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Construction products: Assessment of release of dangerous substances — Determination of biocide residues using liquid chromatography with mass spectrometric detection (LC-MS/MS)

National foreword

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A list of organizations represented on this committee can be obtained on request to its committee manager.

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European foreword

This document (EN 17845:2023) has been prepared by Technical Committee CEN/TC 351 "Construction products: Assessment of release of dangerous substances", the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by May 2024, and conflicting national standards shall be withdrawn at the latest by May 2024.

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Introduction

This document deals with the determination of the content of biocides in construction products and eluates using liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

Following an extended evaluation of available methods for content and eluate analysis in construction products (CEN/TR 16045) and subsequent method evaluation in the robustness validation [Van De Weghe et al., 2018] it was concluded that eluate analysis and content analysis for biocides can be based on EN 15637 after some modifications.

This document is part of a modular horizontal approach and belongs to the analytical step. An overview of all modules which belong to a chain of measurement and the manner how modules are selected is given in CEN/TR 16220.

In the growing amount of product and sector-oriented test methods it was recognized that many steps in test procedures are or could be used in test procedures for many products, materials and sectors. It was supposed that, by careful determination of these steps and selection of specific questions within these steps, elements of the test procedure could be described in a way that can be used for all materials and products or for all materials and products with certain specifications.

In this context a horizontal modular approach is adopted in CEN/TC 351. “Horizontal” means that the methods can be used for a wide range of materials and products with certain properties. “Modular” means that a test standard developed in this approach concerns a specific step in assessing a property and not the whole “chain of measurement” (from sampling to analyses). A beneficial feature of this approach is that “modules” can be replaced by better ones without jeopardizing the standard “chain”.

The use of modular horizontal standards implies the drawing of test schemes as well. Before executing a test on a certain material or product to determine certain characteristics, it is necessary to draw up a protocol in which the adequate modules are selected and together form the basis for the entire test procedure.

1 Scope

This document describes a method for the determination of the content of biocides in construction products, (either finished (dried) or in a ready-to-use state) and in eluates thereof, using liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

For content analysis liquid chromatography with UV-detection can also be used, if sufficient sensitivity and selectivity is ensured (see Annex A (normative)).

The method in this document is validated for the product types listed in Annex B (informative). For eluate analysis quantification limits of 0,1 µg/l can be achieved.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 16637-2, *Construction products: Assessment of release of dangerous substances — Part 2: Horizontal dynamic surface leaching test*

EN 16637-3, *Construction products: Assessment of release of dangerous substances — Part 3: Horizontal up-flow percolation test*

EN 16687:2023, *Construction products: Assessment of release of dangerous substances — Terminology*

EN 17087, *Construction products: Assessment of release of dangerous substances — Preparation of test portions from the laboratory sample for testing of release and analysis of content*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 16687:2023 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
blank value
test result obtained by carrying out the test procedure in the absence of a test portion

[SOURCE: EN 16687:2023, 3.3.1.10; modified – Note 1 to entry removed]

3.2
clean-up
purification of a crude extract to remove interfering compounds

[SOURCE: EN 16687:2023, 3.2.2.27; modified – Note 1 to entry removed]

3.3
external standard
known quantity of the target analytes that is measured in the same series as the solution to be measured and is used for identification and quantification

[SOURCE: EN 16687:2023, 3.2.2.20]

3.4
extract
solution resulting from extraction of a sample with a solvent

[SOURCE: EN 16687:2023, 3.2.2.13]

3.5
extraction
dissolution of substances in a solvent for subsequent chemical analysis

[SOURCE: EN 16687:2023, 3.2.2.14; modified – Note 1 to entry removed]

3.6
final extract
solution that is obtained after clean-up of the Soxhlet extract through a purification stage

[SOURCE: EN 16687:2023, 3.2.2.18]

3.7
internal standard
known quantity of a substance (where applicable deuterated) not present in the sample, which is added to the analysis sample in order to determine the recovery

[SOURCE: EN 16687:2023, 3.3.2.10]

3.8
laboratory sample
sample or subsample(s) sent to or received by the laboratory

[SOURCE: EN 16687:2023, 3.2.2.1; modified – Notes to entry removed]

3.9
method detection limit
MDL
lowest analyte concentration that can be detected with a specified analytical method including sample preparation with a defined statistical probability

[SOURCE: EN 16687:2023, 3.3.1.12; modified – Note 1 to entry removed]

3.10
product matrix
main composition of the product dictating the manner of sample preparation and the type of digestion or extraction for later chemical analysis

[SOURCE: EN 16687:2023, 3.1.1.2; modified – Note 1 to entry removed]

3.11
sample
portion of material selected from a larger quantity of material

[SOURCE: EN 16687:2023, 3.2.1.5; modified – Notes to entry removed]

3.12
Soxhlet extract
solution that is obtained after extraction of a solid subsample by the Soxhlet technique for determining organic compounds

[SOURCE: EN 16687:2023, 3.2.2.17]

3.13
Soxhlet extraction
chemical pre-treatment of a solid subsample, where the organic compounds to be determined are dissolved by the Soxhlet technique

[SOURCE: EN 16687:2023, 3.2.2.16]

3.14
test portion
analytical portion
amount of the test sample taken for testing/analysis purposes, usually of known dimension, mass or volume

[SOURCE: EN 16687:2023, 3.2.2.3; modified – Examples removed]

3.15
test sample
analytical sample
sample, prepared from the laboratory sample, from which test portions are removed for testing or for analysis

[SOURCE: EN 16687:2023, 3.2.2.2]

4 Abbreviations

For the purposes of this document, the following abbreviations apply.

a.u.	Arbitrary units
DTL	Detection limit
HPLC	High performance liquid chromatography NOTE High pressure liquid chromatography is an (outdated) synonym.
ISTD	Internal standard solution
LC	Liquid chromatography
LOD	Limit of detection
MQL	Method quantification limit
MRL	Maximum residue limit

MRM	Multiple reaction monitoring
MS	Mass spectrometry; Mass selective detection
PTFE	Polytetrafluoroethylene
SPE	Solid phase extraction

5 Sample preparation

To obtain test samples for extraction (and analysis), guidance on sample preparation as specified in EN 17087 shall be applied.

