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**Kakovost vode - Določevanje pentabromodifeniletra (PBDE) v celotnem vzorcu vode z ekstrakcijo na trdni fazi (SPE) s SPE-diski v kombinaciji s plinsko kromatografijo/masno spektrometrijo (GC/MS)**

Water quality - Determination of pentabromodiphenyl ether (PBDE) in whole water samples using solid phase extraction (SPE) with SPE-disks combined with gas chromatography - mass spectrometry (GC-MS)

Wasserbeschaffenheit - Bestimmung von Pentabromdiphenylether (PBDE) in Gesamtwasserproben mittels Festphasenextraktion (SPE) mit SPE-Disks in Verbindung mit Gaschromatographie - Massenspektrometrie (GC-MS)

Qualité de l'eau - Dosage du pentabromodiphényléther (PBDE) dans la totalité de l'échantillon d'eau par extraction en phase solide (EPS) avec disques EPS, avec couplage chromatographie en phase gazeuse-spectrométrie de masse (CG-SM)

**Ta slovenski standard je istoveten z: EN 16694:2015**

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**ICS:**

13.060.50	Preiskava vode na kemične snovi	Examination of water for chemical substances
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EUROPEAN STANDARD  
NORME EUROPÉENNE  
EUROPÄISCHE NORM

**EN 16694**

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English Version

**Water quality - Determination of selected polybrominated diphenyl ether (PBDE) in whole water samples - Method using solid phase extraction (SPE) with SPE-disks combined with gas chromatography - mass spectrometry (GC-MS)**

Qualité de l'eau - Dosage du pentabromodiphényléther (PBDE) dans des échantillons d'eau totale - Méthode par extraction en phase solide (SPE) avec disques SPE, avec couplage chromatographie en phase gazeuse - spectrométrie de masse (CG-SM)

Wasserbeschaffenheit - Bestimmung von ausgewählten polybromierten Diphenylethern (PBDE) in Gesamtwasserproben - Verfahren mittels Festphasenextraktion (SPE) mit SPE-Disks in Verbindung mit Gaschromatographie - Massenspektrometrie (GC-MS)

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
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EUROPÄISCHES KOMITEE FÜR NORMUNG

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**EN 16694:2015 (E)****European foreword**

This document (EN 16694:2015) has been prepared 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 March 2016, and conflicting national standards shall be withdrawn at the latest by March 2016.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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## Introduction

**WARNING —** Persons using this European Standard should be familiar with usual laboratory practice. This European Standard 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 European Standard be carried out by suitably trained staff.

Polybrominated diphenyl ethers (PBDE) are priority substances 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 waters as well as other surface waters to protect the aquatic environment against chemical pollution (Directive 2008/105/EC). With the exception of metals, the EQS 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 PBDE 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 PBDE as listed in Annex A of Directive 2008/105/EC.

The priority substances list in Annex X of the WFD includes technical pentabromodiphenyl ether, which is regarded as a mixture of the congeners BDE-28, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154. The annual average environmental quality standard (AA-EQS) for pentabromodiphenyl ether in inland surface waters is 0,5 ng/l and is defined for the whole water sample including suspended particulate matter (SPM) present in the sample. Compounds such as PBDE strongly adsorb to environmental solids resulting in a fraction bound to particles, which may be substantial. The aim of the method is to extract whole water samples in one single step to determine the dissolved as well as the particle bound PBDE fraction. Identification and quantification of BDE congeners at trace level concentrations require both highly sensitive chromatographic equipment and effective enrichment steps and awareness of the potential impact of blanks.

## 1 Scope

This European Standard specifies a method for the determination of six selected polybrominated diphenyl ethers (PBDE) listed in Table 1, representative for technical brominated diphenyl ethers (BDE) in water samples in mass concentrations  $\geq 0,025$  ng/l for each individual congener. The method uses solid-phase extraction with SPE-disks in combination with gas chromatography-mass spectrometry (GC-MS). It is applicable to the analysis of PBDE in surface water containing suspended particulate matter (SPM) up to 500 mg/l (whole water samples), drinking water and groundwater. The limit of quantification (LOQ) was determined according to ISO/TS 13530, on the basis of replicate determinations of the procedural blank, carried out under reproducibility conditions.

