Australian/New Zealand Standard™

Methods for sampling and analysing timber preservatives and preservative-treated timber

Part 3: Analysis methods for determination of preservative retention

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Methods for sampling and analysing timber preservatives and preservative-treated timber

Part 3: Analysis methods for determination of preservative retention

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PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee TM-012, Timber Grading and Preservation, to supersede AS/NZS 1605.3:2006.

The objective of this revision is to add analytical procedures to determine—

(a)   triadimefon and cyproconazole in glueline-treated and surface-treated timber;

(b)   quaternary ammonium compound in wood treated with ACQ preservatives;

(c)   didecyldimethyl ammonium chloride in wood treated with ACQ preservatives;

(d)   zinc borate in reconstituted wood products;

(e)   thiacloprid in glueline-treated plywood and laminated veneer lumber (LVL); and

(f)   deltamethrin in glueline-treated engineered wood products.

The objective of this Standard is to set out methods for the sampling and analysing of preservative treated timber.

AS/NZS

1605	Methods for sampling and analysing timber preservatives and preservative-treated timber	
1605.1	Part 1:	General requirements, sampling, and determination of sapwood and heartwood presence
1605.2	Part 2:	Determination of preservative penetration by spot tests
1605.3	Part 3:	Analysis methods for determination of preservative retention (this Standard)
1605.4	Part 4:	Analysis methods for determination of preservative solution concentration

This Standard is intended to be read in conjunction with AS/NZS 1605.1 and the AS/NZS 1604 series, NZS 3640 or the relevant Standard to which timber is claimed to have been treated. The preservatives incorporated in the AS/NZS 1604 series are required to be accompanied by analytical methods for their determination, which are incorporated in the AS/NZS 1605 series.

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Australian/New Zealand Standard

Methods for sampling and analysing timber preservatives and preservative-treated timber

Part 3: Analysis methods for determination of preservative retention

SECTION 1   SCOPE AND GENERAL

1.1 SCOPE

This Standard specifies analysis methods for determining preservative retention in preservative-treated wood products. The calculations necessary for determining compliance with the requirements specified in the AS/NZS 1604 series and NZS 3640 are outlined.

1.2 APPLICATION

This Standard should be read in conjunction with—

(a)   the AS/NZS 1604 series, NZS 3640 or the relevant Standard to which timber is claimed to have been treated; and

(b)   AS/NZS 1605.1.

Methods for sampling and analysing timber preservatives and preservative-treated timber are categorized into the following parts of the AS/NZS 1605 series, according to the general nature of the tests:

1.3 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS/NZS

1604   Specification for preservative treatment (all parts)
1605   Methods for sampling and analysing timber preservatives and preservative-treated timber (all parts)
1605.1   Part 1: General requirements, sampling, and determination of sapwood and heartwood presence

AS/NZS
4491   Timber—Glossary of terms in timber-related Standards

NZS
3640   Chemical preservation of round and sawn timber

1.4   DEFINITIONS

Refer to AS/NZS 1605.1 for applicable definitions.

1.5   PREPARATION OF TEST PIECES FROM TEST SPECIMEN

Test pieces shall be prepared from test specimens cut according to AS/NZS 1605.1, from samples taken according to AS/NZS 1605.1.

The material for analysis shall be evenly cut from across the full cross-section of the penetration zone for the appropriate hazard class specified in the AS/NZS 1604 series or the relevant Standard.

1.6   USE OF ANALYTICAL METHODS FOR EXAMINATION OF PRESERVATIVE PENETRATION

Analytical methods may be used where no effective spot test is available. In such cases, the sample shall be taken from the central ninth of the sapwood penetration zone.

Where heartwood penetration or an envelope is specified, additional samples shall be taken adjacent to the boundary of the required penetration zone. Each sample taken shall be analysed individually for the presence of preservative. For example, in the case of an H3 sample, the central ninth of the penetration zone is required to be analysed as one sample, and samples taken from the heartwood analysed separately.

1.7   RELATED METHODS OF ANALYSIS

The analytical methods given in this Standard are intended as referee methods for the determination of timber preservative retentions. Alternative methods should be compared with methods presented in this Standard before being routinely used, provided that they are of comparable accuracy, e.g. copper chromium arsenic (CCA) treated timber may be analysed by atomic absorption spectrophotometry (AAS), X-ray diffraction (XRF), or inductively-coupled plasma (ICP) methods, and boron-treated timber by alternative water extracted titration method.

SECTION 2   DETERMINATION OF COPPER, CHROMIUM AND ARSENIC IN PRESERVATIVE-TREATED TIMBER

2.1 PRINCIPLE

Copper, chromium and arsenic compounds are leached quantitatively from the timber sample with a mixture of diluted sulphuric acid and hydrogen peroxide solution. After adding sodium sulphate to the resulting extracts, the elemental concentrations are determined by atomic absorption spectrophotometry.

NOTE: The method may be used for copper alone in determining the copper content in timber treated with copper-based preservatives.

2.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a)   *Sulphuric acid, 2.5 M*   Cautiously add, with stirring and cooling, 280 mL sulphuric acid (sg = 1.84 g/mL) to 1500 mL water. Cool and dilute with water to 2000 mL.

(b)   *Hydrogen peroxide solution, 30% (100 volume).*

(c)   *Sodium sulphate, 3.0% solution*   Dissolve 30 g anhydrous sodium sulphate in water and dilute with water to 1000 mL.

(d)   *Sulphuric acid, 0.5 M/sodium sulphate 3.0% diluent solution*   Dilute 200 mL sulphuric acid solution [(Clause 2.2(a)] plus 100 mL of 3.0% sodium sulphate solution to 1000 mL with water and mix.

(e)   *Standard solution (1 mL = 500 μg of arsenic)*

The standard solution shall be prepared as follows:

(i)   Dissolve 0.9825 g copper sulphate pentahydrate, $CuSO_4 \cdot 5H_2O$, in a little water and transfer the solution to a 500 mL one-marked volumetric flask.

(ii)   Dissolve 1.4135 g potassium dichromate, $K_2Cr_2O_7$, in a little water, add 50 mL sulphuric acid solution [Clause 2.2(a)] and 10 mL hydrogen peroxide solution [Clause 2.2(b)]. Boil, cool and transfer the mixture to the one-marked volumetric flask containing the copper sulphate solution.

(iii)   Dissolve 0.6600 g arsenic trioxide, As_2O_3, by boiling it in a solution containing 50 mL sulphuric acid solution [Clause 2.2(a)] 10 mL hydrogen peroxide solution [Clause 2.2(b)] and 75 mL water. Cool and transfer it to the one-marked volumetric flask. Add 50 mL sodium sulphate solution [Clause 2.2(c)] to the one-marked volumetric flask. Dilute to the mark with water and mix.

2.3 PROCEDURE

2.3.1 Instrument settings and operation

The instrument settings and operating conditions for determining copper, chromium and arsenic shall be as recommended in the instrument user's manual.

Copper shall be determined in a fuel-lean air/acetylene flame at 327.4 nm, chromium in a fuel-lean nitrous oxide/acetylene flame at 357.9 nm, and arsenic in a nitrous oxide/acetylene flame at 193.7 nm, with background correction for non-atomic absorption.

2.3.2 Preparation of calibration solutions

The procedure for the preparation of the calibration solutions shall be as follows:

(a)   Transfer aliquot portions of 2, 4, 8 and 16 mL of the standard solution [Clause 2.2(e)] to a series of 200 mL one-marked volumetric flasks.

(b)   Dilute to the mark with sulphuric acid/sodium sulphate solution [Clause 2.2(d)] and mix. When made up this way, 1 mL aliquot portions of this solution contain 5, 10, 20 and 40 μg, respectively, of copper and 10, 20, 40, and 80 μg, respectively, of chromium and of arsenic.

It is necessary to adjust the number of calibration solutions and the range of concentrations covered to suit the sensitivity of the instrument used.

2.3.3 Analysis of treated timber

The procedure for the analysis of treated timber shall be as follows:

(a)   Comminute the dry timber sample so that it will pass through a 0.1 cm mesh. Oven-dry at 103°C before weighing a representative portion for analysis. Accurately weigh—

    (i)   ≈2 g for H1, H2, or H3;

        or

    (ii)   ≈1 g for all other hazard class treatments.

(b)   Transfer the timber sample to a 250 mL conical flask, add 40 mL sulphuric acid solution [Clause 2.2(a)] and, with caution, 8 mL hydrogen peroxide solution [Clause 2.2(b)].

(c)   Heat at 75°C in a water bath for 30 min with occasional swirling to mix the contents of the flask.

(d)   Remove the flask from the water bath, add 80 mL water and 20 mL sodium sulphate solution [Clause 2.2(c)] and cool the flask and contents to room temperature.

(e)   Transfer the contents quantitatively to a 200 mL one-marked volumetric flask, dilute to the mark with water and mix.

(f)   Filter a portion of this solution through a Whatman No. 541 filter paper, discarding the first 50 mL, to give the test solution.

(g)   Using the operating conditions suitable for the instrument, aspirate the sulphuric acid/sodium sulphate solution [Clause 2.2(d)] to obtain the blank absorbance, followed by a suitable range of calibration solutions and the test solution(s).

(h)   Check the calibration solutions after the last test solution has been run.

(i)   Plot the calibration curves of copper, chromium and arsenic (in μg/mL) against absorbance.

(j)   Determine the contents of copper, chromium and arsenic in the test solutions by comparing the absorbance readings with the calibration curves.

If a number of samples are to be analysed, it is necessary to check the instrument stability by bracketing samples with appropriate standard solutions.

2.4 CALCULATIONS OF THE PERCENTAGES OF METALS IN TREATED TIMBER

The percentage by mass of copper or chromium or arsenic in the dry timber shall be calculated from the following equation:

$$\text{Percent metal} = \frac{X}{50\,m} \qquad \qquad \ldots 2.4$$

where

X = the concentration of the appropriate metal in µg/mL of the test solution (Clause 2.3.3)

m = the oven-dried mass of the timber sample taken, in grams

Total active element concentration is determined by adding the values for the percent metal for each component.

2.5 COMPOSITION OF COPPER CHROMIUM ARSENIC (CCA)

The composition of copper chromium arsenic (CCA) shall be calculated to determine whether it conforms to the element ratios as shown in the AS/NZS 1604 series.

SECTION 3   DETERMINATION OF BORON IN PRESERVATIVE-TREATED TIMBER

3.1 PRINCIPLE

After dry-ashing the timber sample in lime-eschka mixture, boron is determined titrimetrically by using mannitol.

3.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a) *Lime eschka*   Mix 250 g anhydrous sodium carbonate and 750 g anhydrous powdered calcium oxide.

(b) *Mannitol solution*   Dissolve 15 g mannitol in 100 mL water. Prepare freshly before use because of biodegradation.

(c) *Phenolphthalein indicator*   Dissolve 0.25 g phenolphthalein in 50 mL ethanol, then add 50 mL water. Adjust pH by adding dilute sodium hydroxide solution until the faint pink colouration persists.

(d) *Methyl red indicator*   Filtered, saturated solution of methyl red in ethanol.

(e) *Standard sodium hydroxide solutions, 0.05M*   Dissolve 2 g sodium hydroxide in 1000 mL water, and standardize as follows:

    (i)   Accurately weigh three 0.1 g portions of potassium hydrogen phthalate (KHP) and dissolve in 25 mL water. After adding 0.5 mL phenolphthalein indicator, titrate with the sodium hydroxide solution.

    (ii) The molarity (*M*) is given by the following equation:

$$M = \frac{(\text{Weight of KHP}) \times 1000}{(\text{Titre}) \times 204.22} \qquad \qquad \text{. . . 3.2}$$

3.3 PROCEDURE FOR ANALYSIS OF TREATED TIMBER

The procedure for the analysis of treated timber shall be as follows:

(a) Comminute the timber sample so that it will pass through a 0.1 cm mesh. Oven-dry at 70°C before taking a representative portion for analysis.

(b) Weigh 1.5 g lime eschka into a numbered porcelain crucible. Accurately weigh a portion (≈2.5 g) of sample into the crucible and mix immediately with the lime eschka. Ash at 550°C for 1 h and allow to cool.

(c) Transfer the ash to a beaker and rinse out the crucible with small portions of dilute (10%) hydrochloric acid and water.

(d) Transfer the washings to the beaker, making sure the sides of the crucible are rubbed down with a glass rod fitted with a short length (about 15 mm to 20 mm) of rubber tubing.

(e) Carefully add 10 mL concentrated hydrochloric acid to the beaker, covering with a watch-glass to prevent spattering.

    NOTE: More acid may be required if the solid contents are not completely dissolved.

(f)   Wash down the watch-glass and beaker sides with distilled water and add 1 mL phenolphthalein indicator.

(g)   Turn the solution just alkaline with cool, freshly prepared 15% sodium hydroxide solution. Stir vigorously and add drop-wise to achieve the pink-grey precipitate colour required.

(h)   Quantitatively transfer the contents of the beaker to a 100 mL volumetric flask, and make up to the mark when cool. (The precipitate is transferred to the volumetric flask.)

(i)   Filter a portion of the solution through a Whatman No. 542 filter paper.

(j)   Pipette 25 mL of the filtrate into a 250 mL Erlenmeyer flask, dilute to 50 mL with water and add 1 mL phenolphthalein indicator.

(k)   Add dilute hydrochloric acid drop-wise until phenolphthalein turns colourless.

(l)   Add 0.5 mL methyl red indicator, which should turn yellow.

(m)   Add dilute hydrochloric acid drop-wise until methyl red turns red. If the solution stands for 4 h or more, the acid aliquot shall be refluxed to eliminate carbon dioxide, and the solution shall be cooled.

(n)   Add the standard sodium hydroxide solution drop-wise until the red colour is just discharged, leaving the solution straw yellow.

(o)   Add 10 mL 15% freshly prepared mannitol solution, whereupon the solution should again turn to the red colour of methyl red.

(p)   Titrate the solution with the standard sodium hydroxide. During the course of titration the colour will change from *red* to the *yellow* of methyl red, and then to the *pink* of phenolphthalein. The end-point is the first permanent appearance of this pink colour.

(q)   Run a blank determination in conjunction with the above procedure, using 2.50 g ground, untreated sapwood in the initial stages.

(r)   Obtain the titre for this blank determination in exactly the same way as for the sample determination.

3.4 CALCULATIONS

The percentage by mass of boron in the dry timber shall be calculated from the following equation:

$$\text{Percent boron} = 1.7288 \, (T - B) \, (M) \qquad \qquad \ldots 3.4$$

where

T and B = titre and blank volumes of M standard sodium hydroxide solution [Clause 3.2(e)(ii)]

M = molarity [see Clause 3.2(e)(ii)]

SECTION 4 DETERMINATION OF FLUORIDE IN PRESERVATIVE-TREATED TIMBER

4.1 PRINCIPLE

The timber sample is fused with sodium hydroxide. The melt is dissolved in water, and fluoride level is determined by ion-selective electrode.

4.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a) *Sodium hydroxide concentrate, 60%* Dissolve 600 g sodium hydroxide in 1000 mL water. Cool and store in a polyethylene container.

(b) *SSA-EDTA buffer* Dissolve 204 g sulphosalicylic acid (SSA) and 74 g disodium dehydrate EDTA in 700 mL water and adjust the pH to 9.5 with concentrated (s.g.:0.880) ammonia solution before dilution to 1000 mL. This buffer is 0.8M SSA and 0.2M EDTA.

(c) *Fluoride standard, 1000 µg/mL* Dissolve 2.210 g sodium fluoride in water and dilute to 1000 mL. Store in a polyethylene container.