Precautions shall be taken before and during transport of the laboratory sample as well as during the time in which the samples are preserved in the laboratory before being analysed, to avoid alteration of the sample (see CEN/TR 16220).

Extracts are susceptible to change due to physical or chemical reactions which can take place between the time of extraction and the analysis.

It is therefore essential to take the necessary precautions to minimize these reactions and in the case of many parameters to analyse the extract with a minimum of delay. The maximum delay is given in the respective analytical standards.

6 Principle

Quantification of biocides is performed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS), using electrospray ionization. Eluates are injected directly into the LC-MS/MS system.

Solid and pasty samples are extracted with methanol or a methanol/water mixture. Clean-up may be applied when necessary by solid phase supported liquid-liquid extraction.

To achieve the required selectivity the mass spectrometer is operated in the MRM mode.

7 Reagents

Use reagents of recognized analytical grade, unless otherwise specified. Take every precaution to avoid possible contamination of water, solvents, inorganic salts, etc.

7.1 Additive for LC-eluent depending on method used, e.g. ammonium formate, formic acid, acetic acid.

7.2 Water, HPLC quality.

7.3 Dichloromethane, for biocide analysis.

7.4 Methanol, LC-MS/MS quality.

7.5 Internal standard (ISTD) solutions in methanol, mass concentration ρ = 10 µg/ml to 50 µg/ml[1].

Use as much as possible isotope labelled internal standards, if available and obtainable at reasonable price. The internal standard solution should be added at the extraction step and to standard solutions.

If no isotope labelled internal standards are used, it is recommended to check whether matrix compounds are co-eluting with the ISTD to ensure reliable results. Losses of the ISTD during clean-up will result in an overestimation of analyte concentration. Such losses should thus be minimal.

7.6 Biocide stock solutions.

Prepare individual stock solutions of analytical standards at concentrations that are sufficiently high to allow the preparation of complex biocide mixtures. The solvent used shall not negatively influence the stability of the biocides employed.

Usually, store stock solutions at approximately −18 °C. Check the stability of stock solutions during storage regularly. In some cases, the addition of acids or bases is helpful to enhance stability and extend the acceptable storage period.

7.7 Biocide mixtures.

Because of the broad applicability of this method and due to the partly divergent pH-stability of biocides, analyte mixtures of different composition might be needed. These are prepared by mixing together defined volumes of the required biocide stock solutions (7.6) and appropriately diluting them with methanol. The analyte concentrations in this mixture should be sufficient to allow the preparation of the required matrix matched standards (see 7.8.3) with moderate dilution of the blank sample extract (e.g. less than 20 %).

Usually, biocide mixtures are stored at approximately −18 °C. Since the stability of the biocides in the mixture can be lower than in stock solutions, stability has to be checked regularly. In some cases, the addition of acids or bases is helpful to enhance stability and extend acceptable storage times.

7.8 Standard solutions.

7.8.1 Standard solutions prepared in pure solvent (solvent-based standards).

Solvent-based standards are prepared by mixing a certain volume of methanol with known amounts of biocide mixtures (7.7). The preparation of multiple standards of different biocide concentration is useful to cover a broad concentration range.

NOTE The linear range of calibration depends on the sensitivity of the instrument and the MS signal response of the biocide. A calibration range of 0,1 µg/l to 100 µg/l correlates to a biocide content of 0,3 mg/kg to 300 mg/kg when a 10 g sample is employed (see 9.3.1).

7.8.2 Standard solutions with internal standard prepared in pure solvent.

Solvent-based standards with ISTD are prepared by mixing a certain volume of methanol with known amounts of biocide mixtures (7.7) and a fixed volume of internal standard solution (7.5).

The volume used shall result in that concentration of ISTD which is obtained in the final extracts after sample extraction and clean-up (see 9.2 and 9.3). The concentration of internal standard in the final extract ($c_{\mathrm{ISTD}}^{\mathrm{sample}}$) is calculated using Formula (1). The preparation of multiple standards of different biocide concentration but with constant ISTD concentration is useful to cover a broad concentration range.

$$c_{\mathrm{ISTD}}^{\mathrm{sample}} = \frac{V_{\mathrm{ISTD}} \times c_{\mathrm{ISTD}} \times V_1}{V_{\mathrm{ex}} \times V_{\mathrm{end}}} \quad (1)$$

where

$c_{\mathrm{ISTD}}^{\mathrm{sample}}$ is the concentration of internal standard in the final extract, in µg/ml;

V_{ISTD} is the volume of internal standard solution (7.5) added to the test portion, in ml;

c_{ISTD} is the concentration of internal standard solution (7.5), in µg/ml;

V_1 is the volume used for solid phase supported liquid/liquid extraction, in ml (here: V_1 = 5 ml);

V_{ex} is the total volume of extraction solvents, in ml (here: V_{ex} = 30 ml);

V_{end} is the final volume of extract obtained after clean-up, in ml (here: V_{end} = 0,5 ml).

In case no clean-up is applied Formula (1) simplifies to Formula (2).

$$c_{\mathrm{ISTD}}^{\mathrm{sample}} = \frac{V_{\mathrm{ISTD}} \times c_{\mathrm{ISTD}}}{V_{\mathrm{ex}}} \quad (2)$$

7.8.3 Standard solutions prepared in blank matrix extracts (matrix-matched standards).

Prepare matrix-matched standards in the same way as the solvent-based standards, however, instead of pure methanol use extracts of blank samples (prepared as described in 9.3, but without ISTD addition). To minimize errors caused by matrix induced effects during chromatography, it is best to choose similar commodities (e.g. sealant for sealant samples, render for render samples, wood for treated wood samples, insulation foam for insulation foam samples, etc.).

The stability of biocide in matrix-matched standards can be lower than that of standards in pure solvents and has to be checked more thoroughly.

7.9 Cartridge for solid phase supported liquid/liquid extraction of appropriate sample volume (e.g. 5 ml or 20 ml), diatomaceous earth, for example ChemElut CE 1005[1].

