# Thuja occidentalis

Fresh, leafy, one-year-old twigs of Thuja occidentalis L.

### Description

The fresh leaves develop a very strong, balsamic odour when crushed.

One-year-old twigs are still herbaceous or very slightly lignified and are profusely branched. The small, scale-like leaves are arranged in four rows and lie close to the stem. They are narrowly lineal on young trees, broadly triangular on mature trees, closely appressed to the stem and imbricate. Their underside shows little or no concavity and is lighter in colour, without whitish stomatal lines. The dorsal and ventral leaves (middle leaves) each have a dorsal resin gland, which is absent on the lateral leaves. The leafy branches are dark green on top and significantly lighter in colour on the underside.

The ends of the branches may bear very small, spheroidal to ovoid, cone-like, brownish yellow male flowers or yellow-green female flowers.

#### **Dosage forms**

The mother tincture contains minimum 0.10 and maximum 0.50 per cent (m/m) of total thujone, calculated as  $\alpha$ -thujone (C<sub>10</sub>H<sub>16</sub>O;  $M_r$  152.2).

#### Production

Prepare the mother tincture and liquid dilutions according to Method 3a.

#### Characteristics

The mother tincture is a greenish to green-brown liquid.

### Identification

Thin-layer chromatography (2.2.27)

Test solution: the mother tincture

*Reference solution:* Dissolve 10 mg of borneol R and 10µl of thujone R in 10 ml of methanol R.

*Plate:* TLC silica gel plate *R* 

*Mobile phase:* diisopropyl ether *R*, toluene *R* (20:80 *V*/*V*)

Application: 10 µl; as bands of 20 mm

*Development:* over a path of 10 cm

*Detection:* Allow the mobile phase to evaporate, then treat the plate with ethanolic phosphomolybdic acid solution RH, heat at 100 to 105 °C for 5 to 10 min and examine the chromatograms in daylight.

*Results:* See below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Additional zones may be present in the chromatogram obtained with the test solution.

Top of the plate		
Thujone: a violet zone	A blue zone A blue zone An intense violet zone	
Borneol: a blue zone	One to two blue zones One to two blue zones	
Reference solution	Test solution	

## Tests

**Relative density (2.2.5):** 0.905 to 0.925

Dry residue (H 2.2.6): minimum 3.5 per cent

# Assay

Gas chromatography (2.2.28)

*Internal standard solution:* Dissolve 100.0 mg of car-3-ene *R* in ethanol (96 per cent) *R* to 100.0 ml.

*Test solution:* To 0.50 g of the mother tincture add 1.0 ml of the internal standard solution and dilute to 10.0 ml with ethanol (96 per cent) *R*.

*Reference solution:* Dissolve 10.0 mg of (-)- $\alpha$ -thujone *RH* in ethanol (96 per cent) *R* to 20.0 ml. Dilute 3.0 ml of the solution and 1.0 ml of the internal standard solution to 10.0 ml with ethanol (96 per cent) *R*.

Column

- material: fused silica
- size:  $l = 30 \text{ m}, \emptyset = 0.25 \text{ mm}$
- stationary phase: poly(dimethyl)siloxane *R* (film thickness 0.25 μm)

Carrier gas: helium for chromatography R

Flow rate: 1.0 ml/min

Split ratio: 1:25

Temperature

	Time (min)	Temperature (°C)
Column	0-10	80
	10-15	$80 \rightarrow 100$
	15-16	100
	16-21	$100 \rightarrow 240$
	21-31	240
Injection port		250
Detector		250

Detection: flame ionisation

#### Injection: 1 µl

*Relative retentions* (with reference to  $\alpha$ -thujone,  $t_R$  about 10 min)

- car-3-ene: about 0.65
- $-\beta$ -thujone: about 1.05

System suitability

- repeatability: maximum relative standard deviation of 2.0 per cent for the ratio of the area of the  $\alpha$ -thujone peak to the area of the peak due to the internal standard determined on 6 injections of the reference solution

Calculate the per cent content (m/m) of total thujone (sum of  $\alpha$ - and  $\beta$ -thujone), calculated as  $\alpha$ -thujone, according to the following formula:

$$\frac{A_1 \cdot A_4 \cdot p \cdot m_2 \cdot 0.15}{A_2 \cdot A_3 \cdot m_1}$$

- $A_1$  = sum of the areas of the  $\alpha$  and  $\beta$ -thujone peaks in the chromatogram obtained with the test solution
- $A_2$  = area of the  $\alpha$ -thujone peak in the chromatogram obtained with the reference solution
- $A_3$  = area of the car-3-ene peak in the chromatogram obtained with the test solution
- $A_4$  = area of the car-3-ene peak in the chromatogram obtained with the reference solution
- $m_1 = \text{mass of the mother tincture in grams}$
- $m_2 = \text{mass of the reference substance } (-)-\alpha-\text{thujone } RH \text{ in grams}$
- $p = \text{per cent content of } \alpha \text{-thujone in the reference substance } (-)-\alpha \text{-thujone } RH$

## Storage

Store protected from light.