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# Analytical Method for the Analysis of Propiconazole in treated Wood Samples

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TECHNICAL REPORT

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# CEN/TR 16420

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English Version

# Analytical Method for the Analysis of Propiconazole in treated Wood Samples

Méthode d'analyse du propiconazole dans des échantillons de bois traité

Analyseverfahren zum Nachweis von Propiconazol in behandelten Holzproben

This Technical Report was approved by CEN on 27 August 2012. It has been drawn up by the Technical Committee CEN/TC 38.

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**Management Centre: Avenue Marnix 17, B-1000 Brussels**



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# Foreword

This document (CEN/TR 16420:2012) has been prepared by Technical Committee CEN/TC 38 “Durability of wood and wood-based products”, the secretariat of which is held by AFNOR.

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## 1 Scope

This CEN Technical Report specifies a laboratory method for determining the content of propiconazole in treated wood using either Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC).

The method is aiming at determining the treatment quality at the time of treatment.

NOTE 1 Under appropriate circumstances the method is applicable for tebuconazole-treated wood as well as for the analysis of waste timber with respect to its propiconazole content.

The method has a detection limit lower than 1 µg propiconazole/g and a quantification limit corresponding to 30 µg propiconazole/g of wood material expressed as dry matter. It can be used over a measurement range up to a propiconazole content of 600 µg/g of dry matter.

NOTE 2 This method may need some modifications with some wood species such as hardwoods.

## 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 212, *Wood preservatives – General guidance on sampling and preparation for analysis of wood preservatives and treated timber*

EN 351-2, *Durability of wood and wood-based products – Preservative treated solid wood – Part 2: Guidance on sampling for the analysis of preservative-treated wood*

EN 13183-1, *Moisture content of a piece of sawn timber – Part 1: Determination by oven dry method*

## 3 Safety precautions

Persons using this method should be familiar with normal analytical laboratory procedures and practice.

This method does not purport to address all safety problems, if any, associated with its use.

It is the responsibility of the user to establish safety and health practices and to ensure compliance with any European or national regulatory conditions.

# 4 Principle

Propiconazole is quantitatively extracted from the wood material using methanol. The extracted compounds are analysed by GC-ECD, GC-MS, GC-NPD, HPLC-UV or HPLC-DAD.

NOTE Other analytical techniques have been reported to be adequately applicable. However, when using other analytical techniques the comparability to the method described in this CEN Technical Report should be demonstrated. Neither FID[1)] nor NPD[2)] detectors are expected to be appropriate detecting devices.

# 5 Reagents

## 5.1 General

During the analysis, unless otherwise specified, use only reagents of recognised analytical grade that have been checked in advance as to not interfere with the analytical results.

**5.2 Methanol ($CH_3OH$)**, analytical grade.

NOTE Other solvents may be used instead of methanol (e.g. ethylacetate) as some extraction difficulties may occur with some wood species (e.g. hardwoods). It is recommended to cross check the extraction efficiency of any other solvent or solvent mixture with that of methanol.

**5.3 Propiconazole [$C_{15}H_{17}Cl_2N_3O_2$] [CAS 60207-90-1] of certified purity:**

- purity >98 % - mixture of stereo isomers (e.g. Pestanal from Sigma-Aldrich, cat. 45642),
- alternatively, (typical purity 50 %) - mixture of stereo isomers, from Janssen Pharmaceutica, supplied with Certificate of Analysis.

# 6 Apparatus

Ordinary laboratory apparatus and the following:

**6.1 Grinder**, capable of grinding timber samples such that the resultant particles will pass through a 1000 µm sieve.

**6.2 Analytical balance**, accurate to 0,1 mg.

**6.3 Ultra-sonic bath** equipped with a thermostat capable of controlling and maintaining a temperature of (50 ± 1) °C.

**6.4 Adjustable volumetric micropipettes**, 10 µl, 100 µl and 1000 µl full capacity.

**6.5 Volumetric pipettes**, 10 ml capacity.

**6.6 Volumetric flasks**, 50 ml capacity.

**6.7 Glass tubes** 30 ml capacity, with screw caps provided with an insert of polytetrafluoroethylene (PTFE) or alternatively with standard ground stoppers provided with PTFE standard ground sockets and conical joint clips.

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1) Concluded from the 2007 round robin test.

2) Concluded from the 2008 round robin test.

**6.8 PTFE filters** 0,45 µm porosity with supporting device.

**6.9 2 ml GC vials** with sealed caps.

**6.10 Gas chromatograph** equipped with a splitless/split or a non-discriminating injection system and an electron capture detector (ECD) for example.

