitrogen of high purity, i.e. minimum 99,996 % by volume, for concentration by evaporation.

5.4 Solvents for extraction, chromatography and preparation of reference solutions.

— hexane, C_6H_{14}, (boiling point: 69 °C);

— *iso*-octane, C_8H_{18} (boiling point: 99 °C);

— *iso*-propanol, C_3H_7OH (boiling point: 82 °C);

— methanol, CH_3OH (boiling point: 65 °C).

5.5 Acetate buffer solution, solution, $c(CH_3COONa)$ = 4 mol/l.

5.6 Sodium hydroxide, solution, $c(NaOH)$ = 10 mol/l.

5.7 Hydrochloric acid, $w(HCl)$ = 36 % to 38 % mass fraction.

5.8 Nitric acid, $v(HNO_3)$ = 10 % volume fraction.

5.9 Aluminium oxide, activated at 800 °C for 4 h, (10 ± 2) % deactivated.

5.10 Sodiumtetraethylborate, $w(NaEt_4B)$ = 2 % mass fraction, (CAS Registry Number 15523-24-7).

5.11 Reference ethyl-TBT stock solution

This substance is used for the calibration. Use a commercially available solution, e. g. in *iso*-octane or the commercially available pure substance (Table 1). The stock solution is prepared by dissolving, e. g. 5 mg of the reference substance (precision of 0,001 mg) in 20 g of an appropriate solvent (precision of 0,01 g) e. g. hexane or *iso*-octane. Store stock solutions at temperatures between 1 °C and 5 °C, protected from light. Stock solutions with concentrations > 1 mg/g are stable for at least 12 months and at least 3 months for concentrations > 1 µg/g.

5.12 Reference TBT stock solution

This substance is used for spiking water samples for determination of procedural recovery values. Use a commercially available solution of TBT, e. g. TBT-chloride in *iso*-octane or the commercially available pure substance (Table 1). Stock solution is prepared as described in 5.11, with e. g. *iso*-propanol or methanol as solvent. Stock solutions with concentrations > 1 mg/g are stable for at least 12 months and at least 3 months for concentrations > 1 µg/g.

5.13 Derivatization standard stock solution

In order to check the efficiency of the derivatization process, choose a tri-alkylated cation substance not present in the sample with similar physicochemical properties as TBT, e. g. TPrT-Cl (Table 1). The ethylderivative of the derivatization standard, e. g. ethyl-TPrT (Table 1) is used for calibration. Prepare stock solutions of the derivatization standard substances in the same way as specified for the reference stock solutions (5.11 and 5.12). Stock solutions with concentrations > 1 mg/g are stable for at least 12 months and at least 3 months for concentrations > 1 µg/g.

5.14 Internal standard solution

5.14.1 Internal standard for calculation of TBT concentration

As internal standard for quantification of TBT, a deuterium-labelled substance is used. The ethylderivative is used in the calibration. In Table 1 internal standards are listed. Prepare stock solutions of the internal standard substances in the same way as specified for the reference stock solutions (5.11 and 5.12).

^{13}C-enriched TBT might be used as an alternative internal standard, but suitability should be checked, as it has not been used in the method development nor in the interlaboratory comparison.

5.14.2 Internal standard for calculation of the derivatization standard

For calculation of the derivatization standard (5.13) concentration use, e. g. tetrapropyltin (Table 1) as internal standard or choose a substance with similar physicochemical properties as the derivatization standard (extraction behaviour, retention time). The internal standard should be tetra-alkylated. Prepare stock solutions of the internal standard substance in the same way as specified for reference stock solution (5.11).

5.15 Calibration working solutions

Use the ethyl derivative of TBT (5.11) and the derivatization standard (5.13) of a defined concentration, used for gas chromatography and suitable for the preparation of calibration solutions. Prepare a minimum of seven calibration solutions with concentrations according to the detection capability of the mass spectrometer. Combine and dilute internal standards (5.14) with hexane and *iso*-octane to produce solutions for the calibration range in a hexane - *iso*-octane (9:1, volume fraction) solvent. Store calibration solutions for maximum 3 days at temperatures between 1 °C and 5 °C protected from light.

5.16 TBT reference substance solution

Use TBT (5.12) of defined concentration, suitable for the preparation of a reference solution used for spiking water samples. Spike blank water samples for the determination of procedural recovery values (9.4).

5.17 Derivatization standard solution

Use the tri-alkylated derivatization standard (5.13) of defined concentration, suitable for the preparation of reference solutions used for spiking water samples. Spike each sample for calculation of the recovery (9.5).

