

**BLACK SPRUCE
FOR HOMOEOPATHIC PREPARATIONS**

**ABIES NIGRA
FOR HOMOEOPATHIC PREPARATIONS**

***Picea mariana* ad praeparationes homoeopathicas**

DEFINITION

Resin hardened in air, seeping naturally or by tapping trunk or branches of *Picea mariana* (Mill.) B.S.P.

Content: minimum 0.6 per cent of α -pinene ($C_{10}H_{16}$; M_r 136.2).

CHARACTERS

Macroscopic characters described under identification test A.
Aromatic odour.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

- A. More or less hard substance of varied shape and size, composed of sticky, agglomerated tears, light yellow to more or less dark brown, punctuated of purplish-pink. Shining fracture.
- B. Examine the chromatograms obtained in the assay.

Results: the peak of the chromatogram obtained with the test solution is similar in retention time to the peak in the chromatogram obtained with the reference solution (α -pinene).

- C. Examine the chromatograms obtained in the test « Other resins ».

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min. Examine in daylight.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Bornyl acetate: a yellow-brown zone	A purple zone
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Borneol: a yellow-brown zone	A spread out purple zone
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Reference solution	Test solution

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

TESTS

Total ash (2.4.16): maximum 3.0 per cent.

Other resins Thin layer chromatography (2.2.27).

Test solution. Add 30 mL of *ethanol* (90 per cent V/V) R to 3 g of powdered drug (180). Heat under a reflux condenser on a water-bath at 60 °C for 15 min. Allow to cool. Filter. Take 1 mL of this solution and dilute to 10 mL with *ethanol* (90 per cent V/V) R.

Reference solution. Dissolve 10 mg of *bornyl acetate* R and 20 mg of *borneol* R in 20 mL of *ethanol* (96 per cent) R.

Plate: TLC silica gel plate R.

Mobile phase: *ethyl acetate* R, *hexane* R (30:70 V/V).

Application: 20 µL, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: Examine in ultraviolet light at 365 nm.

Results: the presence of very intense, blue, fluorescent zones shows adulteration by other resins.

ASSAY

Gas chromatography (2.2.28).

Internal standard solution. In a 100.0 mL volumetric flask, place 0.100 g of *bornyl acetate* R in *ethanol* (96 per cent) R and dilute with the same solvent.

Test solution. Add 30 mL of *ethanol* (90 per cent V/V) R to 2.500 g of powdered drug (180) and shake for 1 h. Allow to separate. Filter in a 50.0 mL volumetric flask. Rinse the residue and the filter with *ethanol* (90 per cent V/V) R then dilute to 50.0 mL with the same solvent. In a 20.0 mL volumetric flask, place 4.0 mL of this solution, add 2.0 mL of internal standard solution and dilute with *ethanol* (96 per cent) R.

Reference solution. In a 100.0 mL volumetric flask, dissolve 0.150 g of α -*pinene* R in *ethanol* (96 per cent) R and dilute with the same solvent. In a 20.0 mL volumetric flask, place 10.0 mL of this solution and dilute with *ethanol* (96 per cent) R. In a 20.0 mL volumetric flask, place 2.0 mL of this solution, add 2.0 mL of the internal standard solution and dilute with *ethanol* (96 per cent) R.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Column:

- *material:* fused silica,
- *size:* $l = 30 \text{ m}$, $\varnothing = 0.53 \text{ mm}$,
- *stationary phase:* polydimethyl-diphenyl siloxane R (1.5 μm).

Carrier gas: helium for chromatography R.

Pressure: 1 Bar.

Temperature:

	Time (min)	Temperature (°C)
Column	0 – 2,5	40 → 50
	2,5 – 21	50 → 200
	21 – 41	200
	41 – 43	200 → 240
	43 – 48	240
Injection port		220
Detector		250

Detection: flame ionisation.

Injection: 1 μL .

Elution order of the peaks: α -pinene, bornyl acetate.

System suitability: reference solution.

- *resolution:* minimum 1.5 between the major peak and bornyl acetate (test solution).

