temperature of the mixture has fallen 3 °C below $t_1$, stir continuously. Note the highest temperature at which the mixture crystallises ($t_2$). Repeat the operation until 2 highest values obtained for $t_2$ do not differ by more than 0.2 °C. If supercooling occurs, induce crystallisation by adding a small crystal of the complex consisting of 3.00 g of *cineole R* and 2.10 g of melted *cresol R*. If $t_2$ is below 27.4 °C, repeat the determination after the addition of 5.10 g of the complex.

The content of cineole corresponding to the highest temperature observed ($t_2$) is given in Table 2.8.11.-1. If 5.10 g of the complex has been added, calculate the cineole content per cent *m/m* from the expression:

$$2\,(A - 50)$$

where $A$ is the value found in Table 2.8.11.-1.

The content of cineole, corresponding to the highest temperature observed ($t_2$), is obtained, where necessary, by interpolation.

Table 2.8.11.-1

| $t_2$ °C | cineole per cent *m/m* | $t_2$ °C | cineole per cent *m/m* | $t_2$ °C | cineole per cent *m/m* | $t_2$ °C | cineole per cent *m/m* |
|---|---|---|---|---|---|---|---|
| 24 | 45.5 | 32 | 56.0 | 40 | 67.0 | 48 | 82.0 |
| 25 | 47.0 | 33 | 57.0 | 41 | 68.5 | 49 | 84.0 |
| 26 | 48.5 | 34 | 58.5 | 42 | 70.0 | 50 | 86.0 |
| 27 | 49.5 | 35 | 60.0 | 43 | 72.5 | 51 | 88.5 |
| 28 | 50.5 | 36 | 61.0 | 44 | 74.0 | 52 | 91.0 |
| 29 | 52.0 | 37 | 62.5 | 45 | 76.0 | 53 | 93.5 |
| 30 | 53.5 | 38 | 63.5 | 46 | 78.0 | 54 | 96.0 |
| 31 | 54.5 | 39 | 65.0 | 47 | 80.0 | 55 | 99.0 |

**01/2008:20812**
**corrected 6.0**

# 2.8.12. DETERMINATION OF ESSENTIAL OILS IN HERBAL DRUGS

The determination of essential oils in herbal drugs is carried out by steam distillation in a special apparatus in the conditions described below. The distillate is collected in the graduated tube, using xylene to take up the essential oil; the aqueous phase is automatically returned to the distillation flask.

*Apparatus.* The apparatus comprises the following parts:

(a) a suitable round-bottomed flask with a short, ground-glass neck having an internal diameter of about 29 mm at the wide end;

(b) a condenser assembly (see Figure 2.8.12.-1) that closely fits the flask, the different parts being fused into one piece; the glass used has a low coefficient of expansion:

- the stopper *K′* is vented and the tube *K* has an orifice of diameter about 1 mm that coincides with the vent; the wide end of the tube *K* is of ground-glass and has an internal diameter of 10 mm;
- a pear-shaped swelling, *J*, of 3 ml capacity;
- the tube *JL* is graduated in 0.01 ml;
- the bulb-shaped swelling *L* has a capacity of about 2 ml;
- *M* is a three-way tap;
- the junction *B* is at a level 20 mm higher than the uppermost graduation;

(c) a suitable heating device, allowing a fine control;

(d) a vertical support with a horizontal ring covered with insulating material.

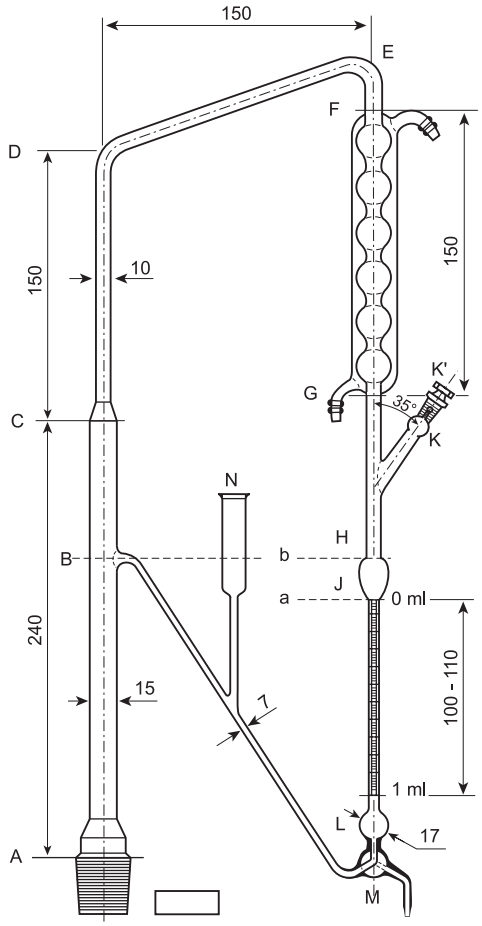


Figure 2.8.12.-1. - *Apparatus for the determination of essential oils in herbal drugs*

*Dimensions in millimetres*

*Method.* Use a thoroughly cleaned apparatus. Carry out the assay according to the nature of the drug to be examined. Place the prescribed volume of distillation liquid in the flask, add a few pieces of porous porcelain and attach the condenser assembly. Introduce *water R* through the filling funnel *N* until it is at the level *B*. Remove the stopper *K′* and introduce the prescribed quantity of *xylene R*, using a pipette with its tip at the bottom of the tube *K*. Replace the stopper *K′* and ensure that the orifice coincides with the vent. Heat the liquid in the flask to boiling and adjust the distillation rate to 2-3 ml/min, unless otherwise prescribed.