7.10 Solid phase extraction (SPE) cartridges, e.g. OASIS HLB[1] 6 ml, 200 mg[3].

8 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following:

8.1 Suitable equipment for sample comminution and grinding.

8.2 Laboratory balance, accuracy: 0,01 g.

8.3 Volumetric flasks, 10 ml and 20 ml.

8.4 Ultrasonic bath.

[1] ChemElut and OASIS HLB are trade names of products. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

8.5 Centrifuge tubes, suitable to withstand acceleration.

8.6 Centrifuge, capable of producing a relative centrifugal force (RCF) of at least 3000 *g* (at the bottom of the tube).

8.7 Round bottom flasks, capacity 50 ml and 250 ml.

8.8 Glass syringe, minimum volume 2 ml.

8.9 Microlitre syringes, for sample fortification.

8.10 Rotary evaporator, with temperature-controlled water bath.

8.11 Syringe filters, 0,45 µm pore size, PTFE membrane.

8.12 Glass vials and caps, 1,8 ml volume, suitable for an autosampler.

8.13 LC-MS/MS system, triple quadrupole mass spectrometer with electrospray interface.

NOTE Depending on construction product, matrix, system interferences, biocide and requirement for limit of quantification, it is possible that reliable results are to be obtained using a less selective detection system, e.g. LC/UV.

8.14 SPE equipment, e.g. vacuum manifold.

9 Procedure

9.1 Preparation and storage of the samples

Sample processing and storage procedures should be demonstrated to have no significant effect on the biocides present in the test sample. Processing should also ensure that the test sample is homogeneous enough so that sub-sampling variability is acceptable. If a single analytical portion is unlikely to be representative of the test sample, larger or replicate portions shall be analysed, to provide a better estimate of the true value. For content analysis, the degree of comminution of solid samples supports a quantitative biocide extraction.

9.2 Preparation of eluates

Make eluates according to EN 16637-2 and EN 16637-3; centrifuge the eluates, take a test portion, add internal standard and proceed to 9.4.

Depending on the sensitivity of the instrument and the required limit of quantification it can be necessary to concentrate the eluates by solid phase extraction (SPE). An example of an SPE procedure is given in Annex B (informative).

9.3 Sample preparation for content analysis

9.3.1 Extraction

The reduction of the sample shall be carried out in such a way that representative portions are obtained (see EN 17087). Follow guidance in this document for any other aspects of sample pre-treatment. This results in the test sample. Calculation of the biocide shall be based on the mass of the original test sample.

For dry sample materials weigh a homogenized portion of 5 g (m_A) into the centrifuge tube (8.5). Add 10 ml of water and 20 ml of methanol (7.4). Homogenize for approximately 2 min (e.g. using a high-speed blender). Take at least 10 ml of the resulting extract of 30 ml (= V_{ex}) and centrifuge at a minimum of 3 000 *g* for at least 10 min.

For pasty products, transfer a representative test portion of m_a = 10 g into a centrifuge tube (8.5). Add 10 ml of water and 20 ml of methanol (7.4). Homogenize for approximately 2 min (e.g. using a high-speed blender). Take at least 10 ml of the resulting extract (V_{ex}) and centrifuge at a minimum of 3 000 *g* for at least 10 min. If the product is partly or completely dissolved in the extraction solvent, the volume V_{ex} has to be corrected, taken the sample volume into account.

As an option an internal standard may be used additionally. In that case add a small volume (<1 % of V_{ex}) of internal standard solution (= V_{ISTD}) to the test portion after addition of 20 ml of methanol.

9.3.2 Clean-up

If necessary, apply 5 ml of the diluted centrifugate (= V_3) from 9.3.1 to a 5 ml cartridge (7.9). After approximately 5 minutes, elute into a 50 ml round bottom flask (8.7), using 12,5 ml of dichloromethane (7.3). Repeat the elution with another 12,5 ml of dichloromethane. Reduce the combined eluates almost to dryness using the rotary evaporator (8.10). Remaining dichloromethane shall be removed with a gentle stream of nitrogen. Add 500 µl of methanol (7.4) to the round bottom flask and weigh with stopper. Carefully dissolve the biocide by swirling the flask in the ultrasonic bath (8.4), but avoid losses of methanol. If losses of methanol occur (re-weighing), add methanol to obtain the previous total weight. Filter the obtained sample test solution of 0,5 ml (= V_{end}) through a 0,2 µm-filter (8.11) into a sample vial (8.12) for injection.

To obtain a larger amount of sample test solution for the preparation of matrix-matched standards (7.8.3) a 20 ml cartridge may be used. In that case, 400 % of all volumes mentioned above have to be used.

NOTE The sample test solution contains the extractable components of 3,33 g sample per ml final extract (or 1,67 g/0,5 ml).

9.4 Determination

For eluate analysis, the sample test solutions (9.2) and standard solutions (7.8.1, 7.8.2 or 7.8.3) are injected into the LC-MS/MS instrument in an appropriate sequence. This may involve introducing calibration solutions at the beginning and the end of the sample extract runs. The LC-MS/MS instrument shall be operated in the MRM mode with transitions selective for the biocides under investigation. Examples of MRM transitions of many biocides are given in CEN/TR 15641. The measurement may be performed using various instruments, instrument parameters and columns. Some example instrument parameters and columns are listed in Annex C (informative). These conditions have been shown to provide satisfactory results. Nevertheless, individual tuning of the compounds on the instrument that is used for measurement usually provides better sensitivities.

For content analysis the same chromatographic conditions and detection method can be applied. It has to be ensured, that the concentration of biocides in the sample solution is within the calibration range of the instrument, otherwise the sample solution has to be diluted.

In addition, the LC-UV method given in Annex A (normative) can be used for the determination of the content, provided that it is ensured that the device has sufficient sensitivity and selectivity.

9.5 Test for interference and recovery

Prepare reagent blanks and carry out spiked recovery tests at appropriate concentrations. The chromatogram of the reagent blank shall not show any significant peak (e.g. 10 % of relevant MRL) at the retention time of the analytes.