4.3 PROCEDURE

4.3.1 Instrument operation

An ion-selective meter shall be used to determine fluoride level.

Several different types of ion-selective meters are available, and each has different operating characteristics. The operating manual shall be followed.

Particular attention shall be given to measuring electrode slope and setting the meter to the current slope value; solutions shall be gently stirred during electrode equilibration.

4.3.2 Preparation of calibration solutions

The procedure for the preparation of the calibration solutions shall be as follows:

(a) Transfer a 200 mL aliquot of the 1000 µg/mL fluoride standard [Clause 4.2(c)] to a 1000 mL one-marked volumetric flask, dilute to the mark and mix well.

(b) Transfer aliquot of 1 and 10 mL of this 200 µg/mL standard solution to 200 mL standard solution to 200 mL one-marked volumetric flasks.

(c) Transfer a 20 mL aliquot of the original 1000 µg/mL fluoride standard [Clause 4.2(c)] to a third 200 mL one-marked volumetric flask.

(d) To each of the three volumetric flasks, add 10 mL sodium hydroxide concentrate [Clause 4.2(a)] and 100 mL SSA-EDTA buffer [Clause 4.2(b)], dilute to the mark and mix well. These calibration solutions contain 1, 10 and 100 µg fluoride/mL, respectively.

(e) Store the calibration solutions in polyethylene containers.

4.3.3 Analysis of treated timber

The procedure for the analysis of treated timber shall be as follows:

(a)   Comminute the timber sample so that it will pass through a 0.1 cm mesh. Oven-dry at 70°C before taking a representative portion for analysis.

(b)   Weigh 2 g ground timber into a 70 mL nickel crucible.

(c)   Add 10 mL sodium hydroxide concentrate, and stir to wet all the material in the crucible.

(d)   Evaporate to dryness in an oven at 80°C.

(e)   Place in a muffle furnace and raise the temperature to 550°C, holding that temperature for 1 h.

(f)   Cool the crucible in a desiccator, add 30 mL water, and warm until the contents dissolve.

(g)   Transfer the solution to a 100 mL volumetric flask and dilute to the mark with water.

(h)   Filter the solution (or allow the solids to settle first) and pipette 25 mL into a small beaker, together with 25 mL SSA-EDTA buffer.

(i)   Using suitable operating procedures for the electrode/meter combination, use calibration solutions (Clause 4.3.2) and set up the instrument calibration at 1, 10 and 100 µg/mL fluoride, ensuring that sufficient equilibration time is allowed, especially at 1 µg/mL.

(j)   Read the fluoride content of the solutions on the calibrated instrument, together with a reagent blank solution.

(k)   If the fluoride content of the timber is expected to be below 0.2% sodium fluoride, reduce the dilution to 100 mL of the crucible contents to 50 mL, and introduce an appropriate factor into the calculations.

4.4 CALCULATIONS

The percentage by mass of fluoride (NaF) in the dry timber, expressed in terms of equivalent sodium fluoride, shall be calculated from the following equation:

$$\text{Percent NaF} = 0.02210\,X \qquad\qquad\qquad \dots 4.4$$

where

X = the fluoride concentration of the test solution, in µg/mL

SECTION 5   DETERMINATION OF
PENTACHLOROPHENOL (PCP) IN
PRESERVATIVE-TREATED TIMBER

5.1 PRINCIPLE

Pentachlorophenol is leached quantitatively from the timber sample with methanol, using ultrasonic agitation. The resulting solution is determined by a high-performance liquid-chromatography system.

5.2 REAGENTS

All reagents shall be of chromatographic reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a) *Methanol.*

(b) *Acetonitrile:water:acetic acid (75:25:0.1)*   Add 1 mL acetic acid to a mixture of 750 mL acetonitrile and 250 mL water. Degas before use.

(c) *Acetonitrile:acetic acid (100:1)*   Add 1 mL acetic acid into acetonitrile. Make up to 1000 mL. Degas before use.

(d) *Pentachlorophenol*   Purified for use as a standard.

(e) *Standard solutions*   Working standards should contain 0.002% m/v and 0.020% m/v pure pentachlorophenol in methanol.

5.3 PROCEDURE

5.3.1 Instruments, settings and operation

A high-performance liquid-chromatography system shall be used. The system shall be capable of step-gradient in solvent composition at a specified run time, with UV detection at 220 nm and peak area integration. Ultrasonic treatment shall be carried out using a thermally insulated ultrasonic bath with a power rating of approximately 50 W/L.

The high-performance liquid-chromatography system consists of the following:

(a) *Detector settings*   0.30 AUFS for Waters 450 at 220 nm.

(b) *Column*   Waters Associates microbondapak C-18, 30 cm × 4 mm steel.

5.3.2 Preparation of samples

The procedure for the preparation of the samples shall be as follows:

(a) Comminute the dry timber sample so that it will pass through a 0.2 cm mesh.

(b) Take 0.5 g to 1 g mass from the specified zone such that its maximum thickness in the longitudinal direction is 4 mm.

   NOTE: Larger samples may be taken provided the thickness is not increased and the total extracting volume is adjusted accordingly.

(c) Place the sample in a 50 mL beaker with 25 mL methanol. Cover and place in an ultrasonic bath at 50°C to 55°C for 1 h.

(d) Decant extract into a 50 mL volumetric flask and re-extract sample with second portion of methanol. Combine extracts, cool, and make up to the mark with methanol.

(e)   Dry the extracted timber sample at 100°C for 4 h before weighing to 0.01 g.

(f)   Submit the extract to the high-performance liquid-chromatography system.

5.3.3 Analysis of treated timber

The procedure for the analysis of the treated timber shall be as follows:

(a)   Filter extracts or standards through a 0.5 µm PTFE filter in an 8 mm Swinnex filter holder.

(b)   Inject 20 µL of extract of standards into the high-performance liquid-chromatography system with an initial solvent flow rate of 2.5 mL/min of 75:25:0.1 acetonitrile: water: acetic acid until 2 min after injection.

(c)   Step to 100:0.1 acetonitrile: acetic acid for 2 min, return to 75:25:01 acetonitrile:water:acetic acid for 4 min.

(d)   Inject 2 or 3 times per sample or standard.

(e)   Compare peak areas or percent mass/volume pentachlorophenol in sample extracts.

5.4 CALCULATIONS

The percent mass/mass of pentachlorophenol (PCP) on an oven-dried timber basis shall be calculated from the following equation:

$$PCP \ (\% \ m/m) = \frac{50 \times E}{m} \qquad \qquad \ldots 5.4$$

where

E = percent PCP mass/volume in the final extract

m = oven-dried mass of timber, in grams

SECTION 6   DETERMINATION OF CREOSOTE OR PIGMENT-EMULSIFIED CREOSOTE IN PRESERVATIVE-TREATED TIMBER

6.1 PRINCIPLE

The retention of creosote, pigment-emulsified creosote, mineral oil and pentachlorophenol, or combinations of these, is measured by extracting these from a weighed timber sample using toluene as a solvent and expressing the mass loss as a percentage of the oven-dried extracted timber mass. The moisture content of the timber shall lie between 8% to 18% of the oven-dried extracted timber mass, or an appropriate volume correction shall be made.

6.2 REAGENT—TOLUENE

NOTE: Laboratory reagent-grade toluene may be used if it has been distilled to remove water.

6.3 PROCEDURE

6.3.1 Determination of moisture content

The moisture content of the test piece is the mass of water recovered during the extraction of preservative and is expressed as a percentage of the mass of extracted timber after oven-drying in a well-ventilated oven at 105 ±2°C to constant mass.

6.3.2 Apparatus

The following apparatus shall be used for the extraction of creosote or mineral oil, or both (see Figure 6.3.2):

(a) *Extraction flask, Soxhlet extractor* The apparatus shall be of glass with standard ground-glass joints open to the atmosphere at the top.

(b) *Water trap, condenser* The apparatus shall be a Dean and Stark side-arm attachment made of glass with standard ground-glass joints, fitted with a water-cooled condenser at the top and open to the atmosphere. The size of the apparatus shall be appropriate to collect within the graduation range of the water trap. The trap shall be chemically clean so that the shape of the meniscus at the end of the extraction is the same as at the beginning. Coating the trap from time to time with silicone resin gives a uniform meniscus.

NOTE: A thin rod of some material to which water does not adhere, such as polytetrafluoroethylene, may be used to transfer water condensed elsewhere to the water layer of the trap.

(c) *Heating apparatus* An electric heating mantle shall be used.
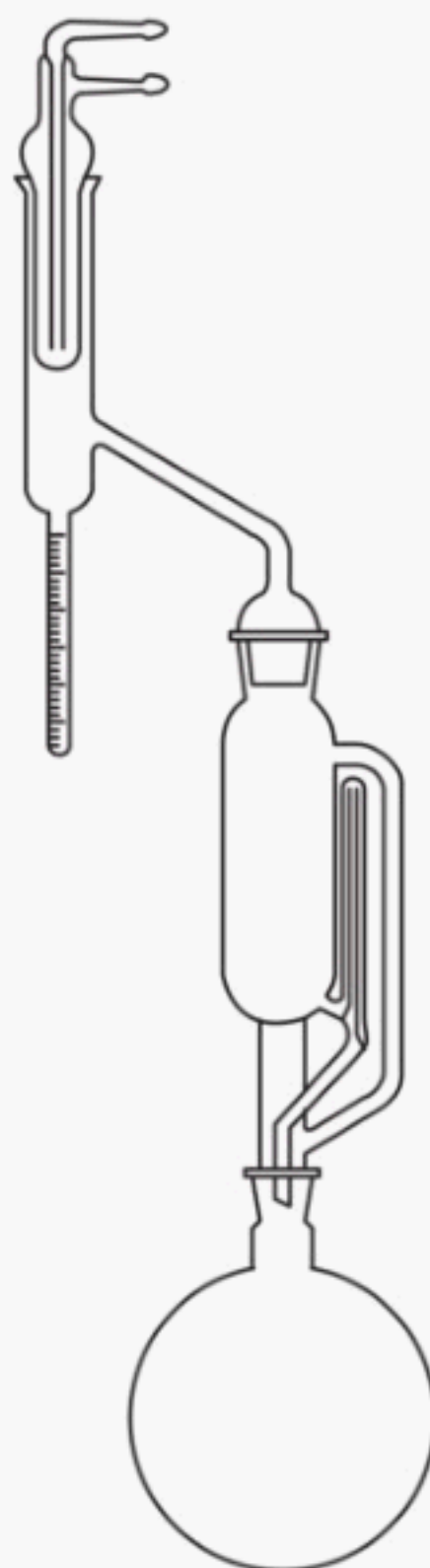
FIGURE 6.3.2 EXTRACTION FLASK, SOXHLET EXTRACTOR, WATER TRAP AND
COLDFINGER (OR CONDENSER) FOR EXTRACTION OF CREOSOTE
AND/OR MINERAL OIL FROM TREATED TIMBER

6.3.3 Extraction of timber

The procedure for the extraction of timber shall be as follows:

(a)   Take a test piece of 5 g to 20 g treated timber. If the test piece to be analysed has been broken into fragments, use the larger of these fragments to make a composite sample of aggregate mass in excess of 10 g. Weigh the test piece in air to an accuracy of 0.02 g.

(b)   Place in the extraction flask sufficient toluene to fill the water trap and Soxhlet extractor and to maintain a reservoir in the extraction flask. Add approximately 0.5 mL distilled water to the toluene. Assemble the apparatus, apply heat and reflux for 30 min. Allow the contents of the water trap to cool to room temperature then, using the polytetrafluoroethylene rod, transfer any water adhering to the walls of the condenser and water trap to the water layer in the trap. Note the volume of water in the trap to the nearest 0.01 mL.

(c)   Transfer the test piece to the Soxhlet extractor in the assembled apparatus.

(d)   Apply sufficient heat to the extraction flask to reflux the toluene at the base of the condenser and to operate the Soxhlet siphon at least once every 12 min. Continue the refluxing for 16 h or until no more colour is extracted from the sample, whichever is greater. Allow the contents of the trap to cool to room temperature before transferring any water adhering to the walls of the condenser or water trap, or both, to the water layer in the trap. Read and record the volume of water in the trap to the nearest 0.01 mL.

The difference between this reading and the first reading represents the mass of water in the test piece.

6.4 CALCULATIONS

The moisture content, mass and percentage of preservative in a test piece shall be calculated from the following equations:

(a)   Moisture content of test piece $= \dfrac{m_3}{m_2} \times 100$ . . . 6.4(1)

(b)   Mass of preservative (creosote or mineral oil, or both) $= m_4 = m_1 - m_2 - m_3$ . . . 6.4(2)

(c)   Percent creosote $\left(\text{or mineral, or both}\right)$ oil $= \dfrac{m_4}{m_2} \times 100$ . . . 6.4(3)

where

$m_1 =$ mass of test piece before extraction, in grams

$m_2 =$ oven-dried mass of extracted test piece, in grams

$m_3 =$ mL of water collected from test piece, in grams

$m_4 =$ mass of toluene-soluble extract, in grams

6.5 CORRECTION

Correction for toluene soluble extract should be applied when the toluene soluble extract from untreated timber exceeds 5% of its oven-dried mass. The extract content of untreated timber may be determined by Soxhlet extraction in the same manner as preservative-treated timber. The calculation for this shall be as follows:

(a) $m_4 = m_1 - m_2 - m_3$ . . . 6.5(1)

(b)   Percent extract $= \dfrac{m_4}{m_2} \times 100$ . . . 6.5(2)

where

$m_1 =$ mass of test piece before extraction, in grams

$m_2 =$ oven-dried mass of extracted test piece, in grams

$m_3 =$ mL of water collected from test piece, in grams

$m_4 =$ mass of toluene-soluble extract, in grams

SECTION 7   DETERMINATION OF ORGANO TIN OXIDE (TBTO) IN PRESERVATIVE-TREATED TIMBER

7.1 PRINCIPLE

Tri-(n-butyl) tin oxide (TBTO) is extracted quantitatively from the timber sample with a mixture of hydrochloric acid and ethanol. The resultant solution is determined by atomic absorption spectrophotometry.

7.2 REAGENTS

All reagents shall be of analytical reagent quality.

The following reagents shall be used:

(a)   *2% hydrochloric acid in ethanol* Add 20 mL of 32% hydrochloric acid to approximately 600 mL ethanol. Mix well and make up to 1000 mL with ethanol.

(b)   *TBTO standard solution*   Weigh exactly 0.2511 g TBTO into a 1000 mL volumetric flask. Make up to the mark with 2% hydrochloric acid in ethanol. This is a 1000 ppm standard solution. Working standards should contain 0, 10, 20, 50 and 100 ppm TBTO made by pipetting 0, 2, 4, 10 and 20 mL of 1000 ppm standard solution to 200 mL volumetrics, making up to the mark with 2% hydrochloric acid in ethanol. Mix well.

7.3 PROCEDURE

7.3.1 Instrument settings and operation

An atomic absorption spectrophotometer shall be used in the determination of tin.

The instrument settings and operating conditions for the atomic absorption spectrophotometer used in the determination of tin shall be as recommended in the instrument user's manual.

Tin shall be determined in a fuel-lean reducing red cone nitrous oxide/acetylene flame at 224.6 nm with deuterium background corrector on.

7.3.2 Sample preparation

The procedure for the preparation of the samples shall be as follows:

(a)   Comminute the timber sample so that it will pass through a 0.5 cm mesh.

(b)   Weigh a portion ($\approx$1 g) out on a 50 mL beaker (m_1).

(c)   Put the beaker in a well-ventilated 105°C oven for 24 h. Reweigh when cool (m_2).