Table 1 — Reference substances, internal and derivatization standards and their ethyl-derivatives

Substance	Abbreviation	Molecular formula	Molar mass g/mol	EC number[a]	CAS RN[b]
Reference substance and ethyl derivative					
Tributyltin chloride (5.12)	TBT-Cl	$C_{12}H_{27}SnCl$	325,51	215–958–7	1461–22–9
Ethyltributyltin (5.11)	Ethyl-TBT	$C_{14}H_{32}Sn$	319,11	not applicable	19411–60–0
Deuterated internal standard and ethyl derivative (5.14.1)					
Tributyltin-d_{27}chloride	d_{27}-TBT-Cl	$C_{12}D_{27}SnCl$	352,51	not applicable	–
Ethyltributyltin-d_{27}	Ethyl-d_{27}-TBT	$C_{14}H_5D_{27}Sn$	346,11	not applicable	–
Tetra-alkyltin standard (5.14.2)					
Tetrapropyltin	TePrT	$C_{12}H_{28}Sn$	291,05	218–536–0	2176–98–9
Derivatization standard and ethyl derivative (5.13)					
Tripropyltin chloride	TPrT-Cl	$C_9H_{21}SnCl$	283,41	218–910–3	2279–76–7
Ethyltripropyltin	Ethyl-TPrT	$C_{11}H_{26}Sn$	277,03	not applicable	3440–79–7

[a] EC Number: European inventory of existing commercial substances (EINECS) or European list of notified chemical substances (ELINCS).

[b] CAS RN: Chemical Abstracts Service Registry Number.

6 Apparatus

6.1 General

Equipment or parts of it which are likely to come into contact with the water sample or its extract shall be free from residues causing interferences. The use of vessels made of glass is recommended. Heat all glassware to 450 °C, or clean the glassware with 10 % (volume fraction) nitric acid solution. Decontaminate overnight and rinse three times with deionized water.

6.2 Sample flasks, e. g. brown glass, flat bottomed, with glass or PTFE coated stoppers, e. g. 250 ml or 500 ml and 1 000 ml.

6.3 Magnetic stirrer, including PTFE-coated magnetic stir bar of suitable size.

6.4 Drying ovens, capable of maintaining temperatures in the ranges of 100 °C to 800 °C for baking and storage of clean-up materials and maintaining temperatures up to 450 °C for baking glassware.

6.5 Solid-phase extraction disks (SPE disks), wide inner diameter: e. g. 45 mm, packed with an appropriate reversed phase adsorbent material, e. g. C_{18}-based or DVB-based adsorbent.

6.6 Vacuum device for solid-phase extraction, e. g. vacubox, extraction box or automated workstation for solid-phase extraction procedure capable for processing SPE-disks or automatic solid disk extractor, capable of extracting water samples up to 1 l with organic solvents and with automatic cleaning cycle.

6.7 Glass columns for chromatographic clean-up.

6.8 Evaporation device, based on nitrogen flow evaporation or rotary evaporator under reduced pressure.

6.9 Syringes, 10 µl, 25 µl, 50 µl, 100 µl, 1 000 µl, volume precision ± 2 %.

6.10 Single volume pipettes, capacities between 100 µl and 50 ml.

6.11 Glass sample vials, dark coloured glass capacity e. g. 2 ml with 300 µl inserts, with inert cap and PTFE-coated septum.

6.12 Capillary gas chromatograph with a triple quadrupole mass spectrometer, (GC-MS/MS) using single GC-MS reaction mode, gas supply in accordance with the respective manufacturer's instructions.

6.13 Non-discriminating GC injector, e. g. splitless mode of a split or splitless injection system and programmable temperature vaporiser (PTV), programmable for large volume injection (LVI).

6.14 Automatic sampler with option for large volume injection (LVI), including syringes for normal injection (e. g. 1 µl and 2 µl) and LVI (e. g. 50 µl and 100 µl).

6.15 Capillary column, for gas chromatography, fused silica column with non-polar low bleed separating phase (e. g. 5 % diphenyl 95 % dimethyl polysiloxane, length 20 m, inner diameter 0,18 mm and film thickness 0,18 µm).

6.16 Pasteur pipettes

6.17 pH indicator strips, for the appropriate pH-ranges.

7 Sampling

For sampling, use thoroughly cleaned, flat bottomed glass flasks (6.2). Fill the bottles completely with the water to be examined. Samples should be brought to pH (2 ± 0,2) with hydrochloric acid (5.7) within 24 h.

If extraction requires the sample flask to be included in the working process, it is recommended to fill the bottle to the shoulder (e.g. about 1 000 ml or 2 000 ml when using a 1 000-ml or 2 000-ml flat bottomed sample flask).

Treat and analyse the samples as soon as possible after sample collection as specified in EN ISO 5667-3. Store the samples at temperatures between 1 °C and 5 °C, protected from light.

NOTE It is best to carry out the extraction as soon as practicable to minimize potential adsorption effects to glass.

8 Procedure

8.1 Sample preparation

In general, samples are examined without pre-treatment, e. g. suspended particulate matter is not removed prior to analysis. Do not filter the sample.