10 Evaluation of results

10.1 Identification

To identify analytes, compare the retention times obtained from the sample test solution with those obtained from the calibration solutions. Positive findings are confirmed by comparing the peak intensity ratios of the first and second compound specific *m/z* transition with the peak intensity ratios found in standards. A different LC column, another eluent or an additional *m/z* transition may be used, if additional measures are necessary.

10.2 Quantification

Use standard solutions (7.8.1 or 7.8.2) or matrix-matched standards (7.8.3) to determine the calibration functions for each active substance by plotting the peak areas (if ISTDs are not used) or peak ratios (if ISTDs are used) of one MRM transition against the analyte concentration (µg/ml) of the standard solution.

Then carry out a linear regression to determine the slope of the curve b and the intercept a. The function for linear regression is given in Formula (3).

$$\Delta\left(\frac{A_i}{A_{\mathrm{ISTD}}}\right) = b \times \Delta\left(\frac{c_i}{c_{\mathrm{ISTD}}^{\mathrm{sample}}}\right) + a \qquad (3)$$

where

A_i is the peak area of compound i, in a.u.;

A_{ISTD} is the peak area of the internal standard, in a.u.;

NOTE 1 $A_{\mathrm{ISTD}} = 1$, if no internal standard is used.

b is the slope of the corresponding calibration graph, in a.u. (without ISTD) or without dimension (with ISTD);

c_i is the concentration of compound i in the calibration solution, in µg/ml;

$c_{\mathrm{ISTD}}^{\mathrm{sample}}$ is the concentration of the internal standard in the calibration solution, in µg/ml;

NOTE 2 $c_{\mathrm{ISTD}}^{\mathrm{sample}} = 1$, if no internal standard is used.

a is the intercept of the corresponding calibration graph, in a.u. (without ISTD) or without dimension (with ISTD);

For some biocides a quadratic calibration equation is more appropriate.

For a first estimate of the content of biocides in construction products or to show their absence, the standard solutions (7.8.1 or 7.8.2) in pure methanol can be used. They can be also used for quantification if preliminary experiments indicate that any suppression or enhancement effects experienced do not significantly affect the results obtained. As soon as relevant biocide concentrations are detected (e.g. suspected MRL violations), a more precise determination using matrix-matched standards (7.8.3) or the standard addition method is preferred.

NOTE 3 Matrix effects influence the response of target analytes in sample extracts compared to the response of standard solutions in pure solvent.

The calibration range shall be appropriate to the biocide concentrations to be quantified. Thus, it can be necessary to construct more than one calibration graph from the results of calibration measurements.

10.3 Quality assurance

When using ISTDs it is important to know that any shift in the ISTD signal will directly influence the calculated concentration of the analytes. Ideally, the ISTD signal should only shift due to volume differences and thus improve the accuracy of measurement. However, there are also other, non-desirable, factors that can also affect the signals of the ISTD thus introducing errors in the analyte quantification. A specific suppression of the ISTD signal, potentially occurring in LC-MS applications due to co-eluting matrix components, will also result in analyte overestimations. Matrix effects depend on whether the extract contains specific components that co-elute with the ISTD and affect its ionization process.

In any case it is always crucial to introduce quality control measures to ensure that any error introduced by the ISTD remains insignificant. Quality control measures may include the use of backup ISTDs and quality control standards that may be added at other stages of the analytical procedure (e.g. to the final extract) and that can help to identify any non-volume related shifts of the ISTD signal. Very helpful for quality control is the observation of the signal intensity of the ISTD in every sample within a sequence. When a significant signal shift occurs, quantification is performed using a backup ISTD or without using ISTD. In the latter case exact liquid transfers and equalization of the volumes of the standard solutions and the sample extracts are mandatory.

11 Calculation of results

11.1 Calculation of biocide concentrations without standard addition

11.1.1 Content analysis

If the standard addition method is not used, the content w_R (in mg/kg) of a biocide in a construction product is calculated from the obtained peak areas using Formula (4).

$$w_R = \frac{\frac{A_i}{A_{ISTD}} - a}{b} \times \frac{V_{ex}}{m_a} \times \frac{V_{end}}{V_1} \times c_{ISTD}^{sample} \quad (4)$$

where

w_R is the content of a biocide in a construction product, in mg/kg;

A_i is the peak area for one MRM transition measured, in a.u.;

A_{ISTD} is the peak area of the internal standard, in a.u.;

NOTE 1 $A_{ISTD} = 1$, if no internal standard is used.

a is the intercept of the corresponding calibration graph, in a.u. (without ISTD) or without dimension (with ISTD);

b is the slope of the corresponding calibration graph, in a.u. (without ISTD) or without dimension (with ISTD);

V_{ex} is the total volume of extraction solvents (here: V_{ex} = 30 ml);

m_a is the initial sample weight, in g;

V_{end} is the final volume of extract obtained after clean-up (here: V_{end} = 0,5 ml);

V_1 is the volume used for solid phase supported liquid/liquid extraction (here: V_1 = 5 ml);

c_{ISTD}^{sample} is the concentration of the internal standard in the final extract, in µg/ml.

NOTE 2 $c_{ISTD}^{sample} = 1$, if no internal standard is used.

In case no clean-up is applied Formula (4) simplifies to Formula (5).

$$w_R = \frac{\frac{A_i}{A_{ISTD}} - a}{b} \times \frac{V_{ex}}{m_a} \times c_{ISTD}^{sample} \quad (5)$$

11.1.2 Eluate analysis

For eluates the biocide concentration c_R (in µg/ml) is derived from Formula (6).

$$c_R = \frac{\frac{A_i}{A_{ISTD}} - a}{b} \times c_{ISTD}^{sample} \quad (6)$$

where

c_R is the biocide concentration in eluates, in µg/ml;

A_i is the peak area for one MRM transition measured, in a.u.;

A_{ISTD} is the peak area of the internal standard, in a.u.;

NOTE 1 A_{ISTD} = 1, if no internal standard is used.

a is the intercept of the corresponding calibration graph, in a.u. (without ISTD) or without dimension (with ISTD);

b is the slope of the corresponding calibration graph, in a.u. (without ISTD) or without dimension (with ISTD);

c_{ISTD}^{sample} is the concentration of the internal standard in the final extract, in µg/ml.

