TESTS

Foreign matter (*2.8.2*): maximum 3 per cent of dark and brown leaves, maximum 5 per cent of stems and maximum 2 per cent of other foreign matter. Cordate or ovate sessile leaves of young branches, with numerous glands on both sides, visible as points in transmitted light, are not present. Determine by using 30 g of the drug to be examined.

Water (*2.2.13*): maximum 100 ml/kg, determined on 20.0 g of powdered drug (355) (*2.9.12*).

Total ash (*2.4.16*): maximum 6.0 per cent.

ASSAY

Carry out the determination of essential oil in herbal drugs (*2.8.12*). Use 10.0 g of the drug, cut immediately before determination, a 500 ml round-bottomed flask, 200 ml of *water R* and 100 ml of *glycerol R* as the distillation liquid and 0.5 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

01/2008:0390

EUCALYPTUS OIL

Eucalypti aetheroleum

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.

CHARACTERS

A colourless or pale yellow liquid with an aromatic and camphoraceous odour and a pungent and camphoraceous taste.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution. Dissolve 0.1 g of the substance to be examined in *toluene R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 50 µl of *cineole R* in *toluene R* and dilute to 5 ml with the same solvent.

Apply to the plate as bands 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *ethyl acetate R* and 90 volumes of *toluene R*. Allow the plate to dry in air and spray with *anisaldehyde solution R* and examine in daylight while heating at 100-105 °C for 5-10 min. The chromatogram obtained with the reference solution shows in the middle a zone due to cineole. The chromatogram obtained with the test solution shows a main zone similar in position and colour to the zone due to cineole in the chromatogram obtained with the reference solution. Other weaker zones may be present.

B. Examine the chromatograms obtained in the test for chromatographic profile. The chromatogram obtained with the test solution shows 5 peaks similar in retention time to the 5 peaks in the chromatogram obtained with the reference solution.

TESTS

Relative density (*2.2.5*): 0.906 to 0.927.

Refractive index (*2.2.6*): 1.458 to 1.470.

Optical rotation (*2.2.7*): 0° to + 10°.

Solubility in alcohol (*2.8.10*). It is soluble in 5 volumes of *ethanol (70 per cent V/V) R*.

Aldehydes. Place 10 ml in a glass-stoppered tube 25 mm in diameter and 150 mm long. Add 5 ml of *toluene R* and 4 ml of *alcoholic hydroxylamine solution R*. Shake vigorously and titrate immediately with *0.5 M potassium hydroxide in alcohol (60 per cent V/V)* until the red colour changes to yellow. Continue the titration with shaking; the end-point is reached when the pure yellow colour 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 the substance to be examined and, as a reference solution for the end-point, the titrated liquid from the first determination to which has been added 0.5 ml of *0.5 M potassium hydroxide in alcohol (60 per cent V/V)*. Not more than 2.0 ml of *0.5 M potassium hydroxide in alcohol (60 per cent V/V)* is required in the second titration.

Chromatographic profile. Examine by gas chromatography (*2.2.28*).

Test solution. The oil to be examined.

Reference solution. Dissolve 80 µl of *α-pinene R*, 10 µl of *β-pinene R*, 10 µl of *sabinene R*, 10 µl of *α-phellandrene R*, 10 µl of *limonene R*, 0.8 ml of *cineole R* and 10 mg of *camphor R* in 10 ml of *acetone R*.

The chromatographic procedure may be carried out using:

- a fused-silica column 60 m long and about 0.25 mm in internal diameter coated with *macrogol 20 000 R* as the bonded phase,
- *helium for chromatography R* as the carrier gas at a flow rate of 1.5 ml/min,
- a flame-ionisation detector,
- a split ratio of 1:100,

maintaining the temperature of the column at 60 °C for 5 min, then raising the temperature at a rate of 5 °C/min to 200 °C and maintaining at 200 °C for 5 min; maintaining the temperature of the injection port and that of the detector at 220 °C.