(d)   Accurately weigh another portion of timber sample ($\approx$1 g) into a 100 mL round-bottomed quick-fit flask.

(e)   Add 12 mL of 2% hydrochloric acid in ethanol solvent and connect the flask to a water-cooled condenser. Place in an electrically operated heating mantle.

(f)   Allow to reflux for 10 min. Cool and remove the solvent to a 25 mL volumetric flask.

(g)   Add an additional 12 mL of solvent and reflux for another 10 min.

(h)   Cool and transfer solvent to the 25 mL volumetric flask.

(i)   Make up to the mark with solvent.

Submit sample to analysis by atomic absorption spectrophotometry.

7.4 CALCULATIONS

Calculations shall be as follows:

(a)   Moisture content (*MC*) shall be calculated from the following equation:

$$MC = \frac{m_1 - m_2}{m_2} \times 100 \qquad \qquad \text{...} \, 7.4(1)$$

where

$m_1 =$ mass of initial portion of timber [Clause 7.3.2(b)], in grams

$m_2 =$ mass of oven-dried timber [Clause 7.3.2(c)], in grams

(b)   Percent tin in oven-dried timber shall be calculated from the following equation:

$$\text{Percent tin} = \frac{\text{ppm tin} \times 25 \times (MC + 100)}{10^6 \times m} \qquad \qquad \text{...} \, 7.4(2)$$

where

$m \;=$ mass of timber, in grams

SECTION 8   DETERMINATION OF ORGANO TIN IN TRI-(n-BUTYL)TIN NAPHTHENATE (TBTN) IN PRESERVATIVE-TREATED TIMBER

8.1 GENERAL

This procedure is suitable for the quantitative determination of tin as tributyltin naphthenate (TBTN) and related compounds in the sapwood of *Pinus* and *Araucaria cunninghamii* timbers.

8.2 PRINCIPLE

Discrete pieces of preservative-treated timber are dried at 70°C for 24 h before being coarsely ground. The TBTN and related compounds are extracted from the timber using a solution of hydrochloric acid in ethanol and ultrasonication. Tin is determined in the combined extracts by atomic absorption spectrophotometry using a nitrous oxide/acetylene flame against standards in a matrix matching the samples prepared using elemental tin.

8.3 METHOD CHARACTERISTICS

The method characteristics shall be as follows:

(a) *Sensitivity*   Limit of detection of tin is 0.006% in the timber (2 g sample extracted by a total of 50 mL).

(b) *Upper limit of quantitation*   With appropriate dilution, from 0.25% to 2.5% in timber.

(c) *Precision*   At the level of 0.06% in timber, precision of 1.0% has been achieved for replicate analysis of spiked samples, and of 1.0% for replicate analysis of commercially treated timber.

(d) *Specificity*   Any tin in a form that can be extracted by the ethanolic hydrochloric acid reagent will be determined. Spectroscopic interferences are negligible.

8.4 REAGENTS

All reagents shall be of analytical reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a) *Reagent 1—Ethanol*   Commercial grade denatured absolute alcohol.

(b) *Reagent 2—Hydrochloric acid*   AR Grade hydrochloric acid, ≈32% w/v, ≈10 M, r_{20} = 1.16 g/mL.

(c) *Reagent 3—Extracting solution*   0.20 M hydrochloric acid in ethanol. Dilute 20 mL hydrochloric acid [reagent 2, Item (b) above] to 1000 mL with ethanol [reagent 1, Item(a) above], in a 1000 mL volumetric flask.

(d) *Reagent 4—Stock standard tin solution (10 000 mg/L Sn)*   Dissolve 10 000 mg metallic tin in 100 mL hot sulphuric acid. Make up to 1000 mL in a volumetric flask with 50 mL sulphuric acid and water to the mark.

(e) *Reagent 5—Stock standard tin solution (250 mg/L Sn)*   Dilute 25 mL of stock standard tin solution (10 000 mg/L Sn) to 1000 mL in a volumetric flask using the extracting solution [reagent 3, Item (c) above] as diluent.

(f) *Reagent 6—Working tin standards* Prepare a series of standards of 5, 10, 25, 50 and 100 mg/L Sn by diluting 5, 10, 25, 50 and 100 mL aliquot of stock standard tin solution [250 mg/L, reagent 5, Item (e) above], to 250 mL with extracting solution [reagent 3, Item (c) above].

8.5 APPARATUS

The apparatus shall be as follows:

(a) *Atomic absorption spectrophotometer.*

(b) *Volumetric flasks* Pyrex, 50 mL.

(c) *Beakers* Pyrex, 50 mL.

(d) *Ultrasonic bath.*

8.6 PROCEDURE

8.6.1 Preparation of timber

Timber samples shall be dried at 70°C for 24 h to 48 h before being comminuted in a cross-beater mill to pass a 5 mm screen.

8.6.2 Extraction procedure

The extraction procedure shall be as follows:

(a) Accurately weigh a portion ($\approx$2 g) of ground timber into a 50 mL tall form Pyrex beaker.

(b) Add 20 mL of extracting solution [reagent 3, Clause 8.4(c)] to the timber sample and place a small watch-glass on the beaker.

(c) Agitate the sample and extracting solution for 1 h in an ultrasonic bath containing water at 45°C.

(d) Allow the extracting solution to cool and settle then decant it into a 50 mL volumetric flask.

(e) Repeat Steps (c) and (d) using two successive 15 mL volumes of extracting solution [reagent 3, Clause 8.4(c)], and combine the extracts with the previous ones in the same flask.

(f) Make up the combined extracts to 50 mL using extracting solution [reagent 3, Clause 8.4(c) above].

(g) Present the extract solution to the AAS analysis (see Clause 8.6.3) for determination of tin.

8.6.3 AAS analysis

The sample shall be aspirated into the atomic absorption spectrophotometer with the following instrument parameters:

(a) Detection mode .... absorbance.

(b) Wave length ....235.5 nm.

(c) Slit width ....0.2 nm.

(d) Lamp current ....7 mA.

(e) Flame .... N$_2$O/acetylene.

(f) Fuel flow ....6 L/min.

(g) Measurement time .... 5 sec.

(h)   Replicates .... 3.

(i)   Background correction..On.

(j)   Re-slope rate .... 5.

(k)   Oxidant flow... 11 L/min.

8.7 CALCULATION OF RESULTS

The concentration of tin (Sn) in the timber (in percent mass/mass) shall be calculated from the following equation:

$$\mathrm{Sn}\left(\%m/m\right) = \frac{X}{50 \times m} \qquad \qquad \ldots 8.7$$

where

X = the concentration of tin in the extract solution, in milligrams per litre

m = the mass of dried timber taken for extraction, in grams

SECTION 9   DETERMINATION OF TOTAL
TIN IN TRI-(n-BUTYL)TIN NAPHTHENATE
(TBTN) IN PRESERVATIVE-TREATED TIMBER

9.1 PRINCIPLE

Tin is extracted from the timber sample using a solution of hydrochloric acid in ethanol. The concentration of tin in the extract is determined by atomic absorption spectrophotometry.

9.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a) *Ethanolic hydrochloric acid*   Dilute 20 mL of 32% hydrochloric acid to 1000 mL in a volumetric flask with ethanol (denatured absolute alcohol).

(b) *Stock tin standard, 10 000 mg/L*   Dissolve 10 000 mg tin metal in 100 mL concentrated hydrochloric acid in a 600 mL beaker. This process is slow. It will need to be done in a fume hood overnight. Transfer the solution quantitatively to a 1000 mL one-marked volumetric flask, add 100 mL concentrated hydrochloric acid and make up to the mark with water.

(c) *Intermediate tin standard, 250 mg/L*   Pipette 25 mL stock tin standard, 10 000 mg/L [Clause 9.2(b)] into a 1000 mL volumetric flask and make up to volume with ethanol.

9.3 PROCEDURE

9.3.1 Instrument settings and operation

Tin shall be determined using a nitrous oxide/acetylene flame with its stoichiometry adjusted to give a reducing red cone 2 cm to 3 cm high.

The instrument settings and operation shall be as follows:

(a) The wavelength used shall be 235.5 nm at a slit width appropriate for the particular instrument (usually 0.2 nm). If available, use background correction.

(b) Fill the nebulizer trap with either ethanol or ethanolic hydrochloric acid [Clause 9.2(a)] to ensure correct vapour pressure in the nebulizer.

(c) After completing the analysis, remove all traces of ethanol from the trap by removing and washing the trap with water.

9.3.2 Preparation of calibration solutions

Calibration solutions shall be prepared as follows:

Prepare a series of standards of 5, 10, 25, 50, and 100 mg(Sn)/L by diluting 5, 10, 25, 50, and 100 mL aliquot of intermediate tin standard, 250 mg/L [Clause 9.2(c)] to 250 mL with ethanolic hydrochloric acid [Clause 9.2(a)] in separate volumetric flasks.

9.3.3 Analysis of treated timber

The procedure for the analysis of the treated timber shall be as follows:

(a) Air-dry the timber such that practically all of the solvent has evaporated.

(b)   Grind the timber to pass a 5 mm screen. (Generally timber that has not been oven-dried will not pass any screen finer than 5 mm.)

(c)   Accurately weigh (to four decimal places) a portion ($\approx$2 g) of timber into a 50 mL tall form Pyrex beaker and another $\approx$5 g of the same timber into a tared crucible.

(d)   Dry the timber in the crucible at 105°C overnight and then reweigh. The weight loss is used to determine the moisture content of the sample and to correct the mass of the sample taken for analysis to an equivalent oven-dried mass.

(e)   Add 20 mL ethanolic hydrochloric acid [Clause 9.2(a)] to the timber in the beaker, cover with a watch-glass and sonicate in an ultrasonic bath for 1 h.

(f)   Decant the supernatant into a 50 mL volumetric flask.

(g)   Add an additional 15 mL ethanolic hydrochloric acid [Clause 9.2(a)] to the timber in the beaker and sonicate for 1 h and then decant the supernatant into the same volumetric flask.

(h)   Add a final portion of 15 mL ethanolic hydrochloric acid [Clause 9.2(a)] to the timber in the beaker and sonicate for 1 h and then decant the supernatant into the same volumetric flask.

(i)   Make the contents of the volumetric flask up to 50 mL with ethanolic hydrochloric acid [Clause 9.2(a)].

(j)   Allow the slight turbidity to settle out before aspirating the solution into the atomic absorption spectrophotometer.

(k)   Fill the nebulizer trap with ethanolic hydrochloric acid [Clause 9.2(a)]. Using the operating conditions suitable for the instrument, aspirate ethanolic hydrochloric acid solution [Clause 9.2(a)] to obtain a blank absorbance reading, followed by a suitable range of calibration solutions and then the test solution(s).

(l)   Check the calibration solutions after the last test solution has been run.

(m)   Plot the calibration curve of tin (in milligrams per litre) against absorbance.

(n)   Determine the contents of tin in the test solutions by comparing the absorbance readings with the calibration curve.

NOTE: If a number of samples are to be analysed it may be advisable to check the instrument stability by bracketing samples with appropriate standard solutions.

9.4 CALCULATIONS

The concentration of tin (Sn) in the timber (in percent mass/mass) shall be calculated from the following equation:

$$Sn \ (\% \ m/m) = \frac{X}{50 \times m_1 \left(\dfrac{m_2 - m_3}{m_2} \right)} \qquad \ldots 9.4$$

where

X = concentration of tin in the extract, in milligrams per litre

m_1 = mass of timber taken for extraction, in grams

m_2 = mass of timber taken for moisture determination, in grams

m_3 = mass of timber after drying at 105°C, in grams

SECTION 10   DETERMINATION OF
ORGANOCHLORINE INSECTICIDES IN
PRESERVATIVE-TREATED TIMBER

10.1 PRINCIPLE

Organochlorines are quantitatively leached from the timber sample with methanol using ultrasonics. The resultant solutions are cleaned up and analysed by gas chromatography using electron capture detection.

10.2 REAGENTS

All reagents shall be of chromatographic solvent purity.

The following reagents shall be used:

(a)   Standard solutions:

(i)   *100 µg aldrin/mL*   Weigh sufficient aldrin of known purity to contain 50.0 mg exactly. Dissolve in hexane and dilute to 500 mL in a one-marked volumetric flask.

(ii)   *100 µg dieldrin/mL*   Weigh sufficient dieldrin of known purity to contain 50.0 mg exactly. Dissolve in hexane and dilute to 500 mL in a one-marked volumetric flask.

(iii)   *100 µg chlordane/mL*   Weigh sufficient chlordane of known purity to contain 50.0 mg exactly. Dissolve in hexane and dilute to 500 mL in a one-marked volumetric flask.

(iv)   *100 µg heptachlor/mL*   Weigh sufficient heptachlor of known purity to contain 50.0 mg exactly. Dissolve in hexane and dilute to 500 mL in a one-marked volumetric flask.

Store these standard solutions in well-sealed screw-top bottles to prevent evaporation.

(b)   *Extractant*   Methanol, to extract the organochlorines from the timber samples.

(c)   *Florisil for clean-up column*   Florisil (60 to 100 mesh), furnaced at 550°C for 4 h and stored in a desiccator over silica gel. To obtain optimum recovery of pesticide residues and optimum chromatographic separation of residue peaks from interfering substances, it is usually necessary to partially deactivate the ignited florisil by adding a small percentage of water, the required amount being determined by experimenting on a sample extract spiked with known amounts of the pesticides of interest.

(d)   *Eluant*   15% diethyl ether in hexane prepared by adding 300 mL diethyl ether to 1500 mL n-hexane. Make up to 2000 mL with n-hexane and store over anhydrous sodium sulphate.

(e)   *Diluent*   Toluene is used for any further dilutions to bring the organochlorines levels into the workable instrument range.

(f)   *Drying agent*   Anhydrous sodium sulphate is used to dry the extracted and reduced methanol extracts.

10.3 PROCEDURE

10.3.1 Instrument settings and operation

The instrument settings and operation shall be as follows:

(a)   A gas chromatography instrument fitted with an electron capture detector shall be used.

NOTE: Columns used may be packed glass (e.g. 1.8 m × 2 mm, Sil XE-60 on a high performance support) or capillary (e.g. DB-5, 15 m fused silica or 12 m × 0.22 mm SGE BP 10).

(b)   Typical temperature settings shall be as follows:

(i)   Injector... 220°C.

(ii)   Oven .... 190°C.

(iii)   Detector .... 260°C.

(c)   Ultrasonic treatment shall be carried out using a thermally insulated ultrasonic bath, with a power rating of approximately 50 W 1 to 1.

Argon/methane (P-10) is recommended as the carrier gas. Typical gas flow rates are 25 mL/min. The detector electrometer output shall be monitored with a suitable integrator using peak area quantization.

10.3.2 Preparation of calibration solutions

Calibration solutions shall be prepared as follows:

Transfer aliquot portions of the standard solutions [Clause 10.2(a)], appropriate to the expected organochlorine content of the timber to be analysed, to two 100 mL one-marked volumetric flasks. Make up to the mark with toluene.

10.3.3 Preparation of clean-up column

The clean-up column shall be prepared as follows:

(a)   Place a layer of baked florisil 10 cm deep in a 1.5 cm i.d. glass column containing 13 cm of hexane.

(b)   Place a 2.5 cm layer of anhydrous sodium sulphate on top of the florisil.

NOTE: Samples should be poured through a porosity 1 sintered glass filter funnel containing an additional 1 cm layer of sodium sulphate.

10.3.4 Recovery check

The recovery check shall be carried out as follows:

(a)   Check the column efficiency by selecting an aliquot of a calibration solution and adding this to the clean-up column.