**6.11 Semi-polar separation column**. One capillary column, or preferably two with a stationary phase of different polarity; length: 25 m to 30 m; internal diameter: 0,18 mm to 0,32 mm; film thickness: 0,25 µm to 0,33 µm.

NOTE DB1, DB5, DB17 (phenyl)-methylpolysiloxane columns are examples of suitable products available commercially and supplied by Agilent J&W. This information is given for the convenience of users of this CEN Technical Report and does not constitute an endorsement by CEN of this product.

**6.12 HPLC vials; volume = 2 ml**

**6.13 High performance liquid chromatograph** equipped with a UV detector or a diode array detector (DAD) for example.

**6.14 HPLC column type C18**

NOTE Analytical HPLC column type C18, particle size: 3 µm to 5 µm; length: 100 mm to 150 mm; internal diameter: 4,0 mm to 4,6 mm or similar. Suitable products available commercially: Purospher STAR RP 18 end capped; 5 µm; 150 mm × 4,6 mm, with pre-column C18, 4 mm × 4 mm or Xterra MS C18; 3,5 µm; 100 mm x 4,6 mm

**6.15** A storage area (such as a refrigerator) capable of storing solutions at a temperature of no greater than 5 °C.

## 7 Test sample

### 7.1 General

Collect at least 5 g of the sample material taken according to e.g. EN 212 or EN 351-2.

Preferably under mild conditions without over heating, grind the sample material (e.g. cryogenic grinding) to particles with an approximate size lower than 1,0 mm diameter (passing through a 1000 µm sieve).

NOTE Wood shavings can also be prepared for analysis. However, it is recommended to cross check the extraction yield with that obtained when working on ground material.

Homogenise the ground material to obtain a representative sample, and store it in a brown glass bottle with screw caps with a PTFE insert. This is the test sample.

### 7.2 Dry matter content

Approximately one half of the test sample is to be used to determine the moisture content of the sample. This is achieved by following the principles described in EN 13183-1.

## 8 Analytical procedure

### 8.1 General

It is recommended to carry out at least two parallel analyses. If the results differ by more than 10 %, an additional analysis shall be made.

## 8.2 Standard solutions

### 8.2.1 Preparation of stock solutions

Weigh approximately 0,025 g of propiconazole to the nearest of 0,0001 g and transfer it quantitatively into a 50 ml volumetric flask and make up to the mark with methanol.

NOTE This stock solution with nominal concentrations of 500 mg/l can be stored at 5 °C for at least twelve months. Avoid any temperature lower than 1 °C.

### 8.2.2 Preparation of calibration standards

To a series of five volumetric vials (6.6) transfer respectively 0,3 ml, 1 ml, 2 ml, 5 ml and 10 ml of the stock solution (8.2.1). Make up to the mark with methanol. This gives a series of calibration standards with a propiconazole concentration of 3 µg/ml, 10 µg/ml, 20 µg/ml, 50 µg/ml and 100 µg/ml respectively.

NOTE Alternative method for calibration standards preparation is to weigh all materials on a 4 decimal place balance rather than by volume. 5 calibration standards are prepared with approximate propiconazole concentration of 3 µg/g, 10 µg/g, 20 µg/g, 50 µg/g and 100 µg/g.

The maximum storage time of these solutions is two weeks at max. 5 °C, e.g. in a refrigerator. Avoid any temperature lower than 1 °C.

## 8.3 Determination

### 8.3.1 Extraction

Weigh, to the nearest 0,1 mg, approximately 1 g of the test sample (6.12) and transfer it to an extraction tube (6.7). With a pipette (6.5) add exactly 20,0 ml of methanol to the test sample, ensure that all the wood is wet. Close the tube with the screw cap. Place the tube in the ultra-sonic bath for 2 h at a temperature of 50 °C. Shake it vigorously every 30 min.

Allow the wood to settle and the solution to cool down. Filter the solution through a 0,45 µm porosity filter (6.8) in order to avoid column contamination by wood particles. Appropriate conditions should be applied in order to minimize solvent evaporation.

Using a Pasteur pipette transfer 1 ml or 2 ml of the solution into a GC vial (6.6) or HPLC vial (6.12). Seal the vial with an appropriate cap.

NOTE Other extraction techniques, like Soxhlet extraction, microwave assisted extraction and pressurized fluid extraction (e.g. ASE®) have been reported to be suitable. However, when using other extraction techniques the comparability to the method described in this CEN Technical Report should be demonstrated.