NOTE 2 c_{ISTD}^{sample} = 1, if no internal standard is used.

If no internal standard is used, c_{ISTD}^{sample} and A_{ISTD} are equal to 1 and Formula (6) simplifies to Formula (7).

$$c_R = \frac{A - a}{b} \quad (7)$$

If the results indicate that the amount of residue approaches or exceeds the maximum residue level, at least one further test portion shall be analysed.

11.2 Calculation of biocide concentrations with standard addition

11.2.1 Content analysis

In case of compounds which are known to be strongly affected by matrix-induced enhancement or suppression phenomena, the procedure of standard additions is recommended provided that the function between response and concentrations at the concentration range in question is linear. In that case several aliquots of the final sample extract are fortified with increasing known amounts of the analyte of interest. This procedure requires the knowledge of the approximate biocide content w_R from preliminary analysis. In Table 1 the pipetting scheme for the standard addition approach is given.

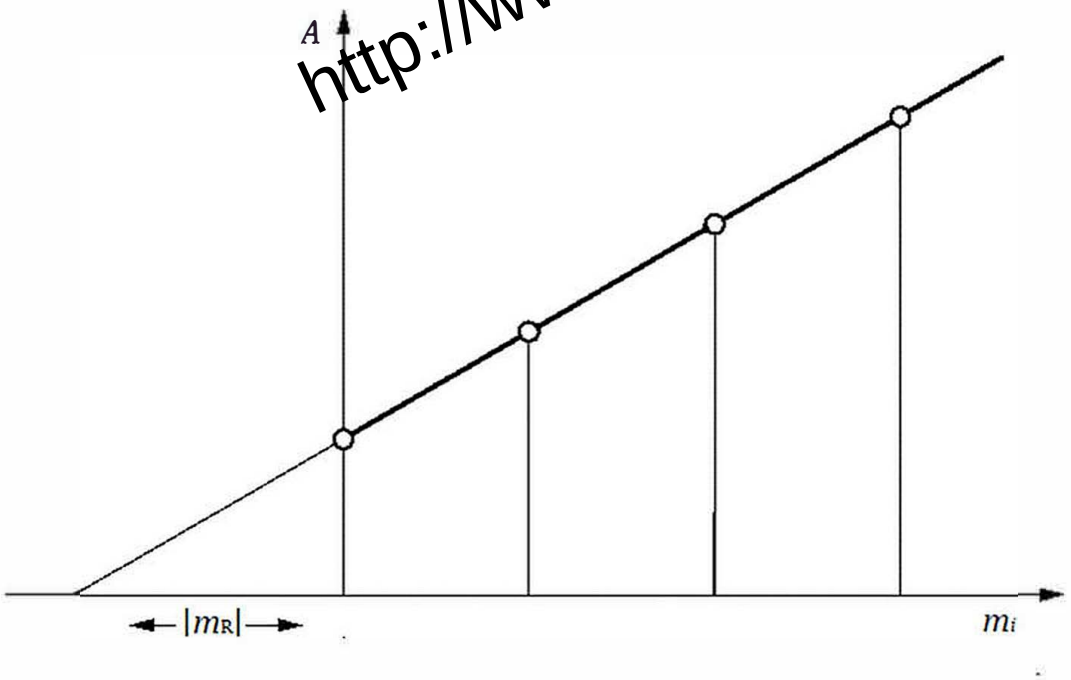
The standard solutions used for standard addition shall have nearly identical solvent composition compared to the sample test solution. Assuming a sample (used sample amount 10 g) with an estimated biocide content w_R = 0,8 mg/kg, the following pipetting scheme can be appropriate.

NOTE In case of other biocide content w_R an adjusted concentration of the analyte standard solution and/or more appropriate volumes of analyte standard solution and solvent are needed.

The amount of analyte in the sample is calculated using a graphical presentation of resulting response data as shown in Figure 1 via linear regression.

Table 1 — Pipetting scheme for the standard addition approach

Additions	Vial 1	Vial 2	Vial 3	Vial 4
Volume of sample extract V_{aliq}	100 µl (= 0,25 g sample)	100 µl (= 0,25 g sample)	100 µl (= 0,25 g sample)	100 µl (= 0,25 g sample)
Volume of analyte standard solution (20 µg/ml)	0 µl	5 µl	10 µl	15 µl
Resulting mass of analyte added	0 µg	0,1 µg	0,2 µg	0,3 µg
Volume of solvent	15 µl	10 µl	5 µl	0 µl
Final volume	115 µl	115 µl	115 µl	115 µl



Key

A Peak area of the analyte, in a.u.;

m_i Added absolute amount of analyte i, in µg;

$|m_R|$ Absolute amount of analyte in the sample extract before standard addition, in µg.

Figure 1 — Internal calibration using the procedure of standard additions, schematically

The peak area A of the analyte is calculated using a graphical presentation of resulting response data as shown in Figure 1 via linear regression (Formula (8)).

$$A = b \times m_i + a \quad (8)$$

where

A is the peak area of the analyte, in a.u.;

b is the slope of the calibration graph of the analyte in question, in a.u./µg;

m_i is the added absolute amount of analyte i, in µg;

a is the y-intercept of the calibration graph of the analyte in question, in a.u.

For A = 0 Formula (8) becomes Formula (9).

$$|m_R| = -\frac{a}{b} \quad (9)$$

where

$|m_R|$ is the absolute amount of analyte in the sample extract before standard addition, in µg;

a is the y-intercept of the calibration graph of the analyte in question, in a.u;

b is the slope of the calibration graph of the analyte in question, in a.u./µg.