(b)   Eluate with 150 mL of 15% diethyl ether in hexane into a 200 mL volumetric flask. Make up to the mark and mix well.

(c)   Compare this with the same aliquot added directly into a 200 mL volumetric flask, adding 150 mL eluant and making up to the mark with hexane.

(d)   Check the organochlorine levels in both recovery volumetric flasks.

NOTE: If levels differ significantly, the column should be remade using less active florisil.

10.3.5 Sample preparation

The samples shall be prepared as follows:

(a)   Comminute the air-dried timber sample so that it passes through a 0.5 cm mesh. Accurately weigh 1 g ground timber in a 100 mL beaker.

(b)   Add 50 mL methanol. Extract in an ultrasonic bath at 50°C to 55°C for 15 min. Decant solvent into another 100 mL beaker. Add an additional 50 mL of methanol.

(c)   Repeat the extraction and decanting two more times, adding the extracting solvent to the same beaker.

(d)   Retain extracted timber. Reduce the volume of extracts to 10 mL by evaporation at ambient temperatures.

(e)   Add 50 mL portions of eluant and dissolve all material in the beaker using a policeman or spatula.

(f)   Pass through clean-up column, adding two further 50 mL portions of eluant. Collect into a 200 mL volumetric flask. Make up to the mark with hexane.

(g)   Make further dilutions (D) by taking aliquot from the 200 mL flask, placing it into another volumetric flask of appropriate volume and making up to the mark with toluene.

(h)   Dry the extracted timber at 103°C and weigh to constant mass (m).

10.3.6 Analysis

Submit the samples recovered after clean-up for gas chromatography using injection volumes and oven program conditions suitable for the column used.

NOTE: In most cases, organochlorines will be expressed as parts per million in the clean-up solution.

10.4 CALCULATION

The percent mass/mass organochlorine shall be calculated from the following equation:

$$\text{Organochlorine } (\% \ m/m \ ) = \frac{0.02 \times y \times D}{m} \qquad \qquad \ldots 10.4$$

where

y = ppm organochlorine in the final sample

m = oven-dried timber mass, in grams

D = further dilutions of extract (after initial 200 mL)

SECTION 11   DETERMINATION OF COPPER IN COPPER NAPHTHENATE IN PRESERVATIVE-TREATED TIMBER

11.1 PRINCIPLE

Copper is leached from the timber sample by the action of dilute sulphuric acid and hydrogen peroxide. The concentration of copper in the leachate is determined by atomic absorption spectrophotometry.

11.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a) *Sulphuric acid, 2.5 M* Cautiously add, with stirring and cooling, 280 mL concentrated sulphuric acid to 1500 mL water. Cool and dilute to 2000 mL with water.

(b) *Hydrogen peroxide solution, 30% (100 vol).*

(c) *Standard solution* Dissolve 0.9825 g copper sulphate pentahydrate, $CuSO_4 \cdot 5H_2O$, in 500 mL water in a volumetric flask.

11.3 PROCEDURE

11.3.1 Instrument settings and operation

Copper shall be determined by using a fuel-lean air/acetylene flame at 327.4 nm.

All other settings and conditions shall be as recommended in the appropriate instrument user's manual.

11.3.2 Preparation of a blank

A blank solution shall be prepared as follows:

(a) Dilute 40 mL sulphuric acid, 2.5 M [Clause 11.2(a)] with 8 mL hydrogen peroxide [Clause 11.2(b)] and 20 mL water in a 250 mL conical flask.

(b) Heat in a water bath at 75°C for 30 min.

(c) After cooling, transfer the solution quantitatively to a 200 mL volumetric flask and make up to the mark with water.

11.3.3 Preparation of calibration solutions

The calibration solutions shall be prepared as follows:

(a) Pipette 2, 4, 8, and 16 mL standard solution [Clause 11.2(c)] into a series of 200 mL one-marked volumetric flasks.

(b) Add 40 mL sulphuric acid, 2.5 M [Clause 11.2(a)].

(c) Dilute to the mark with water.

The resulting solutions have concentrations of 5, 10, 20, and 40 mg/L copper.

NOTE: It may be necessary to adjust the number of calibration solutions and the range of concentrations covered to suit the sensitivity of the instrument used.

11.3.4 Analysis of treated timber

The procedure for the analysis of the treated timber shall be as follows:

(a) Comminute the dry timber sample so that it will pass through a 1 mm mesh. Oven-dry at 70°C overnight.

(b) Accurately weigh a portion (≈4 g) of timber in a clean, dry 250 mL conical flask.

(c) Add 40 mL of the sulphuric acid, 2.5 M [Clause 11.2(a)]. Heat in a water bath at 75°C for 30 min, swirling occasionally to mix the contents of the flask.

(d) Remove the flask from the water bath and add 80 mL of water. Allow to cool.

(e) Transfer the mixture quantitatively to a one-marked 200 mL volumetric flask and make up to the mark with water.

(f) Filter a portion of this solution through a Whatman No. 541 paper, discarding the first 50 mL, to give the test solution.

(g) Using the operating conditions suitable for the instrument, aspirate the blank (Clause 11.3.2) to obtain the blank absorbance, followed by a suitable range of calibration solutions and then the test solution(s).

(h) Check the calibration solutions after the last test solution has been run.

(i) Plot the calibration curve of copper (in milligrams per litre) against absorbance.

(j) Determine the contents of copper in the test solutions by comparing the absorbance readings with the calibration curve.

NOTE: If a number of samples are to be analysed, the instrument stability should be checked by bracketing samples with appropriate standard solutions.

11.4 CALCULATIONS

The concentration of copper (Cu, in percent mass/mass) in the dry timber shall be calculated from the following equation:

$$Cu\ (\%\,m/m) = \frac{X}{50 \times m} \qquad \ldots 11.4$$

where

X = the concentration of copper in the leachate, in milligrams per litre

m = the oven-dried mass of the timber sample taken, in grams

SECTION 12 DETERMINATION OF COPPER IN ALKALINE COPPER QUATERNARY (ACQ) PRESERVATIVE-TREATED TIMBER

12.1 PRINCIPLE

Copper is leached from the timber sample by the action of dilute sulphuric acid and hydrogen peroxide. The concentration of copper in the leachate is determined by atomic absorption spectrophotometry.

12.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for preparing the reagents shall be distilled.

The following reagents shall be used:

(a) *Sulphuric acid, 2.5 M* Cautiously add, with stirring and cooling, 280 mL concentrated sulphuric acid to 1500 mL water. Cool and dilute to 2000 mL with water.

(b) *Hydrogen peroxide solution, 30% (100 vol).*

(c) *Standard solution* Dissolve 0.9825 g copper sulphate pentahydrate, $CuSO_4 \cdot 5H_2O$, in 500 mL water in a volumetric flask.

12.3 PROCEDURE

12.3.1 Instrument settings and operation

Copper shall be determined using a fuel-lean air/acetylene flame at 327.4 nm.

All other settings and conditions shall be as recommended in the appropriate instrument user's manual.

12.3.2 Preparation of a blank

A blank solution shall be prepared as follows:

(a) Dilute 40 mL of the sulphuric acid, 2.5 M [Clause 12.2(a)] with 8 mL hydrogen peroxide [Clause 12.2(b)] and 20 mL water in a 250 mL conical flask.

(b) Heat in a water bath at 75°C for 30 min.

(c) After cooling, transfer the solution quantitatively to a 200 mL volumetric flask and make up to the mark with water.

12.3.3 Preparation of calibration solutions

The calibration solutions shall be prepared as follows:

(a) Pipette 2, 4, 8, and 16 mL of the standard solution [Clause 12.2(c)] into a series of 200 mL one-marked volumetric flasks.

(b) Add 40 mL of the sulphuric acid, 2.5 M [Clause 12.2(a)] and dilute to the mark with water.

The resulting solutions have concentrations of 5, 10, 20, and 40 mg/L copper.

NOTE: It may be necessary to adjust the number of calibration solutions and the range of concentrations covered to suit the sensitivity of the instrument used.

12.3.4 Analysis of treated timber

The procedure for the analysis of the treated timber shall be as follows:

(a)   Comminute the dry timber sample so that it will pass through a 1 mm mesh. Oven-dry at 70°C overnight.

(b)   Accurately weigh a portion ($\approx$4 g) of timber into a clean, dry 250 mL conical flask.

(c)   Add 40 mL sulphuric acid, 2.5 M [Clause 12.2(a)].

(d)   Heat in a water bath at 75°C for 30 min, swirling occasionally to mix the contents of the flask.

(e)   Remove the flask from the water bath and add 80 mL water. Allow to cool.

(f)   Transfer the mixture quantitatively to a one-marked 200 mL volumetric flask and make up to the mark with water.

(g)   Filter a portion of this solution through a Whatman No. 541 paper, discarding the first 50 mL, to give the test solution.

(h)   Using the operating conditions suitable for the instrument, aspirate the blank (Clause 12.3.2) to obtain the blank absorbance, followed by a suitable range of calibration solutions and then the test solution(s).

(i)   Check the calibration solutions after the last test solution has been run.

(j)   Plot the calibration curve of copper (in milligrams per litre) against absorbance.

(k)   Determine the contents of copper in the test solutions by comparing the absorbance readings with the calibration curve.

   NOTE: If a number of samples are to be analysed it may be advisable to check the instrument stability by bracketing samples with appropriate standard solutions.

12.4 CALCULATIONS

The concentration of copper (Cu, in percent mass/mass) in the dry timber shall be calculated from the following equation:

$$Cu\left(\%\,m/m\right) = \frac{X}{50 \times m} \qquad \qquad \ldots 12.4$$

where

X = concentration of copper in the leachate, in milligrams per litre

m = oven-dried mass of the timber sample taken, in grams

12.5 COMPOSITION OF ALKALINE COPPER QUATERNARY (ACQ)

The composition of ACQ shall be calculated to determine whether it conforms to the element ratios as shown in the AS/NZS 1604 series.

SECTION 13   DETERMINATION OF BIFENTHRIN IN PRESERVATIVE-TREATED TIMBER

13.1 PRINCIPLE

Bifenthrin is extracted from ground timber by ultrasonication and heat with acetone. The extracts are combined in a volumetric flask and made up to volume. The extract is analysed by gas chromatography (GC) using an electron capture detector (ECD) and an internal or external standard calibration procedure. Results are expressed on an oven-dried weight basis by correction for the moisture content determined on a parallel sample.

The units reported are percent mass/mass (% m/m) (oven-dried basis).

13.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for the preparation of the reagents shall be distilled water.

The following reagents shall be used:

(a) *Acetone* Laboratory reagent, free of interfering compounds for GC (gas chromatograph) analysis.

(b) *Bifenthrin* Analytical reference-grade material with certificate of purity.

(c) *Nitroxylene* 2-nitro-m-xylene 99%.

(d) *Internal standard, nitroxylene, 500 mg/L in acetone* Dilute 100 μL 2-nitroxylene to 200 mL with acetone.

(e) *Stock standards, 1000 mg/L bifenthrin* Weigh 100 mg bifenthrin standard to nearest 0.1 mg. Dissolve in acetone and make up to 100 mL.

(f) *Working standards, 0.1 mg/L to 10 mg/L* Prepare working standards for GC in acetone. Store standards in fridge.

13.3 SAMPLING

The analytical zone shall be roughly sawn in small pieces (i.e. 1 cm^2) or, where an envelope of treatment is required, the outer 2 mm shall be sawn off.

The sample shall be comminuted in a cross-beater rotary mill to pass a 5 mm screen, and homogenized and dried before a sub-sample shall be taken for analysis.

13.4 PROCEDURE

13.4.1 Instrument settings and operating conditions

The instrument settings and operating conditions shall be in accordance with Table 13.4.1.

TABLE 13.4.1

GAS CHROMATOGRAPH CONDITIONS

Capillary column	Model number	BPX5-0.25
	Nominal length	25.0 m
	Nominal diameter	0.32 mm
Oven	Initial temperature	100°C
	Initial time	1.00 min
	Ramp #1	15°C
	Final temperature	285°C
	Final time	5.00 min
	Run time	18.33 min
	Post time	0 min
	Post temperature	50°C
Inlet	Mode	Splitless
	Initial temperature	250°C
	Total flow	31 mL/min
	Gas type	Helium
Detector	ECD	
	Temperature	350°C
	Mode	Constant column + make-up flow
	Combined flow	30 mL/min
	Make-up gas type	Nitrogen

13.4.2 Weighing and moisture content

The procedure for weighing and determining moisture content shall be as follows:

(a)   Individually label a 100 mL Schott bottle and a 50 mL beaker for each sample using a permanent marker.

(b)   Weigh accurately 1 g of each sample into its corresponding Schott bottle and record the weight to the nearest 0.01 g (*Wet sample*).

(c)   Weigh the correspondingly labelled empty beakers and record the weight to the nearest 0.01 g (*Tare MC*).

(d)   Weigh approximately 5 g of ground timber into the beaker, and record the weight to the nearest 0.01 g (*Wet MC*).

(e)   Place *Wet MC* beaker in the oven (105°C) for a minimum of 18 h.

(f)   Remove the beaker from the oven and cool in a desiccator.

(g)   Weigh the beaker containing the dried sample and record the weight to the nearest 0.01 g (*Dry MC*).

13.4.3 Extraction

The extraction procedure shall be as follows:

(a)   Add acetone (15 mL or 30 mL, see Note below) and screw the lid on the Schott bottle.

NOTE: For timber treated to 0.02%m/m bifenthrin where only the outer 2 mm of timber is analysed, use double the quantities of acetone for each extraction and transfer to a 100 mL volumetric flask and make up to final volume of 100 mL.

(b)   Prepare the ultrasonic bath by filling with warm tap water (40°C to 50°C) to at least the solvent level in the Schott bottle.

(c)   Place 100 mL Schott bottles in the ultrasonic bath, switch on the bath and allow extraction to proceed for 45 min.

(d)   Remove the bottle and then place on absorbant paper. Allow the bottle to cool to room temperature.

(e)   Transfer the liquid contents of each beaker quantitatively to a corresponding labelled receiver (50 mL or 100 mL volumetric flask, see Note above).

(f)   Add another 15 mL (or 30 mL, see Note above) acetone to the sample in the 100 mL Schott bottles. Proceed as in Steps (c) and (e) above.

(g)   Make a final addition of acetone (15 mL or 30 mL, see Note above) to the 100 mL Schott bottles. Proceed as in Steps (c) and (e) above. Rinse the 100 mL Schott bottles with a few millilitres of acetone.

(h)   Make a 50 mL or 100 mL volumetric flask (see Note above) up to the mark with acetone ready for GC analysis.

(i)   Using a pipette, dispense 1 mL of each sample into the corresponding labelled vial.

(j)   Add 20 μL nitroxylene internal standard solution and mix.

(k)   Present samples, designated as *S*, in milligrams per litre, for analysis by gas chromatography.

13.5   DATA HANDLING AND CALCULATIONS

13.5.1   Oven-dried weight

The oven-dried weight shall be calculated from the following equation:

$$\text{Oven-dried weight} = 1 - \frac{(Wet\ MC - Dry\ MC)}{(Wet\ MC - Tare\ MC)} \times wet\ sample \qquad \ldots 13.5.1$$

where *Wet MC*, *Dry MC*, *Wet MC*, *Tare MC*, and *Wet sample* are defined in Clause 13.4.2.