To determine the rate of distillation, during distillation lower the level of the water by means of the three-way tap until the meniscus is at the level of the lower mark (a) (see Figure 2.8.12.-2). Close the tap and measure the time taken for the liquid to reach the upper mark (b). Open the tap and continue the distillation, modifying the heat to regulate the distillation rate. Distil for 30 min. Stop the heating and after at least 10 min read off the volume of xylene in the graduated tube.

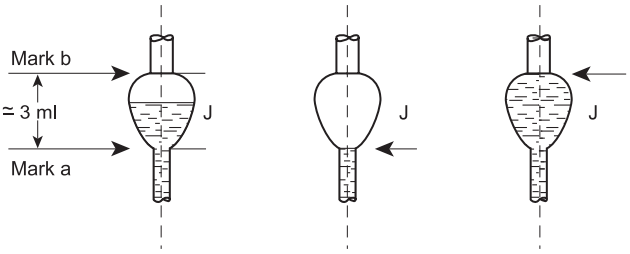


Figure 2.8.12.-2

Introduce into the flask the prescribed quantity of the drug and continue the distillation as described above for the time and at the rate prescribed. Stop the heating and after 10 min read the volume of liquid collected in the graduated tube and subtract the volume of xylene previously noted. The difference represents the quantity of essential oil in the mass of the drug taken. Calculate the result as millilitres per kilogram of drug.

When the essential oil is to be used for other analytical purposes, the water-free mixture of xylene and essential oil may be recovered as follows: remove the stopper *K′* and introduce 0.1 ml of a 1 g/l solution of *sodium fluoresceinate R* and 0.5 ml of *water R*. Lower the mixture of xylene and essential oil into the bulb-shaped swelling *L* by means of the three-way tap, allow to stand for 5 min and lower the mixture slowly until it just reaches the level of the tap *M*. Open the tap anti-clockwise so that the water flows out of the connecting tube *BM*. Wash the tube with *acetone R* and with a little *toluene R* introduced through the filling funnel *N*. Turn the tap anti-clockwise in order to recover the mixture of xylene and essential oil in an appropriate flask.

**01/2008:20813**

# 2.8.13. PESTICIDE RESIDUES

**Definition**. For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of herbal drugs. The item includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

**Limits**. Unless otherwise indicated in the monograph, the drug to be examined at least complies with the limits indicated in Table 2.8.13.-1. The limits applying to pesticides that are not listed in the table and whose presence is suspected for any reason comply with the limits set by European Community directives 76/895 and 90/642, including their annexes and successive updates. Limits for pesticides that are not listed in Table 2.8.13.-1 nor in EC directives are calculated using the following expression:

$$\frac{ADI \times M}{MDD \times 100}$$

*ADI* = acceptable daily intake, as published by FAO-WHO, in milligrams per kilogram of body mass,

*M* = body mass in kilograms (60 kg),

*MDD* = daily dose of the drug, in kilograms.

If the drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms whose preparation method modifies the content of pesticides in the finished product, the limits are calculated using the following expression:

$$\frac{ADI \times M \times E}{MDD \times 100}$$

*E* = extraction factor of the method of preparation, determined experimentally.

Higher limits can also be authorised, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely.

**Sampling**

*Method*. For containers up to 1 kg, take one sample from the total content, thoroughly mixed, sufficient for the tests. For containers between 1 kg and 5 kg, take three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests.

*Size of sampling*. If the number (*n*) of containers is three or fewer, take samples from each container as indicated above under Method. If the number of containers is more than three, take $\sqrt{n} + 1$ samples from containers as indicated under Method, rounding up to the nearest unit if necessary.

The samples are to be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples are stored in airtight containers suitable for food contact, at a temperature below 0 °C, protected from light.

**Reagents**. All reagents and solvents are free from any contaminants, especially pesticides, that might interfere with the analysis. It is often necessary to use special quality solvents or, if this is not possible, solvents that have recently been re-distilled in an apparatus made entirely of glass. In any case, suitable blank tests must be carried out.

**Apparatus**. Clean the apparatus and especially glassware to ensure that they are free from pesticides, for example, soak for at least 16 h in a solution of phosphate-free detergent, rinse with large quantities of *distilled water R* and wash with acetone and hexane or heptane.

**Qualitative and quantitative analysis of pesticide residues**. The analytical procedures used are validated according to the regulations in force. In particular, they satisfy the following criteria:

- the chosen method, especially the purification steps, are suitable for the combination pesticide residue/substance to be analysed, and not susceptible to interference from co-extractives; the limits of detection and quantification are measured for each pesticide-matrix combination to be analysed,
- between 70 per cent to 110 per cent of each pesticide is recovered,
- the repeatability of the method is not less than the values indicated in Table 2.8.13.-2,