Large particles (e. g. leaves, little branches) should be removed using a metal sieve (screening gap 1 mm).

Calculate the exact volume of the water sample by weighing the sample flask before extraction and after emptying.

8.2 Derivatization

8.2.1 General

Bring a magnetic stirring rod (6.3) in the sample container (6.2), e. g. 1 000 ml flat-bottomed flask.

Add a defined amount of internal standard (5.14) and derivatization standard (5.17) dissolved in an appropriate solvent (e. g. *iso*-propanol) and stir for about 1 min.

Stir vigorously for minimum 15 min.

Add 12 ml of acetate buffer solution (5.5) and stir for about 1 min. Check the pH and, if necessary, adjust to between pH 4 and pH 5 using hydrochloric acid (5.7) or sodium hydroxide solution (5.6).

8.2.2 Derivatization procedure

While stirring the samples (about 1 200 min^{-1}) add 2 ml of the derivatization agent (5.10) to the buffered solution (8.2.1) by adding aliquots of 200 µl during a 10 min period. Stir for a further 50 min.

NOTE 1 Adding of the derivatizating agent can be automated by use of e. g. a peristaltic pump and adding at a rate of about 0,2 ml/min

NOTE 2 Adding the reagent at once may result in reduced derivatization efficiencies.

8.3 Extraction with SPE-disks

For washing the SPE disks, add 10 ml of hexane and let it pass through the cartridge in about 20 s, e. g. using a vacuum device.

For conditioning of adsorbent disks add 10 ml of methanol and let it pass through the cartridge in about 20 s, e. g. using a vacuum device. Ensure that the adsorbent does not run dry. Repeat this step once.

Add 10 ml of water and let it pass through the disk in about 20 s, e. g. using a vacuum device. Ensure that the adsorbent does not run dry. Repeat this step twice.

For sample loading and extraction of analytes dissolved in the water phase take, for example, 1 000 ml of the sample to be examined and pass it through the adsorbent conditioned as described above at a flow rate of about 50 ml/min. Rinse the sample reservoir (e. g. the sample bottle) twice with 10 ml of water and pass it through the adsorbent as described above. Dry the adsorbent using a vacuum device or by a gentle stream of nitrogen for at least 5 min.

Perform extraction and elution as follows.

Add 5 ml of methanol, allowing 2 min for the solvent to soak. Collect the eluate by passing it through the cartridge in about 20 s.

Add 5 ml of hexane, allowing 5 min for the solvent to soak. Collect the eluate by passing it through the cartridge in about 20 s. Repeat this step twice.

Collect the combined eluates in a glass vessel. No extract-drying step is required.

Add 200 µl of sodium hydroxide solution (5.6) to the eluate and shake for 1 min.

Check the pH. The pH should be above pH 12 and, if necessary, adjust with sodium hydroxide solution (5.6).

Separate the organic phase by means of a pasteur pipette (6.16). Add 3 ml of hexane to the methanol and separate the organic phase again. Combine the hexane phases.

NOTE For solid-phase extraction, an automatic solid disk extractor can be used. An example of a method, with optimized extraction times for a certain type of automated extractor is described in Annex C.

8.4 Clean-up of the extract

In the case of low polluted samples, the clean-up step may be omitted. Treat the reference solutions in the same way as the samples.

Concentrate the hexane extract carefully to a final volume < 1 ml (e. g. in a gentle stream of nitrogen or on a rotary evaporator under reduced pressure (6.6)). Rinse the wall of the container with a small volume of hexane and evaporate again to a volume < 1 ml.

Prepare a column by bringing 4 g of aluminium oxide (5.9) in the chromatographic column (6.7).

Wash the column with 10 ml of hexane and let the column condition for 30 min.

Bring the hexane extract on top of the column.

Rinse the wall of the container with a small volume of hexane and bring the solvent on top of the column.

Elute the compounds of interest with 15 ml of hexane.

The whole clean-up procedure should be executed with clean glassware (by heating and rinsing with solvents), with maximum protection from light and possible laboratory contamination.

NOTE A silica clean-up is also possible for extract clean-up (see Annex D).

8.5 Solvent concentration step

Add 30 µl of *iso*-octane to the hexane extract. Concentrate the extract carefully to a final volume less than 0,3 ml (e. g. in a gentle stream of nitrogen or on a rotary evaporator under reduced pressure (6.6)). The heating temperature of the evaporator should not be higher than 30 °C during evaporation.

Bring the extract volume up to a 0,3 ml using hexane. Transfer the sample into a glass sample vial (6.11). keep in a cool (3 ± 2 ºC) and dark place until the analysis is carried out. Use an aliquot for the GC-MS determination.

8.6 Gas chromatograph

Optimize the operating conditions of the GC-MS system e. g. according to the manufacturer’s instructions. Examples of the gas chromatographic conditions are given in Annex A.