Calculate the percentage content of α -pinene in the drug, from the expression:

$$\frac{A_1 \times A'_2 \times m_2}{A'_1 \times A_2 \times m_1} \times 12,5$$

A'_1 = area of the peak corresponding to the internal standard in the chromatogram obtained with the test solution,

A'_2 = area of the peak corresponding to the internal standard in the chromatogram obtained with the reference solution,

A_1 = area of the peak corresponding to α -pinene in the chromatogram obtained with the test solution,

A_2 = area of the peak corresponding to α -pinene in the chromatogram obtained with the reference solution,

m_1 = mass of the drug sample, in grams,

m_2 = mass of the sample of α -pinene in the reference solution, in grams.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

STOCK

DEFINITION

Black spruce mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Authority Supplement). The mother tincture is prepared with ethanol (90 per cent V/V), using resin of black spruce, hardened in air.

Content: minimum 0.04 per cent *m/m* of α -pinene ($C_{10}H_{16}$; M_r 136.2).

CHARACTERS

Yellow colour.

Aromatic odour.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Add 1 mL of *water R* to 1 mL of mother tincture of black spruce. An unclear milky coloration appears.

B. Examine the chromatograms obtained in the assay.

Results: the peak of the chromatogram obtained with the test solution is similar in retention time to the peak of the chromatogram obtained with the reference solution (α -pinene).

C. Examine the chromatograms obtained in the test Other resins.

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min. Examine in daylight.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore other faint zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Bornyl acetate: a yellow-brown zone	A purple zone
-----	-----
Borneol: a yellow-brown zone	A purple spread out zone
-----	-----
Reference solution	Test solution

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

TESTS

Ethanol (2.9.10): 85 per cent V/V to 95 per cent V/V.

Dry residue (2.8.16): minimum 7.0 per cent.

Other resins. Thin layer chromatography (2.2.27).

Test solution. Mother tincture diluted to 1/10 in *ethanol (96 per cent) R*.

Reference solution. Dissolve 10 mg of *bornyl acetate R* and 20 mg of *borneol R* in 20 mL of *ethanol (96 per cent) R*.

Plate: TLC silica gel plate *R*.

Mobile phase: *ethyl acetate R*, *hexane R* (30:70 V/V).

Application: 20 µL, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: Examine in ultraviolet light at 365 nm.

Results: the presence of very intense blue, fluorescent zones shows adulteration by other resins.

ASSAY

Gas chromatography (2.2.28).

Internal standard solution. In a 100.0 mL volumetric flask, place 0.100 g of *bornyl acetate R* in *ethanol (96 per cent) R* and dilute with the same solvent.

Test solution. In a 20.0 mL volumetric flask, place 2.000 g of mother tincture, add 2.0 mL of internal standard solution and dilute with *ethanol (96 per cent) R*.

Reference solution. In a 100.0 mL volumetric flask, dissolve 0.150 g of *α-pinene R* in *ethanol (96 per cent) R* and dilute with the same solvent. In a 20.0 mL volumetric flask, place 10.0 mL of this solution and dilute with *ethanol (96 per cent) R*. In a 20.0 mL volumetric flask, place 2.0 mL of this solution, add 2.0 mL of the internal standard solution and dilute with *ethanol (96 per cent) R*.

Column:

- *material:* fused silica
- *size:* $l = 30$ m, $\varnothing = 0.53$ mm,
- *stationary phase:* *polydimethyl-diphenyl siloxane R* (1.5 µm).

Carrier gas: *helium for chromatography R*.

Pressure: 1 Bar.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Temperature:

	Time (min)	Temperature (°C)
Column	0 – 2,5	40 → 50
	2,5 – 21	50 → 200
	21 – 41	200
	41 – 43	200 → 240
	43 – 48	240
Injection port		220
Detector		250

Detection : flame ionisation.

Injection : 1 µL.

Elution order of the peaks: α-pinene, bornyl acetate.

System suitability: reference solution.

– *resolution*: minimum 1.5 between the major peak and bornyl acetate (test solution).

Calculate the percentage content *m/m* of α-pinene in the mother tincture, from the expression:

$$\frac{A_1 \times A'_2 \times m_2}{A'_1 \times A_2 \times m_1}$$

A'_1 = area of the peak corresponding to the internal standard in the chromatogram obtained with the test solution,

A'_2 = area of the peak corresponding to the internal standard in the chromatogram obtained with the reference solution,

A_1 = area of the peak corresponding to α-pinene in the chromatogram obtained with the test solution,

A_2 = area of the peak corresponding to α-pinene in the chromatogram obtained with the reference solution,

m_1 = mass of the mother tincture sample, in grams,

m_2 = mass of the sample of α-pinene in the reference solution, in grams.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

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