01/2008:0405

PEPPERMINT OIL

Menthae piperitae aetheroleum

DEFINITION

Essential oil obtained by steam distillation from the fresh aerial parts of the flowering plant of *Mentha* × *piperita* L.

CHARACTERS

Appearance: a colourless, pale yellow or pale greenish-yellow liquid.

It has a characteristic odour and taste followed by a sensation of cold.

Solubility: miscible with alcohol and with methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A.

A. Examine the chromatograms obtained in the test for mint oil.

Results A: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.

Top of the plate	
———	———
Thymol: a quenching zone	
	Quenching zones may be present (carvone, pulegone)
———	———
Reference solution	**Test solution**

Results B: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intensely coloured zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	An intense violet-red zone (near the solvent front) (hydrocarbons)
	A brownish-yellow zone (menthofuran)
———	———
Menthyl acetate: a violet-blue zone	A violet-blue zone (menthyl acetate)
	A greenish-blue zone (menthone)
Thymol: a pink zone	
	Light pink or greyish-blue or greyish-green zones may be present (carvone, pulegone, isomenthone)
Cineole: a violet-blue to brown zone	A faint violet-blue to brown zone (cineole)
———	———
Menthol: an intense blue to violet zone	An intense blue to violet zone (menthol)
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results: the characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution. Carvone and pulegone may be present in the chromatogram obtained with the test solution.

TESTS

Relative density (*2.2.5*): 0.900 to 0.916.

Refractive index (*2.2.6*): 1.457 to 1.467.

Optical rotation (*2.2.7*): – 10° to – 30°.

Acid value (*2.5.1*): maximum 1.4, determined on 5.0 g diluted in 50 ml of the prescribed mixture of solvents.

Fatty oils and resinified essential oils (*2.8.7*). It complies with the test for fatty oils and resinified essential oils.

Mint oil

A. Thin-layer chromatography (*2.2.27*).

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

Reference solution. Dissolve 50 mg of *menthol R*, 20 µl of *cineole R*, 10 mg of *thymol R* and 10 µl of *menthyl acetate R* in *toluene R* and dilute to 10 ml with the same solvent.

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *ethyl acetate R, toluene R* (5:95 *V/V*).

Application: 10 µl of the reference solution and 20 µl of the test solution, as bands.

Development: over a path of 15 cm.

Drying: in air.

Detection A: examine in ultraviolet light at 254 mm.

Detection B: spray with *anisaldehyde solution R* and heat at 100-105 °C for 5-10 min. Examine immediately in daylight.

Result B: the chromatogram obtained with the test solution shows no blue zone between the zones due to cineole and menthol.

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results: the chromatogram obtained with the test solution does not show a peak with the retention time of isopulegol that has an area of more than 0.2 per cent of the total area.

Chromatographic profile. Gas chromatography (*2.2.28*): use the normalisation procedure.

Test solution. Mix 0.20 g of the substance to be examined with *hexane R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 10 µl of *limonene R*, 20 µl of *cineole R*, 40 µl of *menthone R*, 10 µl of *menthofuran R*, 10 µl of *isomenthone R*, 40 µl of *menthyl acetate R*, 20 µl of *isopulegol R*, 60 mg of *menthol R*, 20 µl of *pulegone R*, 10 µl of *piperitone R* and 10 µl of *carvone R* in *hexane R* and dilute to 10.0 ml with the same solvent.

Reference solution (b). Dissolve 5 µl of *isopulegol R* in *hexane R* and dilute to 10 ml with the same solvent. Dilute 0.1 ml to 5 ml with *hexane R*.

Column:

– *material*: fused silica,
– *size*: *l* = 60 m, Ø = 0.25 mm,
– *stationary phase*: *macrogol 20 000 R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R.*

Flow rate: 1.5 ml/min.

Split ratio: 1:50.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	60
	10 - 70	60 - 180
	70 - 75	180
Injection port		200
Detector		220

Detection: flame ionisation.

Injection: 1 µl.

Elution order: order indicated in the composition of reference solution (a); record the retention times of these substances.

System suitability: reference solution (a):

– *resolution*: minimum 1.5 between the peaks due to limonene and cineole and minimum 1.5 between the peaks due to piperitone and carvone.

Using the retention times determined from the chromatogram obtained with reference solution (a), locate the components of the reference solution in the chromatogram obtained with the test solution (disregard the peak due to hexane).