13.5.2   Calibration and sample calculation

The calibration and sample shall be calculated from the following equation:

$$\text{Bifenthrin}\ (\%\ m/m) = \frac{V \times S}{(\text{Oven-dried wieght}) \times 10000} \qquad \ldots 13.5.2$$

where

     V = volume of extract, in millilitres

     S = sample, in milligrams per litre [see Clause 13.4.3(k)]

13.5.3   Expression of results

The final results shall be expressed as % m/m of bifenthrin, to three decimal places.

SECTION 14   DETERMINATION OF BIFENTHRIN IN GLUELINE-TREATED TIMBER

14.1 PRINCIPLE

Bifenthrin is extracted from a sample of the product by boiling with toluene and formic acid (94/6). A portion of the resulting extract is analysed for bifenthrin content by capillary gas chromatography (GC). The units reported are % m/m (oven-dried basis).

14.2 REAGENTS

All reagents shall be of analytical reagent quality. The water used for the preparation of the reagents shall be distilled water.

The following reagents shall be used:

(a) *Toluene*   Analytical reagent grade.

(b) *Bifenthrin*   Analytical reference-grade material with certificate of purity.

(c) *Formic acid*   Laboratory reagent grade

(d) *Nitroxylene*   2-nitro-m-xylene 99%.

(e) *Anhydrous sodium sulphate*   Analytical reagent grade.

(f) *Internal standard, nitroxylene, 1000 mg/L in toluene*   Dilute 100 μL 2-nitroxylene to 100 mL with toluene.

(g) *Stock standards, 100 mg/L bifenthrin in toluene*   Weigh out to the nearest 0.1 mg approximately 10 mg bifenthrin [Clause 14.2(b)] and dissolve in toluene. Make up to 100 mL with toluene. Record the actual weight in the organic lab standard solutions register.

(h) *Working standards, 0.1 mg/L to 10 mg/L*   Prepare working standards in toluene. Store standards in fridge.

14.3 SAMPLING

The plywood or LVL sub-samples shall be reduced on a band saw to small pieces not greater than 3 mm to 4 mm in size by sawing along the face grain.

   NOTE: In plywood, the grain of alternate sheets runs perpendicular.

14.4 PROCEDURE

14.4.1 Instrument settings and operating conditions

The instrument settings and operating conditions shall be in accordance with Table 14.4.1.

TABLE   14.4.1

GAS CHROMATOGRAPH CONDITIONS

Capillary column	Model number	BPX5-0.25
	Nominal length	25.0 m
	Nominal diameter	0.32 mm
Oven	Initial temperature	100°C
	Initial time	1.00 min
	Ramp #1	15°C
	Final temperature	285°C
	Final time	5.00 min
	Run time	18.33 min
	Post time	0 min
	Post temperature	50°C
Inlet	Mode	Splitless
	Initial temperature	250°C
	Total flow	31 mL/min
	Gas type	Helium
Detector	ECD	
	Temperature	350°C
	Mode	Constant column + make-up flow
	Combined flow	30 mL/min
	Make-up gas type	Nitrogen

14.4.2 Weighing and moisture content

The procedure for weighing and determining moisture content shall be as follows:

(a)   Individually label two 50 mL beakers for each sample using a permanent marker.

(b)   Weigh approximately 5 g sawn plywood into the first set of labelled and pre-weighed beakers. Record the weight to the nearest 0.01 g (*Wet sample*).

(c)   Transfer the sample to a 500 mL round-bottom flask.

(d)   Weigh the correspondingly labelled empty beaker, and record the weight to the nearest 0.01 g (*Tare MC*).

(e)   Weigh approximately 5 g sawn plywood into the beaker and record the weight to the nearest 0.01 g (*Wet MC*).

(f)   Place the beaker in the oven (105°C) for a minimum of 18 h.

(g)   Remove the beaker from the oven and cool in a desiccator.

(h)   Weigh the beaker containing the dried sample and record the weight to the nearest 0.01 g (*Dry MC*).

14.4.3 Extraction

The extraction procedure shall be as follows:

(a)   Dispense 150 mL toluene and 10 mL formic acid into each round bottom flask containing the weighed sample in Clause 14.4.2(c) above.

(b)   Attach the reflux condenser and turn the water on.

(c)   Heat samples under reflux for 4 h.

(d)   Allow samples to cool.

(e)   Filter each extract through a filter funnel containing anhydrous sodium sulphate and a glass wool plug into a correspondingly labelled 200 mL volumetric flask. Wash the flask with toluene and pour it through filter funnel.

(f)   When samples are at room temperature, adjust the volume of each flask to the mark with toluene.

(g)   Using a pipette, dispense 1 mL of each sample into the corresponding labelled vial.

(h)   Using either a syringe or pipette, dispense 100 μL nitroxylene internal standard solution into each sample and standard vial.

(i)   Present samples, designated as S, in milligrams per litre, for analysis by gas chromatography.

14.5   PREPARATION OF STANDARDS

The standards shall be prepared as follows:

(a)   Prepare bifenthrin standards in toluene at the following concentrations: 0.1 mg/L, 1 mg/L, and 10 mg/L.

(b)   Perform calibration with standards in the range 0.1 mg/L to 10 mg/L.

(c)   Re-calibrate every six to eight samples.

(d)   Calculate results were based on equivalent oven-dried weight and an extract volume of 200 mL, as specified in Clause 14.6.

14.6   DATA HANDLING AND CALCULATIONS

14.6.1   Oven-dried weight

The oven-dried weight shall be calculated from the following equation:

$$\text{Oven-dried weight} = 1 - \frac{(Wet\,MC - Dry\,MC)}{(Wet\,MC - Tare\,MC)} \times Wet\,sample \qquad \ldots 14.6.1$$

where *Wet MC*, *Dry MC*, *Wet MC*, *Tare MC*, and *Wet sample* are defined in Clause 14.4.2.

14.6.2   Calibration and sample calculation

The calibration and sample shall be calculated from the following equation:

$$\text{bifenthrin}\,(\text{mg/kg}) = \frac{S \times 200}{\text{Oven-dried weight}} \qquad \ldots 14.6.2$$

where

S =   sample, in milligrams per litre [see Clause 14.4.3(i)]

14.6.3   Expression of results

The final results shall be expressed as milligrams per kilogram, to two decimal places.

SECTION 15 DETERMINATION OF TEBUCONAZOLE AND PROPICONAZOLE IN PRESERVATIVE-TREATED TIMBER BY GAS CHROMATOGRAPHY (GC)

15.1 PRINCIPLE

For extraction of propiconazole and tebuconazole from treated timber, two procedures may be used. One requires a refluxing methanol extraction procedure (see Clause 15.4.1), while the second uses an ultrasonic bath to extract the azoles from timber (see Clause 15.4.2).

15.2 REAGENTS

All reagents shall be of analytical reagent quality and the water used for the preparation of the reagents shall be distilled water. The following reagents shall be used:

(a) *Propiconazole standard.*

(b) *Tebuconazole standard.*

(c) *Azaconazole standard.*

(d) *Methanol* HPLC grade.

15.3 SAMPLING

Select representative samples from the treated timber source and reduce the size to sawdust using a grinding device.

There are two procedures for extracting the azole from treated timber. Because drying the timber prior to extraction can affect the results, the moisture content of the timber shall be determined simultaneously on a separate portion of the sample and used to adjust the extraction sample weight. Therefore, the sampling procedure shall be as follows:

(a) Obtain a representative sample of timber (0.5 g min.) and weigh to the nearest 0.1 mg (designate as W_i).

(b) Dry in an oven at 105°C to constant weight.

(c) Cool in a desiccator.

(d) Weigh to the nearest 0.1 mg (designate as W_f).

The percent moisture shall then be calculated in accordance with Equation 15.7.1.

15.4 PROCEDURE

15.4.1 Extraction by reflux

The procedure for the extraction shall be as follows:

(a) Weigh approximately 2 g of sample to the nearest 0.1 mg and place in a round-bottom flask along with glass beads or boil easers.

(b) Add approximately 50 mL methanol and attach to a water-cooled condenser. Allow the methanol to reflux for 30 min then decant the extract into a separate flask.

(c) Repeat the above process twice.

(d) Combine the extracts and correct (evaporate) to 100 mL in a volumetric flask.

(e) Add 1000 ppm azaconazole internal standard to the extract at the rate of 20 μL internal standard per 1 mL of solution.

15.4.2 Extraction by ultrasonic bath

An ultrasonic bath of sufficient power and capacity is required to extract the timber samples in the specified time.

> NOTE: If complete extraction is uncertain due to the equipment used, individual labs should perform their own study to determine if their ultrasonic bath power and capacity, as well as extraction duration time, are adequate.

The extraction procedure shall be as follows:

(a) Obtain a tare weight on a test tube and cap.

(b) Weigh approximately 0.5 g sample to the nearest 0.01 mg and quantitatively transfer to the test tube.

(c) Add 10 mL HPLC-grade methanol to the test tube and cap.

(d) Place the test tube in an ultrasonic bath that has been preheated to 55°C and sonicate for 3 h.

(e) Remove the sample at 30 min intervals and shake vigorously. It may be necessary to carefully vent the cap to remove any pressure build-up within the test tube.

(f) After 3 h, remove the sample and dry the test tube with a lint-free cloth. Allow it to cool and then reweigh the contents. The final weight of methanol can then be determined by subtracting the weight of the test tube, cap and sample.

(g) Remove any timber particulate from the extract using a 0.45 μm filter disk and 10 cc syringe. Prior to gas chromatography analysis, add 1000 ppm azaconazole standard to the extract at the rate of 20 μL/mL.

15.5 STANDARD SOLUTIONS

15.5.1 Calibration standards

The standards for instrument calibration using tebuconazole and propiconazole of known purity shall be prepared as follows:

(a) Prepare a 1000 ppm stock solution of each of tebuconazole and propiconazole.

(b) Using A-grade pipettes and volumetric flasks, prepare standard solutions of tebuconazole and propiconazole from the 1000 ppm stock solution to give the appropriate concentration ranges. Typical standard concentrations are 25, 50, 100, 150 and 200 ppm azole.

15.5.2 Internal standard

The procedure for the internal standard preparation shall be as follows:

(a) Use azaconazole as an internal standard to ensure accuracy of results.

(b) Prepare a 1000 ppm azaconazole stock solution and add to all standards and samples at the rate of 20 μL/mL solution.

15.5.3 Operation of gas chromatograph

The configuration of the instrument shall be as shown in Table 15.5.3.

TABLE 15.5.3

CONFIGURATION OF INSTRUMENTS

Injector	Split/splitless at 250°C, using silanized glass insert with silanized quartz wool and low bleed silicone septa 1 μL splitless injection Vent valve open at 1 min
Detector	Thermionic—Nitrogen/Phosphorus (NPD) at 280°C
Column	SGE BPX-35, 25 m, 0.32 mm ID, 0.25 μm film, fitted with a 500 mm section of deactivated silica tubing as a retention gap
Carrier gas	Helium U.H.P at 14.8 psig (program start conditions) Split flow at 50 mL/min
Oven	Initial temperature at 100°C, hold for 1 min Increase temperature (at 15°C/min) to 260°C Hold for 5 min
Integrator software	Integrator or data acquisition system capable of performing internal standard type calibration and quantitative analysis

15.6 STANDARDIZATION

The standardization procedure shall be as follows:

Inject the reference and sample solutions and calculate the peak areas.

In case of peak splitting (propiconazole is a mixture of diastereomers), use peak grouping mode for the integrator or add peak areas.

To obtain the calibration curve, perform a linear regression on the ratio of A_s/A_{is} versus concentration of tebuconazole or propiconazole (C_s), as given in the following equation:

$$A_s/A_{is} = m\,C_s + b \qquad \qquad \ldots 15.6$$

where

A_s = area of sample peak

A_{is} = area of internal standard peak

m = slope

C_s = tebuconazole or propiconazole concentration, in milligrams per litre

b = intercept

15.7 CALCULATIONS

15.7.1 Timber samples

The moisture content of the timber sample (MC) shall be calculated as follows:

$$MC = \frac{W_i - W_f}{W_f} \times 100 \qquad \qquad \ldots 15.7.1$$

where

W_i = initial weight of timber sample, in grams

W_f = final weight of dried timber sample, in grams

15.7.2 Azole concentration (C_s)

The azole concentration (C_s) shall be determined as follows:

$$C_s = \frac{(A_s / A_{is}) - b}{m} \qquad \qquad \dots 15.7.2$$

where C_s, A_s, A_{is}, b and m are defined in Clause 15.6.

15.7.3 Concentration of azole in treated timber sample

The concentration of the azole in the treated timber sample shall be calculated as follows:

$$C_s(ppm) = \frac{C_s \times V}{W \times (1 - MC/100)} \qquad \qquad \dots 15.7.3(1)$$

$$C_s(\%\ m/m) = \frac{C_s \times V}{W \times (1 - MC/100) \times 10\,000} \qquad \qquad \dots 15.7.3(2)$$

where

C_s = concentration of azole, in ppm

V = final volume of methanol, in millilitres

W = weight of timber sample, in grams

MC = moisture content of timber sample, in percent (see Equation 15.7.1)

SECTION 16 DETERMINATION OF PERMETHRIN IN TIMBER EXTRACTS—HPLC ANALYSIS

16.1 GENERAL

This Section provides a method to estimate the amount of permethrin in extracts of treated timber.

The method is suitable for extracts of timber treated to hazard class H1, H2 or H3, as defined in the AS/NZS 1604 series, specifically, timber and oriented strand board treated with water-based preparations containing permethrin, and petroleum solvent-based preparations of permethrin or mixtures of permethrin and an approved complementary fungicidal preservative treatment.

The method sets out a procedure for the determination of permethrin using high-performance liquid chromatography, employing a reverse phase separation mode and ultraviolet absorbance detection. Quantification is achieved using an external standard calibration procedure. For some samples, determination of permethrin using gas chromatography with electron capture detection (ECD) may be necessary due to interference from heartwood extractives.

> NOTE: For timber treated for hazard class H1 exposure conditions, concentration of extract may be necessary for HPLC determination.

16.2 PRINCIPLE

Permethrin is extracted from treated timber. The resulting extract is analysed for permethrin content by high-performance liquid chromatography.

> NOTE: In the case that the product is treated to hazard class H1 exposure conditions, only the sapwood of lyctid-susceptible species should be analysed.

Results are expressed as percent mass/mass on an oven-dry basis for timber extracts.

Results are expressed on an oven-dry weight basis by correction for the moisture content determined on a parallel sample.

The analytes are cis-permethrin and trans-permethrin, and the result is expressed as total permethrin (i.e. cis + trans).

16.3 REAGENTS

All reagents shall be of analytical reagent quality. The water used for the preparation of the reagents shall be distilled water. The following reagents shall be used:

(a) *Ethanol* Denatured alcohol formula F3.

(b) *Permethrin* AR, 97% m/m, 23.6% cis-permethrin and 73.4% trans-permethrin.

(c) *Acetonitrile* HPLC grade.

(d) *Stock standard—0.08% permethrin in ethanol* Weigh to the nearest 0.1 mg approximately 0.165 g permethrin [Clause 16.3(b)] and dissolve in ethanol. Make up to 200 mL with ethanol. Record the actual weight in the organic lab standard solutions register. Calculate the actual cis-permethrin and trans-permethrin amounts in each standard.

(e) *HPLC working standards in ethanol* Prepare working standards for HPLC in ethanol in accordance with Table 16.3.

Record details of all standards in the organic lab standard solutions register, and store standards in fridge.

TABLE 16.3

HPLC WORKING STANDARDS IN ETHANOL

Standard number	Standard volume (mL)	Standard used	Final volume (mL)	Cis-permethrin (mg/L)	Trans-permethrin (mg/L)
1	20	Stock	200	20	60
2	5	Stock	200	5	15
3	20	1	200	2	6
4	20	2	200	0.5	1.5

16.4 APPARATUS

The following apparatus shall be used:

(a) *Schott bottles* 100 mL with closures.

