Water (2.5.32). If intended for use in the manufacture of parenteral dosage forms, maximum 0.1 per cent, determined on 5.0 g by the coulometric method. Use a mixture of equal volumes of decanol R and anhydrous methanol R as solvent.

STORAGE
Store in a well-filled container, protected from light, at a temperature not exceeding 25 °C. If intended for use in the manufacture of parenteral dosage forms, store under an inert gas.

LABELLING
The label states:

- where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms,
- the name of the inert gas.

OLIVE OIL, VIRGIN

Oliveae oleum virginale

DEFINITION
Virgin olive oil is the fatty oil obtained by cold expression or other suitable mechanical means from the ripe drupes of Olea europaea L.

CHARACTERS
A clear, yellow or greenish-yellow, transparent liquid with a characteristic odour, practically insoluble in alcohol, miscible with light petroleum (50 °C to 70 °C). When cooled, it begins to become cloudy at 10 °C and becomes a butter-like mass at about 0 °C. It has a relative density of about 0.913.

IDENTIFICATION
Carry out the test for identification of fatty oils by thin-layer chromatography (2.3.2). The chromatogram obtained shows spots corresponding to those in the typical chromatogram for olive oil. For certain types of olive oil, the difference in the size of spots E and F is less pronounced than in the typical chromatogram.

TESTS

Acid value (2.5.1). Not more than 2.0, determined on 5.0 g.

Peroxide value (2.5.5, Method A). Not more than 20.0.

Unsaponifiable matter. Not more than 1.5 per cent. Place 5.0 g (m g) in a 150 ml flask fitted with a reflux condenser. Add 50 ml of 2 M alcoholic potassium hydroxide R and heat on a water-bath for 1 h, shaking frequently. Add 50 ml of water R through the top of the condenser, shake, allow to cool and transfer the contents of the flask to a separating funnel. Rinse the flask with several portions to a total of 50 ml of light petroleum R1 and add the rinsings to the separating funnel. Shake vigorously for 1 min. Allow to separate and transfer the aqueous layer to a second separating funnel. If an emulsion forms, add small quantities of alcohol R or a concentrated solution of potassium hydroxide R. Shake the aqueous layer with 2 quantities, each of 50 ml, of light petroleum R1. Combine the light petroleum layers in a third separating funnel and wash with 3 quantities, each of 50 ml, of alcohol (50 per cent V/V) R. Transfer the light petroleum layer to a tared 250 ml flask. Rinse the separating funnel with small quantities of light petroleum R1 and add to the flask. Evaporate the light petroleum on a water-bath and dry the residue at 100 °C to 105 °C for 15 min, keeping the flask horizontal. Allow to cool in a desiccator and weigh (a g). Repeat the drying for successive periods of 15 min until the loss of mass between 2 successive weighings does not exceed 0.1 per cent. Dissolve the residue in 20 ml of alcohol R, previously neutralised to 0.1 ml of bromophenol blue solution R. If necessary, titrate with 0.1 M hydrochloric acid (b ml).

Calculate the percentage content of unsaponifiable matter from the expression:

\[ \frac{100(a - 0.032b)}{m} \]

If 0.032b is greater than 5 per cent of a, the test is invalid and must be repeated.

Absorbance (2.2.25). Dissolve 1.00 g in cyclohexane R and dilute to 100.0 ml with the same solvent. The absorbance measured at 270 nm is not greater than 0.20. The ratio of the absorbance at 232 nm to that at 270 nm is greater than 8.

Composition of fatty acids (2.4.22, Method A). The fatty acid fraction of the oil has the following composition:

- saturated fatty acids of chain length less than C\textsubscript{16}: not more than 0.1 per cent,
- palmitic acid: 7.5 per cent to 20.0 per cent,
- palmitoleic acid (equivalent chain length on polyethylene glycol adipate 16.3): not more than 3.5 per cent,
- stearic acid: 0.5 per cent to 5.0 per cent,
- oleic acid (equivalent chain length on polyethylene glycol adipate 18.3): 56.0 per cent to 85.0 per cent,
- linoleic acid (equivalent chain length on polyethylene glycol adipate 18.9): 3.5 per cent to 20.0 per cent,
- linolenic acid (equivalent chain length on polyethylene glycol adipate 19.7): not more than 1.2 per cent,
- arachidic acid: not more than 0.7 per cent,
- eicosenoic acid (equivalent chain length on polyethylene glycol adipate 20.3): not more than 0.4 per cent,
- behenic acid: not more than 0.2 per cent,
- lignoceric acid: not more than 0.2 per cent.

Sterols (2.4.23). The sterol fraction of the oil has the following composition:

- sum of contents of \( \beta \)-sitosterol, \( \Delta_5,23 \)-stigmastadienol, \( \alpha \)-ergosteryl, sitostanol, \( \Delta_5 \)-avenasterol and \( \Delta_5,24 \)-stigmastadienol: not less than 93.0 per cent,
- cholesterol: not more than 0.5 per cent,
- \( \Delta_7 \)-stigmastanol: not more than 0.5 per cent,
- campesterol: not more than 4.0 per cent, and the content of stigmasterol is not more than that of campesterol.

Sesame oil. In a ground-glass-stoppered cylinder shake 10 ml for about 1 min with a mixture of 0.5 ml of a 0.35 per cent V/V solution of furfural R in acetic anhydride R and 4.5 ml of acetic anhydride R. Filter through a filter paper impregnated with acetic anhydride R. To the filtrate add 0.2 ml of sulphuric acid R. No bluish-green colour develops.

STORAGE
Store in a well-filled container, protected from light, at a temperature not exceeding 25 °C.