If the standard addition method is used, the content w_R of a biocide in the construction product is calculated from the obtained peak area using Formula (10).

$$w_R = \frac{|m_R|}{m_a} \times \frac{V_{ex} \times V_{end}}{V_1 \times V_{aliq}} \quad (10)$$

where

w_R is the content of a biocide in a construction product, in mg/kg;

$|m_R|$ is the absolute amount of analyte in the sample extract before standard addition, in µg;

m_a is the initial sample weight, in g (here: m_a = 5 g or 10 g);

V_{ex} is the total volume of extraction solvents and water, in ml (here: V_{ex} = 30 ml);

V_{end} is the final volume of extract obtained after clean-up, in ml (here: V_{end} = 0,5 ml);

V_1 is the volume used for solid phase supported liquid/liquid extraction, in ml (here: V_1 = 5 ml);

V_{aliq} is the aliquot of extract used for analysis, in ml (here: V_{aliq} = 0,1 ml).

In case no clean-up is applied Formula (10) simplifies to Formula (11).

$$w_R = \frac{|m_R|}{m_a} \times \frac{V_{ex}}{V_{aliq}} \quad (11)$$

where

w_R is the content of a biocide in a construction product, in mg/kg;

$|m_R|$ is the absolute amount of analyte in the sample extract before standard addition, in µg;

m_a is the initial sample weight, in g (here: m_a = 5 g or 10 g);

V_{ex} is the total volume of extraction solvents and water, in ml (here: V_{ex} = 30 ml);

V_{aliq} is the aliquot of extract used for analysis, in ml (here: V_{aliq} = 0,1 ml).

11.2.2 Eluate analysis

For determination of biocide content in eluates using standard addition, the peak area A is plotted against the resulting concentration c_i of the analyte in the solution analysed in µg/ml. Therefore, the concentration of the analyte in the sample is given by Formula (12).

$$\left|c_R\right| = \frac{A - a}{b} \quad (12)$$

where

$|c_R|$ the absolute value of the biocide concentration in the sample, in µg/ml;

A is the peak area of the analyte, in a.u.;

a is the y-intercept of the calibration graph of the analyte in question, in a.u.;

b is the slope of the calibration graph of the analyte in question, in a.u./µg.

12 Test performance

As the method for content of biocides was still under discussion when the robustness validation was assessed, no results are available for content of biocides in construction products. The performance characteristics of the method are derived from the intercomparison validation of eluate analysis of a selection of construction products as reported in [García-Ruiz et al., 2020]. Table 2 gives the resulting typical values for relative repeatability and reproducibility standard deviations. The typical value is derived from the intercomparison validation data by taking the median value and rounding the numbers after eliminating substances with measurements too close to the detection limit (between DTL and MQL). In Annex D (informative) the performance by substance and matrix is given.

Table 2 — Typical values of the repeatability and reproducibility of the median

Statistical parameter	Median for biocides in eluates
Relative repeatability standard deviation, %	2
Relative reproducibility standard deviation, %	23
NOTE 1 The relative reproducibility standard deviation provides a determination of the differences (positive and negative) that can be found (with a 68 % statistical confidence) between a single test result obtained by a laboratory using its own facilities and another test result obtained by another laboratory using its own facilities, both test results being obtained under the following conditions: The tests are performed in accordance with all the requirements of the present standard and the two laboratory samples are obtained from the same primary field sample and prepared under identical procedures. The relative repeatability standard deviation refers to measurements obtained from the same laboratory, all other conditions being identical. The reproducibility limit and the repeatability standard deviation do not cover sampling but cover all activities carried out on the laboratory sample including its preparation from the primary field sample. NOTE 2 The relative repeatability standard deviation and the relative reproducibility standard deviation as derived from [García-Ruiz et al., 2020] in this table are indicative values of the attainable precision, if the determination of the content of substances in construction products is performed in accordance with this document. NOTE 3 A limited number of materials and parameters were tested. Consequently, for other materials and parameters, performance characteristics can fall outside the limits as derived from the validation of the determination of the content of substances in a construction product. For relatively heterogeneous materials, the repeatability and the reproducibility limits can be larger than the values given in this table.	

13 Test report

The test report shall contain at least the following data:

a) a title (e.g. "Test Report");

b) the name and address of the laboratory, and the location where the tests were carried out, if different from the address of the laboratory;

c) unique identification of the test report (such as the serial number) and on each page the number of the page and the total number of pages of the report;

d) the name and address of the customer;

e) reference to this document and supplementary standards, including its year of publication;

f) date and type of sampling procedure (if possible);

g) date of receipt of sample in the laboratory;

h) date of test;

i) results and the units in which the results have been expressed;

j) the limit of detection and limit of quantification;

k) all information necessary for the identification of the sample;

l) any particular points observed in the course of the test;

m) any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(normative)

LC-UV method for content analysis

A.1 Extraction and clean-up

Extraction is performed according to 9.3.1. If necessary, clean-up is applied according to 9.3.2.

A.2 HPLC analysis

Inject suitable amounts of sample and the standard solutions (7.8.1, 7.8.2 or 7.8.3) into the LC-system.

The peaks shall be quantified with a UV detector. A wavelength of 275 nm is applied.

For some biocides, e.g. isothiazolinones, another wavelength shall be used to ensure detection at the absorption maximum. Therefore, wavelengths of 240 nm and 320 nm have shown to be effective for evaluation.

The peak area for each biocide should fall within the area range of the appropriate calibration line. In case the peak area falls outside the calibration line, the calibration line should be extended so that the peak area of the compound in the sample falls within the area range of the calibration line or the sample must be diluted.

A.3 Example of chromatographic conditions

The analysis conditions given as examples are recommendations for suitable working methods. Technically equivalent or better separation columns and analysis conditions are permissible.

NOTE The information on commercial products is intended solely for the information of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Column:	Synergi 4 µm Hydro RP 80A, 50 × 1 mm, Ea Stationary Phase: C18 with polar end capping Solid Support: Fully Porous Silica
Oven:	35 °C
Eluent:	A: methanol 20 % and demineralized water 80 % and acetic acid 0,4 % (v + v + v) B: methanol 70 % and demineralized water 30 % (v + v)
Injection volume:	1,0 µl
Gradient	See Table A.1
Detector	See Table A.2

Table A.1 — Gradient

Time min	Flow ml/min	Eluent A %	Eluent B %
0	2	100	0
7,9	2	100	0
19,8	2	0	100
35,7	2	0	100
39,7	2	100	0
42	STOP		

Table A.2 — Detector

Channel	Wavelength nm	Bandwidth nm
UV_VIS 1	275	5
UV_VIS 2	320	5

A.4 Calibration

A.4.1 General

Prepare a suitable number of calibration solutions covering a suitable concentration range for the biocides of interest according to Clause 7.8.