Determine the percentage content of the components. The limits are within the following ranges:

– *limonene*: 1.0 per cent to 5.0 per cent,
– *cineole*: 3.5 per cent to 14.0 per cent,
– *menthone*: 14.0 per cent to 32.0 per cent,
– *menthofuran*: 1.0 per cent to 9.0 per cent,
– *isomenthone*: 1.5 per cent to 10.0 per cent,
– *menthyl acetate*: 2.8 per cent to 10.0 per cent,
– *isopulegol*: maximum 0.2 per cent,
– *menthol*: 30.0 per cent to 55.0 per cent,
– *pulegone*: maximum 4.0 per cent,
– *carvone*: maximum 1.0 per cent,
– *disregard limit*: peak area obtained with reference solution (b) (0.05 per cent).

The ratio of cineole content to limonene content is not less than 2.

STORAGE

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

01/2008:0682

PEPSIN POWDER

Pepsini pulvis

[9001-75-6]

DEFINITION

Pepsin powder is prepared from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases, active in acid medium (pH 1 to 5). It has an activity not less than 0.5 Ph. Eur. U./mg, calculated with reference to the dried substance.

PRODUCTION

The animals from which pepsin powder is derived must fulfil the requirements for the health of animals suitable for human consumption.

CHARACTERS

A white or slightly yellow, crystalline or amorphous powder, hygroscopic, soluble in water, practically insoluble in ethanol (96 per cent). The solution in water may be slightly opalescent with a weak acidic reaction.

IDENTIFICATION

In a mortar, pound 30 mg of *fibrin blue R*. Suspend in 20 ml of *dilute hydrochloric acid R2*. Filter the suspension on a filter paper and wash with *dilute hydrochloric acid R2* until a colourless filtrate is obtained. Perforate the filter paper and wash the *fibrin blue R* through it into a conical flask using 20 ml of *dilute hydrochloric acid R2*. Shake before use. Dissolve a quantity of the substance to be examined, equivalent to not less than 20 Ph. Eur. U., in 2 ml of *dilute hydrochloric acid R2* and adjust to pH 1.6 ± 0.1. Add 1 ml of this solution to a test-tube containing 4 ml of the fibrin blue suspension, mix and place in a water-bath at 25 °C with gentle shaking. Prepare a blank solution at the same time and in the same manner using 1 ml of *water R*. After 15 min of incubation the blank solution is colourless and the test solution is blue.

TESTS

Loss on drying (*2.2.32*). Not more than 5.0 per cent, determined on 0.500 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 670 Pa for 4 h.

Microbial contamination. Total viable aerobic count (*2.6.12*) not more than 10^4 micro-organisms per gram, determined by plate-count. It complies with the tests for *Escherichia coli* and *Salmonella* (*2.6.13*).

ASSAY

The activity of pepsin powder is determined by comparing the quantity of peptides, non-precipitable by *trichloroacetic acid solution R* and assayed using the *phosphomolybdotungstic reagent R*, which are released per minute from a substrate of *haemoglobin solution R*, with the quantity of such peptides released by *pepsin powder BRP* from the same substrate in the same conditions.

For the test solution and the reference solution, prepare the solution and carry out the dilution at 0 °C to 4 °C.

Avoid shaking and foaming during preparation of the test and reference solutions.

Test solution. Immediately before use, prepare a solution of the substance to be examined expected to contain 0.5 Ph. Eur. U./ml in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Reference solution. Less than 15 min before use, prepare a solution of *pepsin powder BRP* containing 0.5 Ph. Eur. U./ml in *dilute hydrochloric acid R2*; before dilution to volume, adjust to pH 1.6 ± 0.1, if necessary, using *1 M hydrochloric acid*.

Designate tubes in duplicate T, T_b, S_1, S_{1b}, S_2, S_{2b}, S_3, S_{3b}; designate a tube B.

Add *dilute hydrochloric acid R2* to the tubes as follows:

B: 1.0 ml

S_1 and S_{1b}: 0.5 ml

S_2, S_{2b} and T and T_b: 0.25 ml

Add the reference solution to the tubes as follows:

S_1 and S_{1b}: 0.5 ml

S_2 and S_{2b}: 0.75 ml

S_3 and S_{3b}: 1.0 ml

Add 0.75 ml of the test solution to tubes T and T_b.