Inject about 0.5 µl of the reference solution. When the chromatogram is recorded in the prescribed conditions the components elute in the order indicated in the composition of the reference solution. Record the retention times of these substances.

The assay is not valid unless: the number of theoretical plates calculated for the peak due to limonene at 110 °C is at least 30 000; the resolution between the peaks due to limonene and cineole is at least 1.5.

Inject about 0.5 µl of the test solution. Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution on the chromatogram obtained with the test solution.

Determine the percentage content of these components by the normalisation procedure.

The percentages are within the following ranges:

- *α-pinene*: traces to 9.0 per cent,
- *β-pinene*: less than 1.5 per cent,
- *sabinene*: less than 0.3 per cent,
- *α-phellandrene*: less than 1.5 per cent,

– *limonene*: traces to 12.0 per cent,
– *1,8-cineole*: minimum 70.0 per cent,
– *camphor*: less than 0.1 per cent.

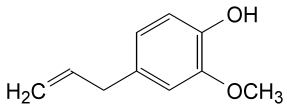
STORAGE

In a well-filled, airtight container, protected from light and at a temperature not exceeding 25 °C.

01/2008:1100

EUGENOL

Eugenolum



$C_{10}H_{12}O_2$ M_r 164.2
[97-53-0]

DEFINITION

2-Methoxy-4-(prop-2-enyl)phenol.

CHARACTERS

Appearance: colourless or pale yellow, clear liquid, darkening on exposure to air.

It has a strong odour of clove.

Solubility: practically insoluble in water, freely soluble in ethanol (70 per cent *V/V*), practically insoluble in glycerol, miscible with ethanol (96 per cent), with glacial acetic acid, with methylene chloride and with fatty oils.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Refractive index (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *eugenol CRS*.

C. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 50 µl of the substance to be examined in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.
Reference solution. Dissolve 50 µl of *eugenol CRS* in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.
Plate: *TLC silica gel F_{254} plate R*.
Mobile phase: *ethyl acetate R, toluene R* (10:90 *V/V*).
Application: 5 µl.
Development: over a path of 15 cm.
Drying: in a current of cold air.
Detection A: examine in ultraviolet light at 254 nm.
Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.
Detection B: spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min.
Results B: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve 0.05 ml in 2 ml of *ethanol (96 per cent) R* and add 0.1 ml of *ferric chloride solution R1*. A dark green colour is produced which changes to yellowish-green within 10 min.

TESTS

Relative density (*2.2.5*): 1.066 to 1.070.

Refractive index (*2.2.6*): 1.540 to 1.542.

Dimeric and oligomeric compounds. Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. The absorbance (*2.2.25*) of the solution at 330 nm is not greater than 0.25.

Related substances. Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution. Dissolve 1.00 g of the substance to be examined in *anhydrous ethanol R* and dilute to 5.0 ml with the same solvent.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *anhydrous ethanol R*.

Reference solution (b). Dissolve 50 mg of *vanillin R* (impurity H) in 1 ml of the test solution and dilute to 5 ml with *anhydrous ethanol R*.

Column:
– *material*: fused silica;
– *size*: *l* = 30 m, Ø = 0.25 mm;
– *stationary phase*: *polymethylphenylsiloxane R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1 ml/min.

Split ratio: 1:40.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	80
	2 - 27	80 → 280
	27 - 47	280
Injection port		250
Detector		280

Detection: flame ionisation.

Injection: 1 µl.

System suitability: reference solution (b):
– *relative retention* with reference to eugenol: impurity H = minimum 1.1.

Limits:
– *any impurity*: for each impurity, maximum 0.5 per cent;
– *sum of impurities with a relative retention greater than 2.0 with reference to eugenol*: maximum 1.0 per cent;
– *total*: maximum 3.0 per cent;
– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Hydrocarbons. Dissolve 1 ml in 5 ml of *dilute sodium hydroxide solution R* and add 30 ml of *water R* in a stoppered test-tube. Examined immediately, the solution is yellow and clear (*2.2.1*).

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

STORAGE

In a well-filled container, protected from light.