Prior to analysis, establish the operating conditions and verify the GC-MS system performance and the calibration for all analytes and their internal standards by analysis of a calibration standard.

For separation, use appropriate capillary columns (6.15) and adjust chromatographic conditions for maximum selectivity (see Annex A for examples).

8.7 Identification of individual compounds by GC-MS

Identify the sample component by matching both retention times and relative intensities of the diagnostic ions (Table 2) of sample components and reference substances (5.11) and derivatization standard (5.13).

The target compound in the sample is to be regarded as identified if:

— the relative or the absolute sample component retention time (RT) measured in the selected ion current chromatogram matches the relative or absolute retention time of the authentic compound within ± 0,2 % in the chromatogram of the latest calibration standard solution (5.15), measured under identical conditions;

— two selected diagnostic ions (see Table 2) are present at the substance-specific retention time;

— relative intensities of all selected diagnostic ions measured in the sample do not deviate by more than ± (0,1 × I + 10) % from the relative intensities determined in the calibration standard solution, where I is the relative intensity of the diagnostic ion of the individual reference substance.

EXAMPLE Three selected diagnostic ions have the following relative intensities: 100 %, 50 % and 15 %. The maximum allowed deviation in the sample is:

— I_1: ± (0,1 × 100 + 10) % = ± 20 %; the relative intensity in the sample shall be between 80 % and 120 %;

— I_2: ± (0,1 × 50 + 10) % = ± 15 %; the relative intensity in the sample shall be between 35 % and 65 %;

— I_3: ± (0,1 × 15 + 10) % = ± 11,5 %; the relative intensity in the sample shall be between 3,5 % and 26,5 %.

No ion of significant intensity should be present in the mass spectrum after background subtraction with a larger mass than the highest possible mass for a compound to be identified.

NOTE Further guidance on identification is given in EN ISO 22892 [2] and the SANCO/12571/2013 guideline [3].

Table 2 — Selected diagnostic ions for mass spectrometric detection and relative abundances

Substance	Mother ion 1 *m/z*	Mother ion 2 *m/z*	Daughter ion 1 *m/z*	Daughter ion 2 *m/z*
Ethyl-TBT	291	291	179 (100)	235 (32)
Ethyl-d_{27}-TBT	254	318	190 (69)	190 (100)
Ethyl-TPrT	247	249	163 (78)	165 (100)
TePrT	247	249	163 (78)	165 (100)

8.8 Blank value measurements

Use periodic blank value measurements (at least one measurement per sequence) to check that both the instrument and chemicals are free from significant contamination. Blank measurements shall comprise all steps of the analytical procedure. If blank values are unusually high (more than 50 % of the lowest reporting level), review every step in the procedure and determine the cause by systematic checks so as to be able to eliminate the contamination source. Try to reduce the blank values as much as possible by applying various measures, such as avoiding contamination by ambient air and using suitable solvents (5.4) as well as checking the analytical instrumentation (e. g. GC-MS, auto sampler, LVI unit).

9 Calibration

9.1 General requirements

Correct calibration requires knowing the retention times of the analytes to be determined (see also Table 1). These shall be determined with reference solutions of individual reference substances at the specified chromatographic conditions.

When setting up the method for the first time, check retention time (RT) and identity of each single compound carefully. It is recommended that each compound of Table 1 is single-injected for checking retention time and/or mass spectrum (for examples of chromatograms see Annex A).

The calibration function determined for a substance applies only to the concentration range covered by it; moreover, it depends on the operating condition of the gas chromatograph and shall be checked at regular intervals.

Design the calibration procedure such that a linear dependence of measurement signal to concentration is achieved for each compound to be determined. Determine the linear working range using at least seven concentration levels (which are distributed as evenly as possible over the working range).

For routine operation, it is sufficient to recalibrate by measuring two concentration levels. Recalibrate at regular intervals within one sample sequence (e. g. after 15 to 20 samples).

For each target compound, calibrate the determination procedure using individual or, more conveniently, multi-component reference solutions. Adjust the calibration range to the existing requirements.

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

Table 3 — Definition of subscripts

Subscript	Meaning
i	Substance
e	Calibration step
g	Total procedure
j	Consecutive figure for pairs of values
I	Internal Standard

9.2 Calibration of the GC-step

For each analyte, establish a calibration function from at least seven points.

For a graphic presentation of the calibration curve, plot the reference function and determine the line of best fit by linear regression according to Formula (1).

$$y_{\mathrm{ie}} = m_{\mathrm{i}} \cdot \rho_{\mathrm{ie}} + b_{\mathrm{i}} \tag{1}$$

where

- $y_{i\mathrm{e}}$ is the measured response (dependent variable) of substance i during calibration as a function of $\rho_{i\mathrm{e}}$, the unit depending on the evaluation, e. g. area unit;
- $\rho_{i\mathrm{e}}$ is the (independent variable) mass concentration of substance i in the reference solution, in nanograms per litre (ng/l);
- m_i is the slope of the calibration function of substance i (response factor);
- b_i is the ordinate intercept of the calibration curve, the unit depending on the evaluation.