This method may be used for the analysis of other BDE congeners not listed in Table 1 or other types of water. However, it is important to verify its applicability before use.

**Table 1 — Polybrominated diphenyl ethers (PBDE) determined by this method**

Congener	Abbreviation <sup>a</sup>	Formula	Molar mass g/mol	CAS RN <sup>b</sup>
2,4,4'-Tribromodiphenyl ether	BDE-28	C <sub>12</sub> H <sub>7</sub> Br <sub>3</sub> O	406,8954	41318-75-6
2,2',4,4'-Tetrabromodiphenyl ether	BDE-47	C <sub>12</sub> H <sub>6</sub> Br <sub>4</sub> O	485,7950	5436-43-1
2,2',4,4',5-Pentabromodiphenyl ether	BDE-99	C <sub>12</sub> H <sub>5</sub> Br <sub>5</sub> O	564,6911	60348-60-9
2,2',4,4',6-Pentabromodiphenyl ether	BDE-100	C <sub>12</sub> H <sub>5</sub> Br <sub>5</sub> O	564,6911	189084-64-8
2,2',4,4',5,6'-Hexabromodiphenyl ether	BDE-154	C <sub>12</sub> H <sub>4</sub> Br <sub>6</sub> O	643,5872	68631-49-2
2,2',4,4',5,5'-Hexabromodiphenyl ether	BDE-153	C <sub>12</sub> H <sub>4</sub> Br <sub>6</sub> O	643,5872	207122-15-4

NOTE EC numbers are not applicable for PBDE congeners.

<sup>a</sup> Numbering analog to IUPAC nomenclature for PCB

<sup>b</sup> CAS RN: Chemical Abstracts Service Registry Number

## 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 5667-1, *Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques (ISO 5667-1)*

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

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

## 3 Principle

A mixture of suitable internal standards (<sup>13</sup>C<sub>12</sub>-labelled BDE or fluorinated BDE) is added to a 1 l water sample, which is then extracted using a solid-phase extraction disk (SPE-disk). The disk is eluted with a



suitable solvent (5.4) or solvent mixture and the resulting extract is concentrated for analysis or further clean-up.

Prior to injection, an injection standard is added to each extract, and an aliquot is injected into the gas chromatograph. The analytes are separated by capillary gas chromatography and detected and quantified by electron ionization high-resolution mass spectrometry (EI-HRMS) or alternatively, by low resolution negative chemical ionization mass spectrometry (NCI-MS) or low resolution tandem mass spectrometry (MS/MS). In the latter cases a clean-up step using e. g. a multilayer-silica column, and/or removal of sulfur, e. g. by gel permeation chromatography, may be necessary.

## 4 Interferences

### 4.1 Interferences with extraction and clean up

To avoid interference, collect samples as specified in Clause 7.

Commercially available SPE-disks can differ frequently in quality. Variations in the selectivity of the materials can occur from batch to batch, and therefore might cause 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 analytical results have a high accuracy and precision, use materials from 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.

### 4.2 Interferences with GC-MS

Interferences may be caused, e. g. by the injection system used or by inadequate separation of the analytes. Experienced operators might be able to minimize this type of interference. Regular checking of the chromatographic and spectrometric system is required to maintain adequate performance. Required system stability should be checked regularly using a GC standard.

## 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**, complying to grade 1 according to EN ISO 3696, or equivalent.

**5.2 Operating gases** for the gas chromatography mass spectrometry, of high purity and according to the 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.**

A variety of solvents may be used depending on the procedural step and the availability of commercial stock solutions, e. g.