(b) *Volumetric flasks* 50 mL and 100 mL.

(c) *Volumetric pipettes* 1 mL, 10 mL, and 20 mL.

(d) *Mohr pipettes* 1 mL.

(e) *Dispenser* 50 mL.

(f) *HPLC*.

(g) *Top pan balances* Capable of weighing to 0.0001 g and 0.01 g.

(h) *Ultrasonic cleaning bath*.

(i) *Syringe* Glass, Leuer Lock, 10 mL.

(j) *Syringe filter holder* Stainless steel with stainless steel screen, Swinex type.

(k) *Syringe filters* PTFE, 0.45 micron, to suit Swinex filter holder.

(l) *Syringe pre-filters* Glass micro-fibre, to suit Swinex filter holder.

(m) *Sample vials and closures* To suit HPLC auto-sampler.

16.5 SAMPLING

All samples shall be at least air-dry, and preferably at equilibrium moisture content.

Samples shall be ground through a 5 mm screen. Samples shall not be oven-dried prior to analysis.

16.6 PROCEDURE

The procedure shall be as follows:

(a) Individually label a 100 mL Schott bottle and a corresponding 50 mL beaker for each sample (e.g. using an adhesive label marked with graphite pencil).

(b) Accurately weigh between 2 g to 3 g of each sample into its corresponding labelled 100 mL Schott bottle. Record the weight to the nearest 0.01 g.
NOTE: For H1 samples, use approximately 4 g of sample.

(c)   Weigh the corresponding labelled empty beaker and record the weight to the nearest 0.01 g (*Tare MC*).

(d)   Accurately weigh 4 g to 5 g of each sample into its corresponding labelled and tared 50 mL beaker. Record the weight to the nearest 0.01 g (*Wet MC*) for moisture content determination.

(e)   Place moisture content samples in a 100°C to 105°C oven for overnight drying (minimum of 12 h).

(f)   Remove the beakers from the oven and cool in a desiccator.

(g)   Weigh the beakers containing the dried sample, and record the weight to the nearest 0.01 g (*Dry MC*).

(h)   Add 50 mL ethanol by calibrated dispenser.

(i)   Prepare the ultrasonic bath by filling with warm tap water (40°C to 50°C) to the estimated solvent level in the Schott bottle.

(j)   Place 100 mL Schott bottles in the ultrasonic bath. Switch on the bath and allow extraction to proceed for a minimum of two 45 min periods.

(k)   Allow samples to cool and solids to settle.

(l)   If necessary, use a glass syringe fitted with the Swinex filter (fitted with 0.45 micron PTFE filter and glass pre-filter) and filter the sample into an HPLC sample vial.

(m)   Rinse the glass syringe and Swinex filter with each sample before collecting 2 mL for HPLC. Change the 0.45 micron PTFE filter and glass pre-filter as required.

(n)   Present the samples for analysis by HPLC.

(o)   Conduct the HPLC analysis in accordance with Table 16.6.

TABLE 16.6

HPLC ANALYSIS

Configuration	Waters 616 pump Waters 717 auto-sampler Waters 916 diaode array detector Waters Millenium32 data handling software
Column	Nova Pak C18 Cartridge 150 × 3.9 mm with Nova Pak C18 Sentry Guard
Column temperature	30°C
Solvent A	Acetonitrile
Solvent B	Water
Solvent program	0 min, 75% acetonitrile: 25% water, 1.4 mL/min 8 min, 100% acetonitrile: 0% B, 1.4 mL/min, Curve 11 10 min, 100% acetonitrile: 0% B, 1.4 mL/min, Curve 11
Acquisition and injection	20 µL; data acquisition delay 3 min Run time 18 min
Detection	232 nm for quantitation by external standard
Retention times	Trans-permethrin: approx. 6.7 min Cis-permethrin: approx. 7.9 min

16.7 DATA HANDLING AND CALCULATIONS

16.7.1 Oven-dry weight

The oven-dry weight shall be calculated from the following equation:

$$\text{Oven-dry weight} = 1 - \frac{\textit{Wet MC} - \textit{Dry MC}}{\textit{Wet MC} - \textit{Tare MC}} \times \textit{Wet sample} \qquad \ldots 16.7.1$$

where *Wet MC*, *Dry MC*, *Wet MC*, *Tare MC*, and *Wet sample* are defined in Clause 16.6.

16.7.2 Calibration and sample calculation

The calibration and sample shall be calculated from the following equation:

$$\text{Total permethrin} \left(\% \text{m/m}\right) = \frac{\left(\text{cis} + \text{trans}\right) \text{ of permethrin} \times V}{\text{Oven-dry weight} \times 10^4} \qquad \ldots 16.7.2$$

where V = final volume of extract (50 mL)

16.7.3 Expression of results

The final results shall be expressed as percent mass per mass of total permethrin, to three decimal places.

NOTES:

1   When HPLC traces show interference from other extractive components it may be necessary to run samples by GC. The extract is analysed by gas chromatography using an ECD and internal standard calibration procedure. Compare cis-permethrin to trans-permethrin results and ratios for permethrin isomers. It may be necessary to calculate results based only on the cis-permethrin or trans-permethrin result using the known ratio of cis-permethrin to trans-permethrin.

2   For low-level samples (i.e. treated timber for hazard class H1), use 4 g of sample.

SECTION 17 DETERMINATION OF IMIDACLOPRID IN TIMBER EXTRACTS—HPLC ANALYSIS

17.1 PRINCIPLE

Timber samples are extracted with acidified-methanol. After solvent evaporation under vacuum and addition of water, the extracts are fractionated on Amberlite XAD-4 to remove resinous substances. Samples are then combined with an internal standard and imidacloprid is quantitated by reverse-phase high-performance liquid chromatography.

17.2 REAGENTS

All reagents shall be of analytical quality. Unless otherwise specified, water used for the preparation of reagents shall be distilled water.

The following reagents shall be used:

(a) *Mobile phase A*   ASTM type 2 water adjusted to pH 3 with phosphoric acid.

(b) *Mobile phase B*   Methanol for HPLC.

(c) *Imidacloprid*   Imidacloprid (99.9%, Bayer).

(d) *Stock imidacloprid standard*   Weigh to the nearest 0.1 mg approximately 0.010 g imidacloprid [Item (c)], dissolve in methanol and make up to 100 mL with methanol in a volumetric flask.

(e) *Stock internal standard*   Weigh approximately 0.030 g etofenprox, dissolve in methanol and make up to 100 mL with methanol in a volumetric flask.

(f) *0.1 M sulphuric acid*   Prepare from sulphuric acid 98% (conc.).

(g) *Filter aid*   Celite 545.

(h) *Amberlite XAD-4*   20–50 mesh.

17.3 SAMPLING AND MOISTURE CONTENT

Timber shall be at least air-dry, and preferably at equilibrium moisture content. Samples to be analysed shall not be oven-dried.

The timber shall be ground to pass through a 3 mm screen (e.g. Wiley Mill).

To determine moisture content, accurately weigh a sample, dry overnight at 105°C, cool the dried sample in a desiccator then reweigh.

17.4 PROCEDURE

17.4.1 Sample preparation

The sample shall be prepared as follows:

(a) Accurately weigh a sub-sample of air dry, ground timber (approximately 10 g) into a plastic bottle.

   NOTE: For example, 400 mL polyethylene terephthalate hexagonal jar, Visy Industrial Plastics 083266.

(b) Add approximately 250 mL methanol and 5 mL 0.1 M sulphuric acid to the sub-sample, cap the bottle and soak at an ambient temperature for at least 3 h.

(c) Blend the mixture using a high-shear mixer for 3 min at 7000 rpm. Rinse wood residues from the mill head with methanol.

NOTE: An example of a suitable mixer is a Silverson L4RT laboratory mixer with square hole high shear screen.

(d) Soak the mixture overnight then repeat the high-shear mixing step using an emulsor screen. Allow to soak for a further 2 h.

(e) Suspend 10 g filter aid in methanol and pour a filter pad on Whatman 541 filter paper. Add a further 10 g filter aid to the blended wood extraction and vacuum filter it through the filter pad. Rinse the remaining wood residue from the jar and use this to wash the filter pad.

(f) Transfer the filtrate and washings to a 1 L round-bottomed flask and evaporate the methanol under reduced pressure using a rotary evaporator (55°C max. bath temperature).

(g) Prepare an amberlite column as follows:

(i) Wash 15 g amberlite XAD-4 resin twice in 50 mL methanol.

(ii) Suspend the washed resin in methanol and pack a column in a 20 mm diameter glass chromatography column.

(iii) Place a glass wool plug over the resin to protect the bed.

(iv) Equilibrate the resin with at least 100 mL distilled water.

(h) Dilute the concentrated timber extract in the round-bottom flask with 50 mL water and pass it through the amberlite column at 3–5 mL/min. Rinse the flask with no more than 50 mL water and use this to wash the column. Drain the remaining water from the resin bed. Discard all aqueous fractions.

(i) Elute bound material from the column with 3 × 30 mL methanol and collect it into a flask.

(j) Into the same flask pipette 5 mL stock internal standard and mix well. Filter a portion of the mixture into an HPLC sample vial using a syringe filter (0.2 mm, regenerated cellulose).

17.4.2 Preparation of working standard solution

The working standard solution shall be prepared as follows:

(a) Accurately pipette 5 mL stock imidacloprid standard into a 50 mL volumetric flask.

(b) Accurately pipette 5 mL stock internal standard into the same volumetric flask. Make up to volume with methanol and mix well.

(c) Filter (0.2 mm regenerated cellulose) a portion of the working standard solution into an HPLC sample vial.

17.4.3 Chromatography

HPLC analysis shall be performed in accordance with the conditions in Table 17.4.3.

TABLE 17.4.3

HPLC CONDITIONS

Column	Luna C18 (5 μm, 100 A) 250 mm × 4.6 mm (Phenomenex 00G-4252-E0) Security guard C18 column (Phenomenx AJO-4287)								
Column temperature	40°C								
Injection volume	25 μL								
Flow rate	1 mL/min								
Solvent program	Time (min)	0	8	16	18	26	32	42	50	65
	% B (v/v)	5	25	50	50	75	100	100	5	5
Detection	270 nm								
Retention times	Imidacloprid	17 min approx.							
	Etofenprox	38 min approx.							

17.5 CALCULATIONS

17.5.1 Moisture content

The percentage moisture content of the timber sample (MC) shall be calculated from the following equation:

$$MC = \frac{W_i \times W_f}{W_f} \times 100 \qquad \ldots 17.5.1$$

where

W_i = initial weight of timber sample, in grams

W_f = final weight of dried timber sample, in grams

17.5.2 Relative response factor

The relative response factor (RRF) shall be calculated from the average of three injections of the working standard solution, from the following equation:

$$RRF = \frac{W_S \times A_{IS}}{A_S} \qquad \ldots 17.5.2$$

where

W_S = weight of imidacloprid standard corrected for percent purity, in grams, in the working standard solution

A_{IS} = area of internal standard peak

A_S = area of imidacloprid peak

17.5.3 Percentage (mass/mass) of imidacloprid

The percentage (mass/mass) of imidacloprid in the treated timber shall be calculated from the following equation:

$$\text{Imidacloprid } (\% \ m/m) = \frac{A_S \times RRF \times \left(1 + \frac{MC}{100}\right) \times 100}{A_{IS} \times W} \qquad \ldots 17.5.3$$

where

W = weight of timber sample analysed, in grams

A_S, RFF, MC, A_{IS}, and W are defined in Clauses 17.5.1 and 17.5.2

SECTION 18 DETERMINATION OF IMIDACLOPRID IN GLUELINE-TREATED TIMBER—ELISA ANALYSIS

18.1 PRINCIPLE

Samples of product are extracted in methanol and the resulting extracts diluted into an aqueous buffer. Imidacloprid is determined by a competitive enzyme-linked immunosorbent assay (ELISA) method using the Envirologix QuantiPlate kit for imidacloprid.

In the ELISA method, imidacloprid in sample extracts competes with enzyme-labelled imidacloprid for a limited number of anti-imidacloprid antibody binding sites on the inner surface of plastic test wells. After a 60 min binding reaction, unbound material is washed from the wells. The outcome of the competition reaction is then quantitated by a colorimetric reaction catalysed by the bound enzyme. The concentration of imidacloprid is inversely proportional to colour development, as in all competitive immunoassays. Imidacloprid is quantitated using external imidacloprid calibration standards.

18.2 REAGENTS AND APPARATUS

All reagents shall be of analytical quality. Unless otherwise specified, water used for the preparation of reagents shall be distilled water.

The following reagents and apparatus shall be used:

(a) *Methanol* Methanol for HPLC.

(b) *ELISA kit* QuantiPlate kit for imidacloprid (Envirologix, EP 006).

(c) *TBS (Tris buffered saline)* 10 mM Tris/HCl, pH 7.5 containing 90 mM NaCl. Dissolve 1.21 g Tris(hydroxymethyl) aminoethane and 5.26 g NaCl in 950 mL distilled water. Adjust the pH to 7.5 with HCl and make the volume up to 1000 mL.

18.3 SAMPLING AND MOISTURE CONTENT

Timber shall be at least air-dry, and preferably at equilibrium moisture content. Samples to be analysed shall not be oven-dried.

The timber shall be ground to pass through a 3 mm screen (e.g. Wiley Mill).

Moisture content shall be determined by accurately weighing a sample, drying it overnight at 105°C, cooling the dried sample in a desiccator then reweighing.

18.4 PROCEDURE

18.4.1 Sample preparation

The procedure for preparing the sample shall be as follows:

(a) Accurately weigh a sub-sample of air-dry, ground timber (approximately 5 g) into a plastic bottle

> NOTE: For example, 400 mL polyethylene terephthalate hexagonal jar, Visy Industrial Plastics 083266.

(b) Add approximately 150 mL methanol to the sub-sample, cap the bottle and soak at ambient temperature for at least 3 h.

(c) Blend the mixture using a high-shear mixer for 3 min at 7000 rpm. Rinse wood residues from the mill head with methanol.

NOTE: An example of a suitable mixer is a Silverson L4RT laboratory mixer with square hole high-shear screen.

(d)   Soak the mixture overnight then repeat the high-shear mixing step using an emulsor screen. Allow to soak for a further 2 h.

(e)   Transfer the extraction mixture to a 250 mL volumetric flask and make up to volume with methanol. Mix well then allow the solids to settle. Filter a sample of clear supernatant into a glass vial with a syringe filter (0.2 mm, regenerated cellulose).

(f)   Pipette 0.05 mL of the filtrate into a 15 mL polypropylene screw-cap tube containing 9.95 mL TBS. This corresponds to an extract dilution factor (DF) equal to 200.

18.4.2   ELISA procedure

The QuantiPlate Kit contains eight 12-well strips coated with anti-imidacloprid antibody, and vials containing a negative control (no imidacloprid), calibration standards (0.2, 1, 5 and 6 ppb imidacloprid in water), imidacloprid-enzyme conjugate solution, substrate solution and stop solution. A detailed set of instructions for use is supplied with each kit.

The ELISA procedure shall be performed as follows:

(a)   Remove imidacloprid ELISA kit reagents from refrigeration and equilibrate at ambient temperature (20°C to 25°C) for at least 30 min. Just before use remove the required number of strips (12 wells per strip) from the sealed pouch and place them in an empty plate frame. Ten wells are required for the negative control and calibration standards, and two wells are required for each sample.