Table 4 — Example concentrations in solutions for evaluating the linear range

Substance	Solution 1 pg/ml	Solution 2 pg/ml	Solution 3 pg/ml	Solution4 pg/ml	Solution 5 pg/ml	Solution 6 pg/ml	Solution 7 pg/ml
Ethyl-TBT	50	100	500	1 000	5 000	10 000	20 000
Ethyl-TPrT	50	100	500	1 000	5 000	10 000	20 000
Internal standards							
Ethyl-d_{27}-TBT	1000	1000	1000	1000	1000	1000	1000
TePrT	1000	1000	1000	1000	1000	1000	1000

9.3 Calibration of the total procedure using the internal standard

As internal standard, choose a substance with similar physicochemical properties (extraction behaviour, retention time) as the substance to be determined (see 5.14). The internal standard should not be present in the sample to be analysed. The choice of a substance may be difficult and it depends on the nature of sample matrix to be analysed; in any case, the suitability should be checked.

The use of an internal standard helps to minimize unavoidable minor errors which may occur throughout the procedure.

The determination of the concentrations will become, to a certain degree, independent of matrix effects in the water sample.

Precision by GC-measurement is independent from minor deviations during probe injection.

Minor sample losses throughout sample preparation as well as insufficient adjusting of small sample extract volumes to a precise level do not cause problems in reproducibility.

Add a known mass of the internal standard I to the water sample prior to analysis (see 8.2.1).

The mass concentration, ρ_I, shall be the same for both calibration and the sample series. All multi-component reference solutions suitable for multipoint calibration (5.15) should contain the same mass concentration of the internal standard.

For calibration covering the total procedure, add e. g. 100 µl of multi-component reference solution (5.15) to e. g. 1 000 ml water (5.1) and treat and analyse the sample as described in Clause 8.

Based on the values obtained from the ratios of $y_{i\text{egj}}/y_{\text{Iegj}}$ and $\rho_{i\text{egj}}/\rho_{\text{Iegj}}$, plot the reference function and determine the line of best fit by linear regression according to Formula (2).

$$\frac{y_{i\text{eg}}}{y_{\text{Ieg}}} = m_{i\text{Ig}} \frac{\rho_{i\text{eg}}}{\rho_{\text{Ieg}}} + b_{i\text{Ig}} \quad (2)$$

where

$y_{i\text{eg}}$ is the measured value (dependent variable) of substance i during calibration as a function of $\rho_{i\text{e}}$, the unit depending on the evaluation, e. g. area unit;

y_{Ieg} is the measured value of internal standard I during calibration, the unit depending on the evaluation, e. g. area unit; all reference solutions contain equal concentrations of the internal standard;

$\rho_{i\mathrm{eg}}$ is the (independent variable) mass concentration of substance i in the reference solution, in nanograms per litre (ng/l);

ρ_{Ieg} is the (independent variable) mass concentration of internal standard I, in nanograms per litre (ng/l);

$m_{i\mathrm{Ig}}$ is the slope of the reference line of $y_{i\mathrm{eg}}/y_{\mathrm{Ieg}}$ as a function of the ratio $\rho_{i\mathrm{eg}}/\rho_{\mathrm{Ieg}}$ (response factor);

$b_{i\mathrm{Ig}}$ is the ordinate intercept of the reference line, the unit depending on the evaluation.

9.4 Determination of procedural recovery values

Reliable recovery data are obtained from analysis of spiked water samples at different concentration levels equidistantly spread over the working range. From these individual results a mean specific recovery $\bar{A}_i$ is calculated.

Using the calibration function in 9.2, calculate the single mass concentration $\rho_{\mathrm{i.N.fnd}}$ for each concentration level N and for each substance i.

Calculate the single recovery $A_{i.\mathrm{N}}$ according to Formula (3).

$$A_{\mathrm{i,N}} = \frac{\rho_{\mathrm{i.N.fnd}}}{\rho_{\mathrm{i.N.nom}}} \cdot f \qquad (3)$$

where

$A_{\mathrm{i.N}}$ is the recovery of substance i on the concentration level N in percent (%);

$\rho_{i.\mathrm{N.fnd}}$ is the recovered mass concentration of substance i on the concentration level N, calculated according Formula (1), in nanograms per litre (ng/l);

$\rho_{i\cdot\mathrm{N.nom}}$ is the original mass concentration of substance i on the concentration level N, in nanograms per litre (ng/l);

f is the conversion factor, here: f = 100.