Perform the LC analysis in the same way as the sample analysis (see A.2 and A.3).

A.4.2 Calibration curve

Plot the peak area A of the investigated biocide(s) versus the concentration of the appropriate compound (c_i).

Then carry out a linear regression to determine the slope b of the curve. The function for linear regression is given by Formula (A.1).

$$A = b \times c_i + a \quad \text{(A.1)}$$

where

- A is the peak are of the investigated biocide(s), in a.u.;
- b is the slope of the calibration graph of the analyte in question, in a.u./µg;
- c_i is the concentration of compound i in the calibration solution, in µg/ml;
- a is the y-intercept of the calibration graph of the analyte in question, in a.u.

A.5 Calculation of biocide content in the construction product

The concentration of the analysed biocide is calculated from Formula (A.2).

$$c_R = \frac{A - a}{b} \quad (A.2)$$

where

c_R is the concentration of biocide in the sample solution, in µg/ml;

A is the peak area of the biocide peak, in a.u.;

a is the y- intercept of the calibration curve, in a.u.;

b is the slope of the calibration curve, in a.u./µg.

The content of biocide in the sample is further calculated using Formula (A.3).

$$w_R = \frac{c_R \times V_{ex}}{m_a} \times \frac{V_{end}}{V_1} \quad (A.3)$$

where

w_R is the content of a biocide in a construction product, in mg/kg;

c_R is the concentration of biocide in the sample solution, in µg/ml;

V_{ex} is the total volume of extraction solvents, in ml (here: V_{ex} = 30 ml);

m_a is the initial sample weight, in g;

V_{end} is the final volume of extract obtained after clean-up, in ml (here: V_{end} = 0,5 ml);

V_1 is the volume used for solid phase supported liquid/liquid extraction, in ml (here: V_1 = 5 ml).

If no clean-up is applied Formula (A.3) simplifies to Formula (A.4).

$$w_R = \frac{c_R \times V_{ex}}{m_a} \quad (A.4)$$

where

w_R is the content of a biocide in a construction product, in mg/kg;

c_R is the concentration of biocide in the sample solution, in µg/ml;

V_{ex} is the total volume of extraction solvents, in ml (here: V_{ex} = 30 ml);

m_a is the initial sample weight, in g.

Annex B
(informative)

Solid phase extraction of eluates (example)

The SPE procedure described below is applicable for OASIS HLB. Other SPE phases can be used. Refer to the manufacturer instructions for a proper use of the SPE phase. The suitability of the SPE phase for the biocides of interest should be verified by spiking experiments. Take a test portion of e.g. 100 ml, centrifuge and add internal standards. Place an SPE cartridge on a vacuum manifold.

Condition the SPE cartridge with 6 ml of methanol followed by 6 ml water. Load the test portion on the cartridge and elute slowly by applying vacuum.

Switch off the vacuum and apply 5 % methanol in water wash solvent. Pull vacuum to remove wash solvent and then pull vacuum for another minute. Switch off the vacuum; add 2 ml of methanol, let it flow through by gravity, collect and pull vacuum. Repeat this step two times.

Evaporate/reconstitute or dilute the collected methanol fractions as needed. Transfer the extract to a vial for analysis.

Annex C
(informative)

Example conditions for suitable LC-MS/MS systems

C.1 Example LC-MS/MS operating conditions

The example LC-MS/MS operating conditions given in C.2 to C.3 have been proven to be satisfactory.

NOTE The information on commercial products as given in this Annex is intended solely for the information of users of this European Standard and does not constitute an endorsement by CEN of the products named. Equivalent products may be used if they can be shown to lead to the same results.

C.2 HPLC system 1

For most LC-amenable compounds:

Column	Phenomenex Aqua 5 µm C18 125 Å, 50 mm × 2 mm
Mobile phase A	Methanol/water 2+8 (v + v) with 5 mmol/l ammonium formate
Mobile phase B	Methanol/water 9+1 (v + v) with 5 mmol/l ammonium formate
Column temperature	20 °C
Flow rate and gradient	See Table C.1.

Table C.1 — Flow rate and elution gradient

Time	Flow rate	Mobile phase A	Mobile phase B
min	µl/min	%	%
0	200	100	0
11	200	0	100
23	200	0	100
25	200	100	0
33	200	100	0

C.3 HPLC-System 2

For most LC-amenable compounds:

Column	Zorbax XDB C18, length 150 mm, inner diameter 2,1 mm, particle size 3,5 µm
Mobile phase A	Ammonium formate solution in water, molar concentration c = 5 mmol/l
Mobile phase B	Ammonium formate solution in methanol, c = 5 mmol/l
Column temperature	40 °C
Injection volume	5 µl
Flow rate and gradient	See Table C.2.

Table C.2 — Flow rate and elution gradient

Time	Flow rate	Mobile phase A	Mobile phase B
min	µl/min	%	%
0	300	50	50
20	300	0	100
25	300	0	100
26	300	50	50
30	300	50	50

C.4 HPLC-System 3

For polar compounds that show low retention at reversed-phased columns:

Column	Phenomenex Aqua, length 150 mm, inner diameter 2 mm, filled with 125 A C18-material, particle size 3 µm
Mobile phase A	Ammonium formate solution in water, c = 5 mmol/l
Mobile phase B	Ammonium formate solution in methanol, c = 5 mmol/l
Column temperature	40 °C
Injection volume	3 µl, automatically diluted with 3 µl of mobile phase A during injection procedure
Flow rate and gradient	See Table C.3

Table C.3 — Flow rate and elution gradient

Time	Flow rate	Mobile phase A	Mobile phase B
min	µl/min	%	%
0	100	100	0
3	100	30	70
6	300	15	85
9	300	10	90
20,5	300	10	90
21	300	100	0
32	300	100	0

When the possibility for an automated dilution of the solutions in the instrument injector does not exist, these shall be manually diluted with mobile phase A (1:1), and 6 µl thereof shall be injected.