(b)   Pipette, in duplicate, 0.1 mL negative control, calibration standards and diluted wood extracts into the test kit wells.

(c)   Immediately pipette 0.1 mL of imidacloprid-enzyme conjugate into each well.

(d)   Mix the well contents by moving the strip holder in a rapid circular motion on the benchtop for 20 s to 30 s. Cover the wells with Para-film to prevent evaporation and allow to stand at ambient temperature.

(e)   After 60 min, tip out the contents of the wells and flood the wells with cool tap water, then shake to empty. Repeat this wash step four times then slap the plate onto a paper towel to remove as much water as possible.

(f)   Pipette 0.1 mL substrate solution into each well and mix thoroughly as described in Step (d) above. Cover the wells with Para-film and allow to stand at ambient temperature.

(g)   After 30 min, pipette 0.1 mL stop solution into each well and mix thoroughly as described above.

(h)   Measure the absorbance at 450 nm, designated as A_{450}, in each well using a multi-well plate reader or a strip reader.

18.4.3   Conversion of absorbance data to imidacloprid concentrations

The absorbance data to imidacloprid concentrations shall be converted as follows:

(a)   Calculate the average for each pair of A_{450} values.

(b)   Calculate percentage initial binding ($\%B_0$) according to the following equation:

$$\%B_0 = \frac{\text{Average } A_{450} \text{ of calibration standard or diluted extract}}{\text{Average } A_{450} \text{ of negative control}} \times 100$$

(c)   Plot a graph of %B_0 versus log of imidacloprid concentration for the calibration standards.

(d)   Read the concentration of imidacloprid in diluted extracts (C_S, in ppb) from the graph. This is best performed using a spreadsheet and a calculated line of best fit.

18.5 CALCULATIONS

18.5.1 Moisture content

The percentage moisture content of the timber sample (MC) shall be calculated from the following equation:

$$MC = \frac{W_i \times W_f}{W_f} \times 100 \qquad \qquad \ldots 18.5.1$$

where

$\quad$ W_i = initial weight of timber sample, in grams

$\quad$ W_f = final weight of dried timber sample, in grams

18.5.2 Sample extract dilution factor

The sample extract dilution factor (DF) shall be calculated from the following equation:

$$DF = \frac{10}{V} \qquad \qquad \ldots 18.5.2$$

where

$\quad$ V = volume of extract diluted, in millilitres

18.5.3 Percentage (m/m) of imidacloprid

The percentage (mass/mass) of imidacloprid in the treated timber shall be calculated from the following equation:

$$\text{Imidacloprid} \left(\% m/m \right) = \frac{C_S \times DF \times 250 \times \left(1 + \frac{MC}{100} \right)}{W \times 10^7} \qquad \qquad \ldots 18.5.3$$

where

$\quad$ C_S = concentration of imidacloprid in the diluted extract determined by ELISA, in ppb

$\quad$ DF = sample extract dilution factor

$\quad$ W = weight of timber sample analysed, in grams

$\quad$ MC = moisture content of the timber sample, in percent

SECTION 19   DETERMINATION OF TRIADIMEFON AND CYPROCONAZOLE IN GLUELINE-TREATED AND SURFACE TREATED TIMBER

19.1 PRINCIPLE

The triadimefon and cyproconazole are extracted with methanol using Soxhlet extraction or an equivalent automated extraction system. The extract is analysed for triadimefon and cyproconazole by capillary gas chromatography (GC) with FID detection.

> NOTE: To determine the concentration of triadimefon and cyproconazole, separate samples and analyses should be carried out on glueline cross-section and surface-treated timber.

Quantification is carried out using an internal standard.

19.2 REAGENTS

The following reagents shall be used:

(a)   *Methanol*   Analytical reagent grade.

(b)   *Triadimefon*   Analytical reference-grade material with certificate of purity.

(c)   *Cyproconazole*   Analytical reference-grade material with certificate of purity.

(d)   *Dibutyl phthalate*   Laboratory reagent grade satisfactory.

19.3 SAMPLING AND MOISTURE CONTENT

Samples shall be at least air-dry, and preferably at equilibrium moisture content. Samples to be analysed shall not be oven-dried.

Cross-sectional samples shall be dry milled to pass through a 2 mm screen (e.g. Wiley mill). Greater than 50% of the sample shall be retained on a 1 mm screen.

Surface samples shall be removed with a planer or similar and blended in a bench top blender for 15 to 30 s to reduce the size of any long shavings.

To determine moisture content, accurately weigh a sample, dry overnight at 105°C, cool the dried sample in a desiccator then reweigh.

19.4 PROCEDURE

The following procedure shall be used:

(a)   Weigh approximately 5 g of sample to the nearest 0.1 mg into a cellulose extraction thimble (W_S).

> NOTE: A small amount of glass wool should be in the top of the thimble.

(b)   Place the extraction thimble into a Soxhlet extractor and add 80 mL of methanol to the collection flask along with some boiling chips.

(c)   Set up the Soxhlet extraction and run for 2.5 h.

> NOTE: Automated Soxhlet extraction may be used in which case sample weights, solvent volumes and extraction time will vary depending on the extraction equipment used.

(d)   Determine the final volume of methanol extract obtained, V (mL), and quantitatively transfer 40 mL of this methanol extract to a 50 mL bottle.

(e)   Add 5 mL of dibutyl phthalate internal standard with a volumetric pipette and mix well.

(f)   Filter an aliquot through a 0.2 μm syringe filter into a vial.

19.5   STANDARD SOLUTIONS

19.5.1   Internal standard

The procedure for preparing the internal sample shall be as follows:

(a)   Weigh 0.15 g of dibutyl phthalate to the nearest 0.1 mg into a 100 mL volumetric flask.

(b)   Make up to the mark with methanol.

19.5.2   Triadimefon and cyproconazole standards

The procedure for preparing the triadimefon and cyproconazole standards shall be as follows:

(a)   Weigh approximately 0.01 g of triadimefon standard to the nearest 0.1 mg into a 50 mL bottle.

(b)   Weigh approximately 0.01 g of cyproconazole standard to the nearest 0.1 mg into the same 50 mL bottle.

(c)   Add 5 mL of dibutyl phthalate internal standard with a volumetric pipette.

(d)   Make up to 50 mL with methanol.

(e)   Filter an aliquot through a 0.2 μm syringe filter into a vial.

19.6   QUANTITATION

The following requirements apply:

(a)   A 1 μL aliquot of each standard and sample solution shall be injected into the GC with FID detection.

(b)   The order of elution shall be—

   (i)   dibutyl phthalate;

   (ii)   triadimefon; and

   (iii)   cyproconazole.

19.7   CHROMATOGRAPHY

The conditions for the gas chromatograph shall be as shown in Table 19.7:

TABLE 19.7

GAS CHROMATOGRAPH CONDITIONS

Capillary column	(a)	Zebron ZB-5, or equivalent
	(b)	15 m, 0.25 mm ID, 0.25 μ film thickness
Injector	(a)	1 µL splitless injection
	(b)	Temperature 280°C
	(c)	Sampling time 1.5 minutes
	(d)	Carrier gas helium
	(e)	Total flow 27.2 mL/min
Oven	(a)	Initial temperature 40°C, hold for 1 min
	(b)	Increase temperature at 5°C/min to 80°, then 10°C/minute to 280°C
	(c)	Hold at 280°C for 10 minutes
Detector	FID at 280°C	

19.8 CALCULATIONS

19.8.1 Moisture content

The percentage moisture content (*MC*) of the timber sample shall be calculated from the following equation:

$$MC = \frac{W_\mathrm{i} - W_\mathrm{f}}{W_\mathrm{f}} \times 100 \qquad \ldots 19.8.1$$

where

W_i = initial weight of timber sample, in grams

W_f = final weight of dried timber sample, in grams

19.8.2 Corrected sample weight

The sample weight in grams corrected for moisture content (W_c) shall be calculated from the following equation:

$$W_\mathrm{c} = \frac{W_\mathrm{S}}{\left(1 + \dfrac{MC}{100}\right)} \qquad \ldots 19.8.2$$

where

W_S = initial weight of timber sample, in grams

MC = Percentage moisture content of the initial timber sample

19.8.3 Relative response factor

The relative response factor (RRF) shall be calculated from the following equation:

$$RRF = \left(\frac{A_{IS}}{A_{std}}\right) \times \left(\frac{wt_{IS}}{1}\right) \qquad \qquad \ldots 19.8.3$$

where

A_{IS} = peak area of internal standard in standard chromatogram

A_{std} = peak area of standard in standard chromatogram

wt_{IS} = weight of active corrected for percent purity in standard, in grams

19.8.4 Percentage (mass/mass) of active ingredients

The percentage (mass/mass) of triadimefon or cyproconazole in the treated timber shall be calculated from the following equation:

$$\text{Active concentration}\,(\%\,\text{m/m}) = \frac{\left(A_S \times RRF \times V \times 100\right)}{\left(A_{IS} \times 40 \times W_C\right)} \qquad \ldots 19.8.4$$

where

A_S = peak area of active in sample chromatogram

V = final volume of methanol extract (mL)

A_{IS} = peak area of internal standard in sample chromatogram

RRF and W_C are defined in Clauses 19.7.2 and 19.7.3.

NOTES:

1   The percent mass/mass (%m/m) of triadimefon or cyproconazole should be calculated separately.

2   Separate results should be obtained for each active ingredient and for the glueline cross-section and surface treated timber zones.

SECTION 20   DETERMINATION OF THIACLOPRID IN GLUELINE-TREATED TIMBER—ELISA ANALYSIS

20.1 PRINCIPLE

Samples of product are extracted in methanol and the resulting extracts diluted into an aqueous buffer. Thiacloprid is determined by a competitive enzyme-linked immunosorbent assay (ELISA) method using the EnviroLogix QuantiPlate kit for imidacloprid. The EnviroLogix QuantiPlate kit for imidacloprid is used because the manufacturer of the kit has determined that it does not distinguish between imidacloprid and thiacloprid. The 50% B_0 value for imidacloprid is 1.05 ppb and the 50% B_0 value for thiacloprid is 1.3 ppb (see Clause 20.4.3), therefore the kit very slightly under-estimates the concentration of thiacloprid in a sample.

In the ELISA method, thiacloprid in sample extracts competes with enzyme-labelled imidacloprid for a limited number of anti-imidacloprid antibody binding sites on the inner surface of plastic test wells. After a 60 min binding reaction, unbound material is washed from the wells. The outcome of the competition reaction is then quantitated by a colorimetric reaction catalysed by the bound enzyme. The concentration of thiacloprid is inversely proportional to colour development, as in all competitive immunoassays. Thiacloprid is quantitated using external imidacloprid calibration standards.

20.2 REAGENTS AND APPARATUS

All reagents shall be of analytical quality. Unless otherwise specified, water used for the preparation of reagents shall be distilled water.

The following reagents and apparatus shall be used:

(a)   *Methanol* Methanol for HPLC.

(b)   *ELISA kit* QuantiPlate kit for imidacloprid (Envirologix, EP 006).

(c)   *TBS (Tris buffered saline)* 10 mM Tris/HCl, pH 7.5 containing 90 mM NaCl. Dissolve 1.21 g Tris(hydroxymethyl) aminoethane and 5.26 g NaCl in 950 mL distilled water. Adjust the pH to 7.5 with HCl and make the volume up to 1000 mL.

20.3 SAMPLING AND MOISTURE CONTENT

Timber shall be at least air-dry, and preferably at equilibrium moisture content. Samples to be analysed shall not be oven-dried.

The timber shall be ground to pass through a 3 mm screen (e.g. Wiley Mill).

To determine moisture content, accurately weigh a sample, dry overnight at 105°C, cool the dried sample in a desiccator then reweigh.

20.4 PROCEDURE

20.4.1 Sample preparation

The sample shall be prepared as follows:

(a)   Accurately weigh a sub-sample of air-dry, ground timber (approximately 5 g) into a plastic bottle.

NOTE: For example, 400 mL polyethylene terephthalate hexagonal jar, Visy Industrial Plastics 083266.

(b) Add approximately 150 mL methanol to the sub-sample, cap the bottle and soak at ambient temperature for at least 3 h.

(c) Blend the mixture using a high-shear mixer for 3 min at 5000 rpm. Rinse wood residues from the mill head with methanol.

NOTE: An example of a suitable mixer is a Silverson L4RT laboratory mixer with square hole high-shear screen.

(d) Soak the mixture overnight then repeat the high-shear mixing step for 3 min at 7000 rpm using an emulsor screen. Allow to soak for a further 2 h.

(e) Transfer the extraction mixture to a 250 mL volumetric flask and make up to volume with methanol. Mix well then allow the solids to settle. Filter a sample of clear supernatant into a glass vial with a syringe filter (0.2 mm, regenerated cellulose).

(f) Pipette 0.05 mL of the filtrate into a 15 mL polypropylene screw-cap tube containing 9.95 mL TBS. This corresponds to an extract dilution factor (DF) equal to 200.

20.4.2 ELISA procedure

The QuantiPlate Kit contains eight 12-well strips coated with anti-imidacloprid antibody, and vials containing a negative control (no imidacloprid), calibration standards (0.2, 1, and 6 ppb imidacloprid in water), imidacloprid-enzyme conjugate solution, substrate solution and stop solution. A detailed set of instructions for use is supplied with each kit.

The ELISA procedure shall be performed as follows:

(a) Remove imidacloprid ELISA kit reagents from refrigeration and equilibrate at ambient temperature (20°C to 25°C) for at least 30 min. Just before use remove the required number of strips (12 wells per strip) from the sealed pouch and place them in an empty plate frame. Eight wells are required for the negative control and calibration standards, and two wells are required for each sample.

(b) Pipette, in duplicate, 0.1 mL negative control, calibration standards and diluted wood extracts into the test kit wells.

(c) Immediately pipette 0.1 mL of imidacloprid-enzyme conjugate into each well.

(d) Mix the well contents by moving the strip holder in a rapid circular motion on the benchtop for 20 s to 30 s. Cover the wells with Para-film to prevent evaporation and allow to stand at ambient temperature.

(e) After 60 min, tip out the contents of the wells and flood the wells with cool tap water, then shake to empty. Repeat this wash step four times then slap the plate onto a paper towel to remove as much water as possible.

(f) Pipette 0.1 mL substrate solution into each well and mix thoroughly as described in Step (d) above. Cover the wells with Para-film and allow to stand at ambient temperature.

(g) After 30 min, pipette 0.1 mL stop solution into each well and mix thoroughly as described above.

(h) Measure the absorbance at 450 nm, designated as A_{450}, in each well using a multi-well plate reader or a strip reader.

20.4.3 Conversion of absorbance data to thiacloprid concentrations

The absorbance data to thiacloprid concentrations shall be converted as follows:

(a) Calculate the average for each pair of A_{450} values.

(b) Calculate percentage initial binding (%B_0) according to the following equation:

$$\%\,B_0 = \frac{\text{Average } A_{450} \text{ of calibration standard or diluted extract}}{\text{Average } A_{450} \text{ of negative control}} \times 100$$

(c)   Plot a graph of $\%B_0$ versus log of imidacloprid concentration for the calibration standards.

(d)   Read the concentration of thiacloprid in diluted extracts (C_S, in ppb) from the graph. This is best performed using a spreadsheet and a calculated line of best fit.