- toluene, C<sub>7</sub>H<sub>8</sub> (boiling point: 111 °C),
- acetone, C<sub>3</sub>H<sub>6</sub>O (boiling point: 56 °C),
- *iso*- or *n*-hexane, C<sub>6</sub>H<sub>14</sub>, (boiling point: 60 °C),

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- *iso*-octane (2,2,4-trimethylpentane), C<sub>8</sub>H<sub>18</sub> (boiling point: 99 °C),
- nonane, C<sub>9</sub>H<sub>20</sub> (boiling point: 151 °C),
- dichloromethane, CH<sub>2</sub>Cl<sub>2</sub>, (boiling point: 39,7 °C),
- ethyl acetate, C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> (boiling point: 77 °C),
- *iso*-propanol, C<sub>3</sub>H<sub>8</sub>O (boiling point: 82 °C)

for residual analysis. A suitable keeper is recommended (e. g. toluene), when reducing the volume of the extract to less than 0,5 ml. For large volume injection a more volatile solvent, e. g. hexane, should be used.

**5.5 Clean up material**, according to Annex C.

**5.6 Sodium sulfate**, anhydrous, Na<sub>2</sub>SO<sub>4</sub>, powdered.

## **5.7 Standards**

### **5.7.1 Reference substances**

PBDE listed in Table 1 are used for calibration. Solutions of the reference substances are commercially available.

### **5.7.2 Internal standards**

Solutions of reference substances for use as internal standards for electron impact ionization (see Table 2) are commercially available.

**Table 2 — Examples of internal standards for GC-EI-MS analysis**

Standard	Abbreviation	Formula	Molar mass g/mol
2,4,4'-Tribromo[ <sup>13</sup> C <sub>12</sub> ]diphenyl ether	<sup>13</sup> C-BDE-28	<sup>13</sup> C <sub>12</sub> H <sub>7</sub> Br <sub>3</sub> O	418,8038
2,2',4,4'-Tetrabromo[ <sup>13</sup> C <sub>12</sub> ]diphenyl ether	<sup>13</sup> C-BDE-47	<sup>13</sup> C <sub>12</sub> H <sub>6</sub> Br <sub>4</sub> O	497,7035
2,2',4,4',5-Pentabromo[ <sup>13</sup> C <sub>12</sub> ]diphenyl ether	<sup>13</sup> C-BDE-99	<sup>13</sup> C <sub>12</sub> H <sub>5</sub> Br <sub>5</sub> O	576,5995
2,2',4,4',5,5'-Hexabromo[ <sup>13</sup> C <sub>12</sub> ]diphenyl ether	<sup>13</sup> C-BDE-153	<sup>13</sup> C <sub>12</sub> H <sub>4</sub> Br <sub>6</sub> O	655,4955

Solutions of reference substances for use as internal standards for negative ion chemical ionization (see Table 3) are commercially available.

**Table 3 — Examples of internal standards for GC-NCI-MS analysis**

Standard	Abbreviation	Formula	Molar mass g/mol
2-Fluoro-2,4,4'-tribromodiphenyl ether	F-BDE-28	C <sub>12</sub> H <sub>6</sub> Br <sub>3</sub> OF	424,8858
6-Fluoro-2,2',4,4'-tetrabromodiphenyl ether	F-BDE-47	C <sub>12</sub> H <sub>5</sub> Br <sub>4</sub> OF	503,7819
3-Fluoro-2,2',4,4',6-pentabromodiphenyl ether	F-BDE-100	C <sub>12</sub> H <sub>4</sub> Br <sub>5</sub> OF	582,6779
4'-Fluoro-2,3,3',4,5,6-hexabromodiphenyl ether	F-BDE-160	C <sub>12</sub> H <sub>3</sub> Br <sub>6</sub> OF	661,5740

### 5.7.3 Injection standard

To determine recovery rates for the internal standards in each sample e. g. dibromooctafluorobiphenyl (C<sub>12</sub>Br<sub>2</sub>F<sub>8</sub>) is used as an injection standard.

### 5.7.4 Preparation of standard stock solutions

#### 5.7.4.1 Stock solutions of the single reference substances/internal standards

Use either commercially available solutions or prepare stock solutions by dissolving e. g. 10 mg of each of the reference substances in toluene (5.4) in a 100 ml volumetric amber flask and bring to volume, resulting in a final concentration of 100 µg/ml. Correct the concentration for purity if this is < 99 %. Store stock solutions at temperatures between 1°C and 5°C according to EN ISO 5667-3, protected from light. They are stable for at least 12 months.