Calculate with these single results the mean recovery $\bar{A}_i$ according to Formula (4).

$$\overline{A_i} = \frac{\sum_{N=l}^{n} A_{i,\mathrm{N}}}{n} \qquad (4)$$

where

$\overline{A_i}$ is the mean recovery of substance i, in percent, %;

$A_{i,\mathrm{N}}$ is the recovery of substance i, on the concentration level N in percent (%);

n is the number of individual measurement values.

With the described procedure stated in Clause 8, recoveries (>20 % up to 50 %) are usually achieved. Lower or unstable recoveries indicate matrix effects or difficulties during derivatization (9.5) or extraction.

9.5 Determination of derivatization standard recovery

Calculate the mass concentration $\rho_{i\mathrm{g}}$ of the derivatization standard substance (5.17) i in the water sample according to Formula (5), taking into account Formula (2) using the internal standard.

$$\rho_{ig} = \frac{\frac{y_{ig}}{y_{Ig}} - b_{iIg}}{m_{iIg}} \cdot \rho_{Ig} \tag{5}$$

where

ρ_{ig}	is the mass concentration of the target substance i in the water sample, in nanograms per litre (ng/l);
y_{ig}	is the measured value of the target substance i in the water sample, e. g. in area units;
y_{Ig}	is the measured value of internal standard I in the water sample, the unit depending on the evaluation, e. g. area unit;
ρ_{Ig}	is the mass concentration of internal standard I in the water sample, in nanograms per litre (ng/l);
b_{iIg}, m_{iIg}	see Formula (2).

Calculate the single recovery $A_{i,N}$ according to Formula (6).

$$A_{i,N} = \frac{\rho_{i.N.fnd}}{\rho_{i.N.nom}} \cdot f \tag{6}$$

where

$A_{i.N}$	is the recovery of substance i on the concentration level N in percent (%);
$\rho_{i.N.fnd}$	is the recovered mass concentration of substance i on the concentration level N, calculated according Formula (5), in nanograms per litre (ng/l);
$\rho_{i \cdot N.nom}$	is the original mass concentration of substance i on the concentration level N, in nanograms per litre (ng/l);
f	is the conversion factor, here: f = 100.

With the described procedure stated in Clause 8, recoveries >70 % up to 120 % are usually achieved. Lower or unstable recoveries indicate matrix effects or difficulties during derivatization or extraction.

10 Calculation

Calculate the mass concentration ρ_{ig} of substance i in the water sample according to Formula (5), taking into account Formula (2) using the internal standard.

$$\rho_{ig} = \frac{\frac{y_{ig}}{y_{Ig}} - b_{iIg}}{m_{iIg}} \cdot \rho_{Ig} \tag{5}$$

where

ρ_{ig}	is the mass concentration of the target substance i in the water sample, in nanograms per litre (ng/l);
y_{ig}	is the measured value of the target substance i in the water sample, e. g. in area units;
y_{Ig}	is the measured value of internal standard I in the water sample, the unit depending on the evaluation, e. g. area unit;
ρ_{Ig}	is the mass concentration of internal standard I in the water sample, in nanograms per

litre (ng/l);

b_i/g, m_i/g see Formula (2).

11 Expression of results

The mass concentration, in nanograms per litre (ng/l) or picograms per litre (pg/l), of TBT shall be reported to two significant figures.

The mass concentration shall be reported on the mass of the TBT. If relevant, the blank value shall be subtracted from the result.

12 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this Technical Specification (CEN/TS 16692);

b) identification of the sample;

c) sample preparation and extraction;

d) expression of the results, according to Clause 11;

e) any details not specified in this Technical Specification or which are optional, as well as any factor which may have affected the results.

