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Eucalyptus Oil

CAS RN®: 8000-48-4.

DEFINITION

Eucalyptus Oil is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus* rich in 1,8-cineole. The species mainly used are *Eucalyptus globulus* Labill., *Eucalyptus polybractea* R.T. Baker, and *Eucalyptus smithii* R.T. Baker. It contains NLT 70.0% and NMT 95.0% of eucalyptol (1,8-cineole, $C_{10}H_{18}O$).

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197F**▲ (CN 1-May-2020)

• **B. Identity by Aroma Substance Profile:** The retention times of the (+)-α-pinene, β-pinene, (*R*)-(−)-α-phellandrene, (*R*)-(+)-limonene, and eucalyptol peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Content of Aroma Substances* in the *Assay*.

ASSAY

• **Content of Aroma Substances**

Standard solution A: 1.0 µL/mL of (+)-α-pinene, 0.5 µL/mL of β-pinene, 0.5 µL/mL of sabinene, 0.5 µL/mL of (*R*)-(−)-α-phellandrene, 1.0 µL/mL of (*R*)-(+)-limonene, 5 µL/mL of USP Eucalyptol RS, and 5 mg/mL of USP Camphor RS in heptane

Standard solution B: 0.01 µL/mL of (*R*)-(+)-limonene in heptane

Sample solution: 20 µL/mL of Eucalyptus Oil in heptane

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 60-m fused-silica capillary; 0.25-µm layer of phase G16

Temperatures

Injection port: 220°

Detector: 220°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	—	60	5
60	5	200	5

Carrier gas: Helium

Flow rate: 1.5 mL/min

Injection type: Split ratio, 1:50

Injection volume: 1 µL

System suitability

Sample: *Standard solution A*

[Note—The relative retention times are listed in *Table 2*.]

Table 2

Name	Relative Retention Time
(+)-α-Pinene	0.64
β-Pinene	0.80
Sabinene	0.83
(*R*)-(−)-α-Phellandrene	0.91
(*R*)-(+)-Limonene	0.98
Eucalyptol	1.00
Camphor	1.58

Suitability requirements

Resolution: NLT 1.5 between (*R*)-(+)-limonene and eucalyptol

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the peaks in the *Sample solution* based on those in *Standard solution A* as well as in *Table 3*.

Table 3

Name	Relative Retention Time
β-Myrcene	0.86–0.90
γ-Terpinene	1.05–1.07
p-Cymene	1.12–1.13
Terpinen-4-ol	1.68
α-Terpineol	1.82

β-Myrcene elutes before (*R*)-(−)-α-phellandrene and after sabinene; γ-terpinene and *p*-cymene elute between eucalyptol and camphor; and terpinen-4-ol and α-terpineol elute after camphor.

Calculate the percentage of (+)-α-pinene [β-pinene, sabinene, (*R*)-(−)-α-phellandrene, (*R*)-(+)-limonene, or eucalyptol] in the portion of Eucalyptus Oil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of (+)-α-pinene [β-pinene, sabinene, (*R*)-(−)-α-phellandrene, (*R*)-(+)-limonene, or eucalyptol] from the *Sample solution*

r_S = peak response of (+)-α-pinene [β-pinene, sabinene, (*R*)-(−)-α-phellandrene, (*R*)-(+)-limonene, or eucalyptol] from the *Standard solution*

C_S = concentration of (+)-α-pinene [β-pinene, sabinene, (*R*)-(−)-α-phellandrene, (*R*)-(+)-limonene, or USP Eucalyptol RS] in the *Standard solution* (µL/mL)

C_U = concentration of Eucalyptus Oil in the *Sample solution* (µL/mL)

Calculate the percentage of camphor in the portion of Eucalyptus Oil taken:

$$\text{Result} = (r_U/r_S) \times [C_S/(C_U \times D)] \times 100$$

r_U = peak response of camphor from the *Sample solution*

r_S = peak response of camphor from the *Standard solution*

C_S = concentration of USP Camphor RS in the *Standard solution* (mg/mL)

C_U = concentration of Eucalyptus Oil in the *Sample solution* (µL/mL)

D = density of Eucalyptus Oil (mg/µL)

Calculate the percentage of β-myrcene (γ-terpinene, *p*-cymene, terpinen-4-ol, or α-terpineol) in the portion of Eucalyptus Oil taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of β-myrcene (γ-terpinene, *p*-cymene, terpinen-4-ol, or α-terpineol) from the *Sample solution*

r_T = sum of the total peak responses, except for the peaks due to solvent, from the *Sample solution*

Acceptance criteria: See *Table 4*. Disregard any peak with an area less than the major peak area from *Standard solution B*, corresponding to 0.05%.

Table 4

Name	Acceptance Criteria, NMT (%)
(+)-α-Pinene	0.2–10.0
β-Pinene	0.05–1.5
Sabinene	0.3
(*R*)-(−)-α-Phellandrene	0.05–1.5
(*R*)-(+)-Limonene	2.0–15.0
Eucalyptol	70.0–95.0
Camphor	0.1

Percentage of γ-terpinene: 0.1%–6.0%

Percentage of *p*-cymene: 0.5%–15.0%

Total percentage of all identified aroma substances [(+)-α-pinene, β-pinene, sabinene, (*R*)-(−)-α-phellandrene, (*R*)-(+)-limonene, eucalyptol, camphor, β-myrcene, γ-terpinene, *p*-cymene, terpinen-4-ol, and α-terpineol]: NLT 98.0%

IMPURITIES

• **Test for Aldehyde**

Alcoholic hydroxylamine solution: Dissolve 3.5 g of hydroxylamine hydrochloride in 95 mL of 60% alcohol (v/v) and add 0.5 mL of a 2-mg/mL solution of methyl orange in 60% alcohol (v/v) and sufficient 0.5 M potassium hydroxide in 60% alcohol (v/v) to give a pure yellow color. Dilute with 60% alcohol (v/v) to 100 mL.

Analysis: In a ground-glass-stoppered tube, 25 mm in diameter and 150 mm long, add 10 mL of Eucalyptus Oil. Then add 5 mL of toluene and 4 mL of *Alcoholic hydroxylamine solution*. Shake vigorously, and titrate immediately with 0.5 M potassium hydroxide in 60% alcohol (v/v) until the red color changes to yellow. Continue the titration with shaking; the endpoint is reached when the pure yellow color of the indicator is permanent in the lower layer after shaking vigorously for 2 min and allowing separation to take place. The reaction is complete in about 15 min.

Repeat the titration using a further 10 mL of Eucalyptus Oil and, as a reference solution for the endpoint, the titrated liquid from the first determination, to which has been added 0.5 mL of 0.5 M potassium hydroxide in 60% alcohol (v/v).

Acceptance criteria: NMT 2.0 mL of 0.5 M potassium hydroxide in 60% alcohol (v/v) is required in the second titration.

SPECIFIC TESTS

• **Specific Gravity ⟨841⟩:** 0.906–0.927, at 20°

• **Refractive Index ⟨831⟩:** 1.450–1.470, at 20°

• **Optical Rotation ⟨781⟩:** 0°–10°, at 20°

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Do not store above 25°.

• **USP Reference Standards ⟨11⟩**

USP Camphor RS

USP Eucalyptol RS

USP Eucalyptus Oil RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
EUCALYPTUS OIL	Documentary Standards Support	CE2020 Complex Excipients

Chromatographic Database Information: Chromatographic Database

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