#### 5.7.4.2 Stock solution of the injection standard

Use a commercially available solution or prepare a stock solution by dissolving e. g. 10 mg of the reference substance in toluene (5.4) in a 100 ml volumetric amber flask and bring to volume, resulting in a final concentration of 100 µg/ml. Make appropriate dilutions in hexane or toluene allowing to spike e. g. 100 pg accurately to the final extract of a water sample. Store stock solutions at temperatures between 1°C and 5°C according to EN ISO 5667-3, protected from light. They are stable for at least 12 months.

#### 5.7.4.3 Multicomponent stock solution of reference substances

Accurately transfer between 100 µl to 500 µl of each single standard solution (5.7.4.1) into a 10 ml volumetric amber flask and bring to volume, resulting in final concentrations between 1 µg/ml and 5 µg/ml per substance. Store multicomponent stock solutions at temperatures between 1 °C and 5 °C, protected from light. They are stable for at least 6 months.

#### 5.7.4.4 Multicomponent stock solution of internal standards

Prepare a stock solution of the internal standards (5.7.4.1) at an appropriate concentration in hexane or toluene (e. g. 1 ng/ml). Store multicomponent stock solutions at temperatures between 1°C and 5°C, protected from light. They are stable for at least 6 months.

#### 5.7.4.5 Calibration control standard solution

Use a commercially available standard solution of reference substances from an independent supplier or prepare an independent standard solution from the pure reference substances. Store the calibration control standard solution at temperatures between 1°C and 5°C, protected from light. It is stable for at least 6 months.

## 6 Apparatus

Equipment or parts of it which have contact with the water sample or its extract shall be free from residues causing interferences. The use of vessels made of glass, stainless steel or polytetrafluoroethylene (PTFE) is recommended. To minimize the blank contribution the vessels shall be rinsed with organic solvent prior to use.

**6.1 Sample flasks**, e. g. brown glass, flat bottomed, with glass or PTFE coated stoppers, e. g. 1 000 ml or 2 000 ml.

**6.2 One-mark volumetric flasks**, capacity e. g. 10 ml, 25 ml, 50 ml and 100 ml.

**6.3 Single volume pipettes**, capacities between 1 ml and 50 ml.

**6.4 Capillary gas chromatograph** with high resolution mass spectrometric detector using electron ionization (GC-HRMS), or **capillary gas chromatograph** with mass spectrometric detector using chemical ionization in negative mode (GC-NCI-MS).

The gas supplies shall be in accordance with the respective manufacturer's instructions.

**6.5 Non-discriminating GC injector**, e. g. splitless mode of a split/splitless injection system or programmable temperature vaporizer (PTV), programmable for large volume injection (LVI).

A cold injection technique is recommended; depending on the sensitivity of the instrument large volume injection may be necessary.

**6.6 Automatic sampler with option for large volume injection (LVI)**, including syringes for normal injection (e. g. 1 µl and 2 µl) and for LVI (e. g. 10 µl, 50 µl and 100 µl).

**6.7 Capillary columns**, for gas chromatography (example chromatograms are given in Annex A). It is advantageous to use non-polar columns (e. g. low-bleed 5 %-phenylsiloxane column), providing thermal stability up to 400 °C; e. g. inner diameter ≤ 0,25 mm, length 15 m, film thickness of 0,1 µm is recommended.

**6.8 Solid-phase extraction disks (SPE-disks)**, wide inner diameter: e. g. 40 mm to 60 mm, packed with an appropriate reversed phase adsorbent material, e. g. C<sub>18</sub>-based or SDB-based adsorbent (for examples, see Annex C).

**6.9 Vacuum device for solid-phase extraction**, e. g. extraction box or automated workstation for solid-phase extraction procedure capable for processing SPE-disks.

**6.10 Evaporation device**, such as rotary evaporator, turboevaporator or vacuum concentration device.

**6.11 Syringes**, 2 µl, 5 µl, 10 µl and 50 µl, volume precision ± 2 %.

**6.12 Sample vials**; amber glass with fluoropolymer-lined screw-cap is most suitable.

**6.13 Glass columns** for chromatographic clean-up.

**6.14 Pasteur pipettes**, made of glass.