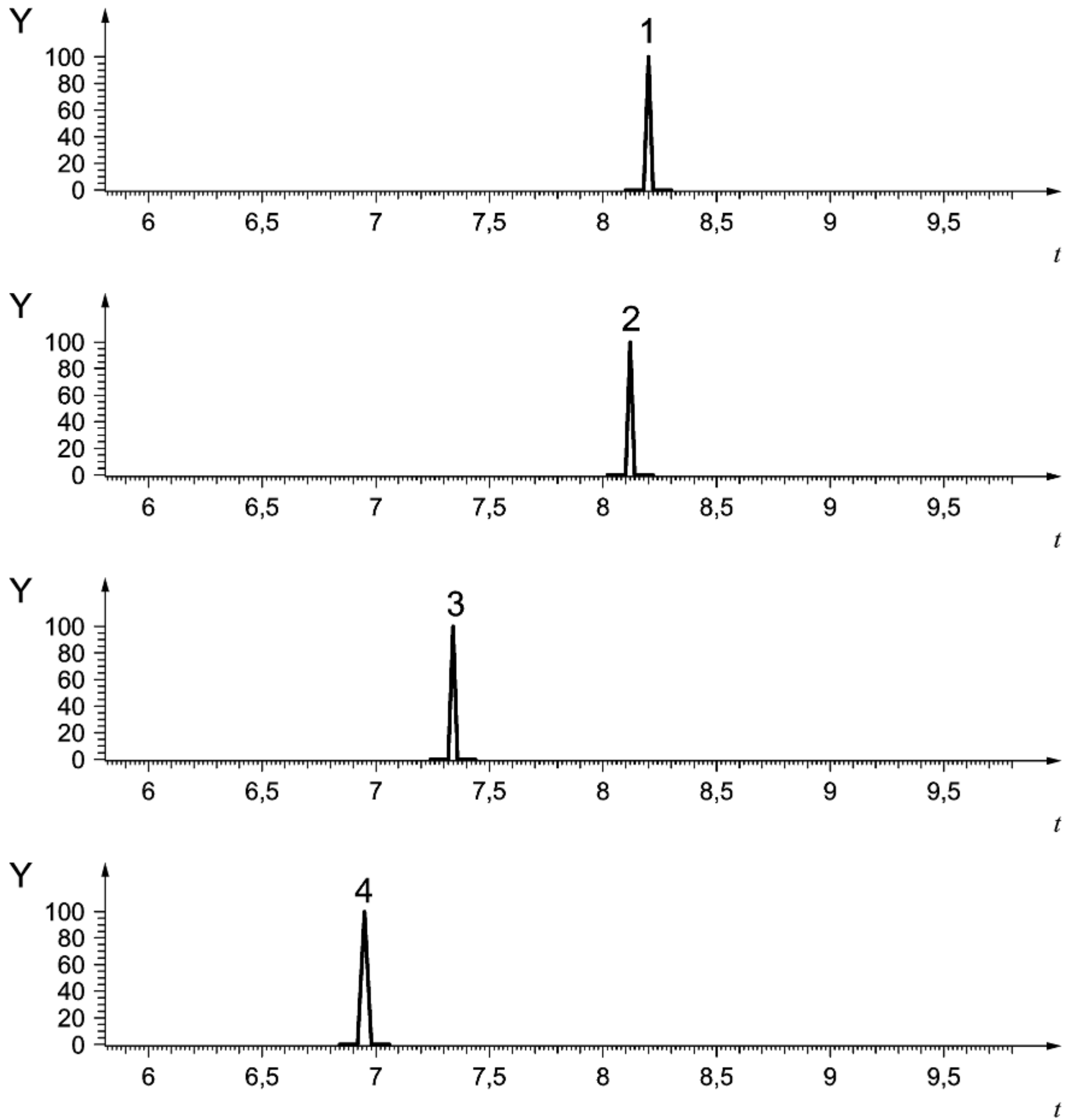
Annex A
(informative)

Suitable gas chromatographic conditions and example chromatograms

Table A.1 — Chromatographic conditions

GC equipment:	Trace GC Ultra Thermo[1)]
Injection	20 µl LV-PTV injection in glass sintered liner (desactivated) at a rate of 1,7 µl/s with injection needle with side-hole. The inlet temperature was 35 °C. The injection step was programmed for 0,05 min, with a flow of 30 ml/min. Evaporation was performed at a pressure of 20 kPa at 35 °C for the duration of 1 min, with a flow of 30 ml/min. The transfer of the compounds was at a pressure of 250 kPa and the temperature was raised to 280 °C at 14,5 °C/min. This temperature was held for 1 min. Then, a cleaning phase was started by heating the PTV-injector to 320 °C at a rate of 14,5 °C/min. This temperature was held for 10 min and the flow was 150 ml/min. The solvent valve temperature was 150 °C.
Capillary column:	Restek Rxi-5sil-MS[1)], 20 m × 0,18 mm ID × 0,18 µm df
Range of concentration:	0,04 ng to 20 ng /ml
Carrier gas:	Helium; 1 ml/min
Temperature programme:	30 °C hold for 2 min; 25 °C/min to 175 °C; 50 °C/min to 330 °C hold for 1 min.
MS detector:	TSQuantum XLS[1)]

[1)] Trace GC Ultra Thermo, Restek Rxi-5sil-MS, TSQuantum XLS are examples of suitable products which are commercially available. These examples are given only as information for the users of this Technical Specification and do not constitute an endorsement by CEN of these products.



Key

Y relative abundance

t time in minutes (min)

1 Ethyltributyltin (ethyl-TBT), R.T. 8,20 min

2 Ethyltributyltin-d_{27} (ethyl-d_{27}-TBT), R.T. 8,12 min

3 Tetrapropyltin (TePrT), R.T. 7,34 min

4 Ethyltripropyltin (ethyl-TPrT), R.T. 6,95 min

Figure A.1 — Example chromatogram

Annex B
(informative)

Repeatability and reproducibility data

The performance data on repeatability and reproducibility given in Table B.1, were determined in a European inter laboratory trial for validation carried out in June 2014 on surface water. The water used was mineral water (SPA Reine).