C.5 HPLC-System 4

For acidic compounds:

Column	Zorbax XDB C18, length 150 mm, inner diameter 2,1 mm, particle size 3,5 µm
Mobile phase A	Acetic acid solution in water, σ = 0,1 ml glacial acetic acid /l
Mobile phase B	Acetic acid solution in acetonitrile, σ = 0,1 ml glacial acetic acid /l
Column temperature	40 °C
Injection volume	5 µl
Flow rate and gradient	See Table C.4

Table C.4 — Flow rate and elution gradient

Time	Flow rate	Mobile phase A	Mobile phase B
min	µl/min	%	%
0	300	80	20
20	300	0	100
22	300	0	100
22,1	300	80	20
30	300	80	20

C.6 UHPLC system

Column	Waters Acquity BEH Shield RP 18, 1,7µm, 2,1 × 100 mm
Column temperature	40°C
Mobile phase A	Water + 0,1 % formic acid + 4 mmol/l ammonium acetate
Mobile phase B	ACN + 0,1 % formic acid
Injection volume	10 µl
Flow rate and gradient	See Table C.5.

Table C.5 — Flow rate and elution gradient

Time	Flow rate	Mobile phase A	Mobile phase B
min	µl/min	%	%
0	500	99	1
1	500	99	1
12	500	1	99
13	500	1	99
15	500	99	1

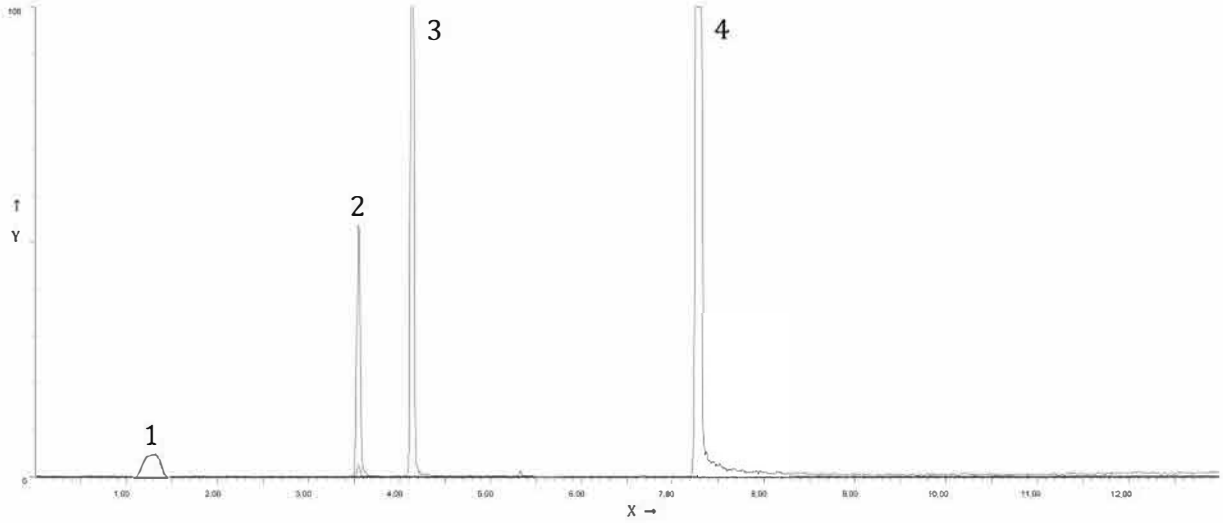
C.7 MS/MS system 1

MS/MS instrument	Waters XEVO TQS
Ion source	ESI+
General parameters	See Table C.6.

Table C.6 — Ion source and general parameters

Cone gas flow	nitrogen, 150 l/h	MS1 LM Resolution	2,7
Desolvation gas flow	nitrogen, 800 l/h	MS1 HM Resolution	15,0
Desolvation temp.	400 °C	MS2 LM Resolution	2,7
Capillary voltage	3 000 V	MS2 HM Resolution	15 014,7
Nebuliser gas flow	7,00 bar	Source temp	150 °C

An example chromatogram is given in Figure C.1.



Key

X	time, in min	2	cMIT – 3,6 min
Y	intensity in counts	3	BIT – 4,1 min
1	MIT – 1,3 min	4	OIT – 7,3 min

Figure C.1 — Example chromatogram: Total ion chromatogram (TIC) of a standard solution of MIT, CMIT, BIT and OIT, all at the concentration of 35 ng/ml

C.8 MS/MS system 2

MS/MS instrument	Agilent 610
Ion source	ESI
General parameters	See Table C.7.

Table C.7 — Ion source and general parameters

Nebulizer	35 psi
Gas flow	nitrogen, 10 l/min
Gas temperature	325 °C
Capillary voltage	2 500 V
Polarity	positive

Annex D
(informative)

Validation results for biocides in eluates from construction products

The method in this document is applicable to the eluate analysis of biocides in organic render.

The results from the intercomparison validation are given in Table D.1.

Table D.1 — Precision data for eluate analysis of renders, after elimination of statistical outliers

	Diuron	Terbutryn	Methylisothiazolinone, MIT	Benzisothiazolinone, BIT	Octylisothiazolinone, OIT	Carbendazim[a]
Mean, mg/l	2 708	712	10 358	15 289	3 105	1 398
Reproducibility S_R	1 119	169	1 666	4 715	749	202
Repeatability S_r	41	17	928	1 814	43	14
Rel. reproducibility S_R, %	41	22	16	31	24	14
Rel. repeatability S_r, %	1,5	2,2	9,0	11,9	1,4	1,0
No. laboratories with results > LOD	3	3	3	3	3	2
No. outliers	–	–	–	–	–	–
No. laboratories after outlier elimination	3	3	3	3	3	2
No. values > LOD after outlier elimination	8	9	9	9	9	6

[a] These results are not taken along in the overall performance evaluation due to a concentration in the eluate too close to the actual detection limit or too low number of remaining test results.

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