### 8.3.2 Gas chromatography (GC)

#### 8.3.2.1 GC conditions

Guidance on the gas chromatographic conditions is given in the following:

— Carrier gas: helium (minimum purity 99,9996 %);

— Gas flow: 20 cm/s to 30 cm/s;

— Make-up: argon-methane 95:5

— Injection mode: preferably splitless/split, 1 µl (any other non-discriminating injection technique is applicable);

— Injector temperature: 250 °C;

— Detector temperature: 350 °C;

— Oven temperature programme: 90 °C for 2 min; 45 °C /min to 300 °C hold for 5 min.

Please consult the user instructions of the manufacturer for the optimal GC conditions.

### 8.3.3 High Performance Liquid Chromatography (HPLC)

#### 8.3.3.1 HPLC conditions

Guidance on the HPLC conditions is given in the following:

Temperature: 25 °C

Injection volume: 15,0 µl

Wavelength: 223 nm

Elution solvents: A acetonitrile

B 0,5 % (m/v) ammonium carbonate solution

Gradient:

| **Time** (min) | **Flow** (ml/min) | **A** | **B** |
|---|---|---|---|
| 0 | 1,50 | 50 | 50 |
| 4 | 1,50 | 50 | 50 |
| 6 | 1,50 | 100 | 0 |
| 7 | 1,50 | 100 | 0 |
| 9 | 1,50 | 50 | 50 |
| 12 | 1,50 | 50 | 50 |

Retention time: Propiconazole about 7,5 min (Purospher STAR column see NOTE 1)

NOTE 1 If the expected content of the treated wood sample is higher than 1000 µg propiconazole / g wood, the extract should be diluted before the HPLC analysis in order to keep the propiconazole concentration within the calibration range (5 µg/ml to 100 µg/ml).

NOTE 2 It may be necessary to change the wavelength or ratio of the elution solvents to avoid interfering wood extractives.

### 8.3.4 Calibration

#### 8.3.4.1 General

The linear calibration function is based on five calibration points as described in (8.2.2). The calibration curve is established by plotting the ratio of the peak area of the propiconazole standard in function of the corresponding concentration.

NOTE 1 The calibration can also be carried out using the internal standard method.

NOTE 2 A Quality Control (QC) procedure is added to validate calibration. The QC procedure can be a set of calibration solutions from another supplier.

NOTE 3 For some column types avoid elution with 100 % organic solvent.

#### 8.3.4.2 Validity check of the calibration curve

The validity of the calibration curve shall be checked within each batch of samples by analysis of calibration solutions as unknown analytical samples (e.g. two for every ten samples). If the linear function calculated from these control measurements falls within the 95 % confidence interval of the actual calibration function, this function is assumed to be valid. If not, a new calibration function shall be established.

## 9 Calculation and expression of results

The propiconazole concentration in the wood sample, in milligrams per kilogram of the test sample is calculated from the multiple point linear calibration curve using the following relation:

$$C_{\text{propico}} = \frac{C_{\text{extract}} \times V}{m}$$

where

$C_{\text{propico}}$ is the concentration of propiconazole, expressed in mg/kg dry wood;

$V$ is the extraction volume, expressed in ml (usually 20 ml);

$m$ is the sample dry mass, expressed in g (usually 1 g).

$C_{\text{extract}}$ is the concentration of propiconazole in µg/ml in the extract given by the calibration curve or calculated from $C_{\text{extract}} = \frac{(A_{\text{propico}} - b)}{a}$ where:

$A_{\text{propico}}$ is the peak area measured for propiconazole;

$a$ and $b$ are respectively the slope and the offset of the regression curve.

A peak splitting is observed on the chromatogram because propiconazole is a mixture of two diastereomers. Because the ratio can vary significantly, the total area of both peaks has to be considered here for the calculation.

## 10 Quality assurance

For each series of test samples to be analysed, a blank measurement of the whole extraction shall be carried out with samples prepared from the same wood species as to be analysed known to be free of the searched propiconazole.

## 11 Precision

**11.1** Calibration curve (curve linear fit): $r^2 > 0{,}985$.

**11.2** This method was validated in two interlaboratory comparisons carried out in 2007 and 2008 respectively. The 2007 and the 2008 comparison involved 12 and 15 laboratories respectively and covered both GC and HPLC methods. The data collected from the last comparison test was carefully evaluated according to the recommendation of ISO 13528 and lead to the conclusion that the observed robust standard deviation was in the range 8 % to 12 %.

## 12 Test report

The test report shall contain the following information:

a) Description and identification of the material sampled and tested;

b) Reference to this CEN Technical Report;

c) Result of the analysis for propiconazole expressed as a concentration (mg/kg dry wood);

d) The date of sampling;

e) The sampling procedure followed based on EN 351-2;

f) The date of testing;

g) Any deviation from the procedure described in this Technical Report.

# Bibliography

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[5] 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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