20.5 CALCULATIONS

20.5.1 Moisture content

The percentage moisture content of the timber sample (MC) shall be calculated from the following equation:

$$MC = \frac{W_i - W_f}{W_f} \times 100 \qquad \qquad \ldots 20.5.1$$

where

    W_i = initial weight of timber sample, in grams

    W_f = final weight of dried timber sample, in grams

20.5.2 Sample extract dilution factor

The sample extract dilution factor (DF) shall be calculated from the following equation:

$$DF = \frac{10}{V} \qquad \qquad \ldots 20.5.2$$

where

    V = volume of extract diluted, in millilitres

20.5.3 Percentage (m/m) of thiacloprid

The percentage (mass/mass) of thiacloprid in the treated timber shall be calculated from the following equation:

$$\text{Thiacloprid}\,(\%\,m/m) = \frac{C_S \times DF\,250\left(1 + \dfrac{MC}{100}\right)}{W \times 10^7} \qquad \qquad \ldots 20.5.3$$

where

    C_S = concentration of thiacloprid in the diluted extract determined by ELISA, in ppb

    DF = sample extract dilution factor

    W = weight of timber sample analysed, in grams

    MC = moisture content of the timber sample, in percent

SECTION 21   DETERMINATION OF
QUATERNARY AMMONIUM COMPOUND IN
WOOD TREATED WITH ACQ PRESERVATIVES

21.1 PRINCIPLE

Quaternary ammonium compounds are cationic surfactants and their determination is based on back titration with an anionic surfactant (sodium lauryl sulphate) titrated against a standard cationic surfactant (Hyamine 1622).

Sodium lauryl sulphate is titrated with Hyamine 1622 in a chloroform and water two-phase system, containing dimidium bromide (a cationic dye) and erioglaucine (an anionic dye) to visually determine the end-point: excess sodium lauryl sulphate results in a pink chloroform-soluble complex with the Hyamine 1622. In the titration, Hyamine 1622 forms a relatively stable complex with the sodium lauryl sulphate and displaces the dimidium bromide from the sodium lauryl sulphate/erioglaucine complex in the chloroform phase. The first excess of Hyamine 1622 reacts with the erioglaucine to form a blue coloured chloroform-soluble complex.

21.2 REAGENTS

The following reagents shall be used:

(a)   0.004M sodium lauryl sulphate.

(b)   0.004M Hyamine 1622.

(c)   Erioglaucine.

(d)   Dimidium bromide.

(e)   Mixed indicator stock.

(f)   Acid indicator stock.

(g)   Chloroform, reagent grade.

(h)   36% hydrochloric acid, reagent grade.

(i)   0.01N HCl extraction solution.

21.3 SAMPLING

The sample shall be prepared as follows:

(a)   Comminute an air-dried wood sample so that it will pass through a 30 mesh screen.

(b)   Oven dry and then transfer 1.25 g of the ground wood sample to a 30 mL screw-capped vial and add 25 mL of 0.01N HCl. Seal tightly.

(c)   Agitate the sealed vial in an ultrasonic bath and agitate for 3 hours or sufficient time to facilitate the extraction process.

(d)   Allow the mixture to cool and the wood meal to settle prior to analysis.

21.4 PROCEDURE

21.4.1 Standardization of sodium lauryl sulphate

The procedure for standardization shall be as follows:

(a)   Add 5 mL of 0.004M sodium lauryl sulphate to a 125 mL titration flask.

(b)   Add 20 mL distilled water, 15 mL chloroform, and 10 mL acid indicator.

(c)   Titrate with 0.004M Hyamine 1622 solution, agitating or stirring after each addition of titrant. The chloroform layer will be coloured pink initially but as the end-point is reached the chloroform/water emulsion will break and a grey-green colour develops in the water phase.

(d)   The end-point is when there is no pink colour and the chloroform layer is a faint grey-blue colour. With excess Hyamine 1622 the chloroform layer becomes blue.

(e)   Record this volume as V_o.

21.4.2   Analysis of sample extract

The procedure for analysis of the sample shall be as follows:

(a)   Place 5 mL of the wood extract into the titration flask containing 20 mL distilled water.

(b)   Add 15 mL chloroform, 10 mL acid indicator, and 5 mL 0.004M sodium lauryl sulphate.

(c)   Agitate thoroughly—the chloroform layer should be pink.

(d)   The excess 004M sodium lauryl sulphate is titrated with 0.004M Hyamine 1622 to the grey-blue end-point. Record the volume used as V.

21.5   CALCULATION OF QUATERNARY AMMONIUM COMPOND RETENTION

The percentage (mass/mass) of quaternary ammonium compound in the wood sample shall be calculated as follows:

$$\text{Molarity Hyamine } 1622 = \frac{\text{Mass Hyamine } 1622 (g)}{448.1} \qquad \ldots 21.5(1)$$

$$\text{QAC}(\%) = \frac{(V_o - V)(\text{Molarity Hyamine } 1622)(\text{MWQAC})(0.5)}{\text{g sample mass}} \times 100 \qquad \ldots 21.5(2)$$

where

V_o = volume (mL) of Hyamine 1622 required in blank titration

V = volume (mL) of Hyamine 1622 required in sample titration

MW = molecular weight of QAC (DDAC is 362.1, and BAC is 354)

SECTION 22 DETERMINATION OF DIDECYLDIMETHYL AMMONIUM CHLORIDE IN WOOD TREATED WITH ACQ PRESERVATIVES

22.1 PRINCIPLE

The method for determining didecyldimethyl ammonium chloride (DDAC) content in treated wood samples uses HPLC with indirect UV detection. Monovalent cations interfere with the chromatographic peaks or troughs.

22.2 REAGENTS

The following reagents shall be used:

(a)   HPLC grade methanol.

(b)   Water, HPLC grade.

(c)   Denatured ethanol, reagent grade.

(d)   Formic acid, reagent grade.

(e)   Acetic acid, reagent grade.

(f)   Benzyltrimethylammonium chloride, reagent grade.

(g)   Didecyldimethylammonium chloride (DDAC), analytical standard.

(h)   HPLC mobile phase: Mix HPLC grade water and HPLC grade methanol in a 1:5 ratio (v/v).

Add 0.75 g of benzyltrimethylammonium chloride and 10 mL of acetic acid into a 1 L flask then add water/methanol solution to 1 L volume. Stir to fully dissolve. Filter through a 0.45 μm PTFE membrane filter.

22.3 SAMPLING

The procedure for preparing the sample shall be as follows:

(a)   Comminute an oven-dried wood sample so that it will pass through a 30 mesh screen and then weigh out a 500 mg sample and place in a PTFE-lined vial.

(b)   Add 20 mL of an extraction solution made from denatured ethanol adjusted to pH 5.0 ±0.1 with formic acid.

(c)   Place the vial in an ultrasonic bath and extract by sonication for 3 hours then allow to cool and settle out.

(d)   Filter the extract and sample for injection into the HPLC.

22.4 CALIBRATION

A calibration should be performed with each analysis batch as follows:

(a)   Equilibrate the HPLC system at a mobile phase flow rate of 3 mL/min.

(b)   Prepare DDAC standards of 50, 100, 500, 1000 ppm in pH5 denatured ethanol.

(c)   Measure the chromatographic peak (retention time of 3 min.) height of the standards. Alternatively use the peak heights determined from an integrator.

(d)   Plot the peak height or area versus concentration and calculate the regression equation for calibration.

22.5 SAMPLE ANALYSIS

The procedure for analysing the sample shall be as follows:

(a)   Filter an aliquot of the sample extracts for injection into the HPLC.

(b)   Run the samples on the HPLC and measure the sample peak heights.

(c)   Calculate the DDAC concentration (ppm) in the extract from the peak heights.

22.6 CALCULATION OF DDAC CONCENTRATION

The percentage of quaternary ammonium compound in the wood sample shall be calculated as follows:

$$DDAC\,(\%) = \frac{(\text{ppm DDAC in extract}) \times 0.02\,L}{\text{g sample mass} \times 10} \times 100 \qquad \ldots 22.6$$

SECTION 23   DETERMINATION OF
DELTAMETHRIN IN PRESERVATIVE-TREATED
TIMBER

23.1 PRINCIPLE

Deltamethrin is extracted from a sample of the treated product (wood, composite wood or surface veneers) with acetone in a sealed bottle with the aid of sonication. A portion of the resulting extract is analysed for Deltamethrin content by capillary gas chromatography.

(a)   The reported analyte is ....Deltamethrin.

(b)   The analyte determined is ....Deltamethrin.

(c)   The reporting units are .... % m/m.

23.2 REAGENTS

The following reagents shall be used:

(a)   *Acetone*   Laboratory reagent grade.
       NOTE: For example, Ajax UNIVAR.

(b)   *Deltamethrin*   Analytical reference grade material with certificate of purity.

(c)   *Nitroxylene*   2-nitro-m-xylene, 99%.

(d)   *Toluene*   AR grade.

(e)   *Internal standard*   Nitroxylene, 1000 mg/L in acetone or toluene: Dilute 100 µL of 2-nitroxylene to 200 mL with acetone or toluene.

(f)   *Stock standard*   500 mg/L Deltamethrin in acetone or toluene. Weigh out to the nearest 0.1 mg about 50 mg of deltamethrin and dissolve in acetone or toluene and make up to 100 mL with acetone. Record actual weight in organic lab standard solutions register.

(g)   *Working standards*   Prepare working standards in acetone or toluene. Record details of all standards in organic lab standard solutions register. Store standards in refrigerator and label them as shown in the table below:

Standard number	Volume standard	Standard used	Final volume	Deltamethrin mg/L
1	5 mL	Stock	50 mL	50
2	5 mL	1	50 mL	5
3	10 mL	2	50 mL	1
4	5 mL	2	50 mL	0.5
5	5 mL	3	50 mL	0.1

23.3 APPARATUS

The following apparatus shall be used:

(a)   *100 mL, borosilicate glass, GL45, screw top bottles*   Commonly Schott or Pyrex brand.

(b)   *GL45, screw top lids with teflon faced liners*   Commonly 'red' autoclavable lids for Schott or Pyrex bottles.

(c)   *Volumetric pipette*   50 mL; B grade or better.

(d)   *Volumetric pipette*   20 mL, 10 mL, 2 mL, 1 mL; A grade.

(e)   *Volumetric flasks*   100 mL; A grade.

(f)   *Ultrasonic bath*   Standard laboratory ultrasonic cleaning bath.

(g)   *Sample vials*   To suit GC sampler in use.

(h)   *Sample vial closures*   Closures to suit GC sample vials.

(i)   *Adhesive labels*.

(j)   *Pipette filling device*.

(k)   *Dispenser*   50 mL.

(l)   *Balance*   analytical, 0.1 mg tolerance.

(m)   *Mechanical pipette*   To dispense 1 mL, fitted with glass Pasteur tips.

(n)   *Syringe or pipette*   Solvent resistant construction, to dispense 20 µL.

(o)   *Gas chromatography system*   (see Clause 23.8).

23.4 SAMPLING

Samples of treated timber should be at equilibrium moisture content, i.e. seasoned. All samples shall be air dry before sample preparation can proceed.

Samples of treated timber shall be reduced on a band saw to small cubes no greater than 10 mm in size. The timber pieces are ground on a cross-beater mill fitted with a 1 mm screen (e.g. Retsch SK-1).

23.5 PROCEDURE

The following procedure shall be used:

(a)   Individually label a 100 ml Schott bottle, and a corresponding 50 ml beaker for each sample.

(b)   Label Schott bottles and Schott bottle lids.

(c)   Label beakers.

(d)   Weigh approx. 2 g of ground timber into each labelled screw top bottle, and record the weight to the nearest 0.01 g (*WET SAMPLE*).

(e)   Weigh the correspondingly labelled empty beaker, and record the weight to the nearest 0.01 g (*TARE MC*).

(f)   Weigh approx. 5 g of ground timber into the beaker, and record the weight to the nearest 0.01 g (*WET MC*).

(g)   Place beakers in the oven (105°C) for a minimum of 18 hours.

(h)   Remove the beakers from the oven and cool in a desiccator.

(i)   Weigh the beakers containing the dried sample, and record the weight to the nearest 0.01 g (*DRY MC*).

23.6 EXTRACTION

The procedure for extraction shall be as follows:

(a)   Dispense 50 mL of acetone into each screw top bottle, using a volumetric pipette with pipette filling device or dispenser.

(b)   Cap firmly with the teflon lined lid.

(c)   Transfer the capped bottles to the ultrasonic bath.

Fill the bath with warm tap water (approx. 500°C) until the level in the bath is between one half and two thirds the height of the bottles, turn on the ultrasonic bath and sonicate for two hours.

(d)   Switch off the ultrasonic bath and allow the bath to stand for 30 minutes, then turn on the ultrasonic bath and sonicate for a further 2 hours.

(e)   Switch off the ultrasonic bath, remove the samples and allow the samples to equilibrate to room temperature.

(f)   Label the required number GC sample vials, one for each sample extract.

(g)   Filter sample if necessary. Pipette 1 mL of extract into its corresponding labelled GC vial.

(h)   Add 20 microL of nitroxylene internal standard solution and mix.

OR

Evaporate 1 mL of extract to dryness under nitrogen at about 45°C.

(i)   Dissolve residue in 1 mL of toluene. Add 20 µL of nitroxylene internal standard solution and vortex mix.

(j)   Cap vials firmly.

(k)   Present samples for analysis by GC, using standards prepared in same solvent.

23.7   DATA HANDLING AND COLLECTION

The percentage of Deltamethrin in the wood sample shall be calculated as follows:

(a)   Oven dry weight:

$$\text{Oven dry weight} = \left(1 - \frac{\text{Wet } MC - \text{Dry } MC}{\text{Wet } MC - \text{Tare } MC}\right) \times \text{wet sample}$$

(b)   Calibration and sample calculations:

The calibration procedure involves the use of an internal standard, which is added to samples and standards at a constant rate. The basis of calibration is the response ratio of the peak areas of analyte compared to the internal standard. The calibration model used is a second order polynomial. The calculations are performed by the data acquisition software.

(c)   Expression of results:

The final results shall be expressed as % m/m, to three decimal places, where % m/m is equal to mg/kg divided by 10 000.

$$\text{Deltamethrin} \left(\% \text{ m/m}\right) = \frac{\text{mg/L deltamethrin} \times 50}{\left(\text{Oven dry weight}\right) \times 10^4} = \frac{0.005 \times \text{mg/L}}{\text{Oven dry weight}}$$

23.8 GC CONFIGURATION

The GC shall be configured as follows:

Pyreth.m (for toluene matrix)		
Capillary column	Model No.	25Qc3/BPX5-0.25 (SGE 054119)
	Nominal length	25 metres
	Nominal diameter	0.32 mm
	Mode	Constant flow
	Initial flow	3.3 mL/min
	Nominal initial pressure	14.65 psi
Oven	Initial temperature	90°C
	Initial time	1 minute
	Ramp #1	15°C per minute
	Final temperature	285°C
	Final time	5 minutes
	Run time	19 minutes
	Post time	0 minute
	Post temp	50°C
Inlet	Mode	Splitless
	Initial temperature	250°C
Column 1	Pressure	14.9 psi
	Purge flow	25 ml/min
	Purge time	1 min
	Total flow	30 mL/min
	Gas type	Helium
	Gas saver	On
Detector	ECD	
	Temperature	300°C
	Mode	Constant column + makeup flow
	Combined flow	30 mL/min
	Makeup gas type	Nitrogen
Typical retention times	Nitroxylene	2.3 minutes
	Deltamethrin	13.4 minutes

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Standards Australia is an independent company, limited by guarantee, which prepares and publishes most of the voluntary technical and commercial standards used in Australia. These standards are developed through an open process of consultation and consensus, in which all interested parties are invited to participate. Through a Memorandum of Understanding with the Commonwealth government, Standards Australia is recognized as Australia's peak national standards body.

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