Sample 1 and sample 2 were spiked with different amounts of suspended particulate matter (SPM). The SPM material used was a TBT-containing SPM derived from the TBT model SPM developed in ENV 08 by IRMM. Sample 3 was spiked with TBT according to Table B.1. Sample 4 was a blank sample. Evaluation process of data was carried out according to ISO 5725-2 [4].

The physico-chemical interactions taking place when adding model SPM to pre-filled water bottles are not known in detail. Therefore all concentrations in the final water samples based on slurry addition are estimated.

Table B.1 — Performance data for TBT samples

Sample	l	n	o %	X ng/l	$\bar{\bar{x}}$ ng/l	η %	s_R ng/l	$C_{V,R}$ %	s_r ng/l	$C_{V,r}$ %
1	7	14	0,0	3,8	4,21	110,8	2,25	53,4	1,05	24,9
2	7	14	0,0	3,4	3,51	103,2	1,10	31,3	0,42	12,0
3	6	12	14,3	17	17,4	102,5	2,18	12,5	1,12	6,4
4	7	13	0,0	-	0,32	-	0,20	62,5	0,10	31,3

Explanation of symbols

l number of laboratories after outlier rejection

n number of individual test results after outlier rejection

o percentage of outliers

X assigned value

$\bar{\bar{x}}$ overall mean of results (without outliers)

η recovery rate

s_R reproducibility standard deviation

$C_{V,R}$ coefficient of variation of reproducibility

s_r repeatability standard deviation

$C_{V,r}$ coefficient of variation of repeatability

NOTE X for sample 1 and 2 represents the estimated reference value, for sample 3 it refers to the spiked concentration of TBT.

Annex C
(informative)

Example of conditions for automated solid-phase extraction

Table C.1 — Programme of a purge cycle with an Automated Extraction System

Solvent	**Soak (time)**		**Airdry (time)**	
1. Program prewet cycle				
Hexane		10 s		20 s
MeOH		10 s		20 s
Reagent water		10 s		20 s
Reagent water		10 s		20 s
2. Sample airdry cycle: 2 min				
3. Program elution cycle				
MeOH	1 min		1 min	
Hexane	1 min			20 s
Hexane	1 min			20 s
Hexane	1 min		1 min	

Table C.2 — Programme of an extraction cycle with an Automated Extraction System

Solvent	**Soak (time)**		**Airdry (time)**	
1. Program prewet cycle				
Hexane	1 min		1 min	
MeOH	1 min		1 min	
Reagent water	1 min			30 s
Reagent water	1 min			30 s
2. Sample input cycle				
3. Sample airdry cycle: 2 min				
4. Program elution cycle				
MeOH	3 min		3 min	
Hexane	3 min		3 min	
Hexane	3 min		3 min	
Hexane	3 min		3 min	

NOTE The automated extraction system was a SPE-DEX® 4790[2].

[2] SPE-DEX® 4790 by Horizon Technology, Salem, NH, United States, is an example of a suitable product which is commercially available. This example is given only as information for the users of this Technical Specification and do not constitute an endorsement by CEN of these products..

Annex D
(informative)

Silica clean-up

D.1 Silica for the clean-up column

It is recommended to prepare batches of no more than 120 g per batch.

Heat silica (grain size 0,2 mm to 0,063 mm (200 mesh to 63 mesh)) for at least 12 h at 500 °C in a muffle furnace (the temperature should not exceed 520 °C).

Allow to cool in the oven to about 200 °C, transfer the silica to a wide-necked glass bottle and allow to cool to room temperature in a dessicator. Add water to the cooled silica until a mass fraction of 3 % is reached. Close the bottle and homogenize the contents for 2 h on a shaker.

D.2 Clean-up column

Add about 5 g of silica to the column and add about 3 g of sodium sulfate. Rinse with 30 ml of hexane and let the solvent pass through the column to the level of the upper surface of the column bed.

The column is now ready for use.

Commercially available pre-packed columns may be used.

D.3 Eluent for cleaning extract

To ensure quantitative elution of all organotin from the clean-up column, use hexane. Using an appropriate standard solution prior to applying the clean-up procedure, determine the volume of hexane necessary for complete elution.

D.4 Clean-up of extract

Transfer the concentrated extract to the clean-up column. After the extract has reached the silica surface, add cautiously 1 ml of eluent onto the column. After penetration of the eluent, elute with the appropriate amount of eluent and collect the eluate. Reduce the volume of the eluate to about 1 ml.

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[1] SMEDES F., DE JONG A.S., DAVIES I.M. Determination of (mono-, di- and) tributyltin in sediments: analytical methods. *J. Environ. Monit.* 2000, **2** pp. 541–549

[2] EN ISO 22892, *Soil quality — Guidelines for the identification of target compounds by gas chromatography and mass spectrometry (ISO 22892)*

[3] SANCO/12571/2013 *Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed*

[4] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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