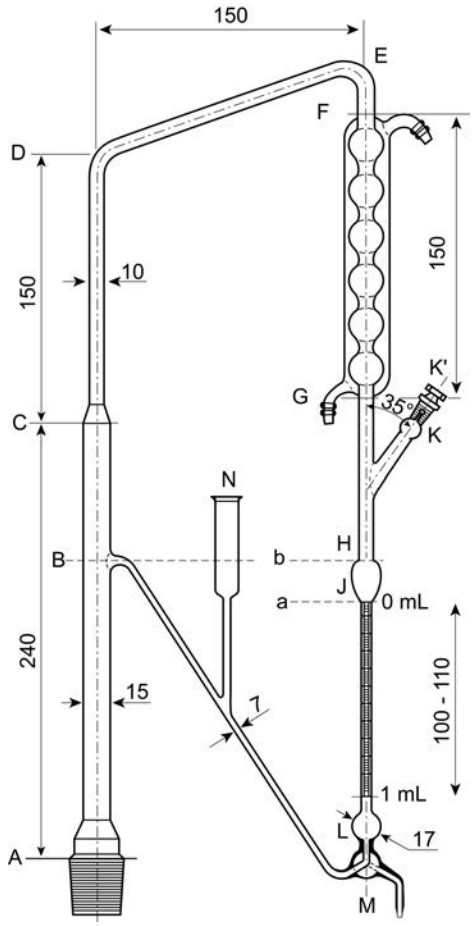

Figure 2.8.12.-1. – *Apparatus for the determination of essential oils in herbal drugs*

Dimensions in millimetres

PROCEDURE

Use a thoroughly cleaned apparatus. Carry out the determination according to the nature of the herbal 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 reaches level *B*. Remove the stopper (*K'*) and introduce the prescribed quantity of the solvent indicated in the monograph using a pipette with its tip placed at the bottom of tube *K*. Re-insert the stopper (*K'*) and ensure that the vent is unobstructed. Heat the liquid in the flask to boiling and adjust the distillation rate to 2-3 mL/min, unless otherwise prescribed.

Determination of the rate of distillation. During distillation, determine the rate of distillation by lowering the level of the water by means of tap *M* until the meniscus reaches the level of the lower mark (*a*) (see Figure 2.8.12.-2). Close tap *M* and measure the time taken for the liquid to reach the upper mark (*b*). Modify the heat to obtain the target distillation rate. If the distillation rate is still not within the prescribed range, repeat the operation. If the distillation conditions are not changed, it is sufficient to determine the distillation rate at regular intervals rather than before each test.

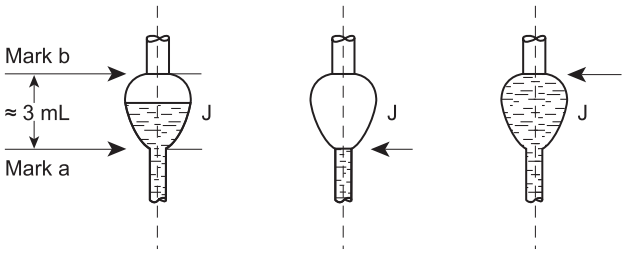

Figure 2.8.12.-2

Determination of the solvent volume after blank distillation. If using *xylene R* or *trimethylpentane R*, distil for 30 min. Ensure that tubes *BM* and *JM* are connected via tap *M* during distillation. Stop heating and wait at least 10 min before reading the volume of solvent in the graduated tube.

If using *1,2,4-trimethylbenzene R*, the 30 min blank distillation step is not necessary. Stop heating after adjusting the distillation rate and wait at least 10 min before reading the volume of solvent in the graduated tube.

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

Recovery of the mixture of solvent and essential oil. When the essential oil is to be used for other analytical purposes, the water-free mixture of solvent 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*. Run the mixture of solvent and essential oil into the bulb-shaped swelling (*L*) by opening tap *M*, allow to stand for 5 min and lower the level of the mixture slowly until it just reaches the level of tap *M*. Open tap *M* clockwise so that the water flows out of the connecting tube (*BM*). Wash the tube with *acetone R* introduced through the filling funnel (*N*). Turn tap *M* anti-clockwise in order to recover the mixture of solvent and essential oil in an appropriate flask.

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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. Pesticide residues can be present and are controlled in herbal drugs and herbal drug preparations.

Limits. Unless otherwise indicated in the monograph, the herbal 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 Table 2.8.13.-1 and whose presence is suspected for any reason comply with the limits (levels) cross-referred to by Regulation (EC) No. 396/2005, including

annexes and successive updates. Limits for pesticides that are not listed in Table 2.8.13.-1 or in European Union texts are calculated using the following expression:

$$\frac{ADI \times M}{MDD_{HD} \times 100}$$

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

M = body mass in kilograms (60 kg);

MDD_{HD} = daily dose of the herbal drug, in kilograms.

The limits for pesticides in herbal drug preparations are calculated using the following expressions:

If *DER* ≤ 10: $MRL_{HD} \times DER$

If *DER* > 10: $\frac{ADI \times M}{MDD_{HP} \times 100}$

MRL_{HD} = maximum residue limit of the pesticide in the herbal drug as given in Table 2.8.13.-1 or in EU texts or calculated using the expression mentioned above;

DER = drug/extract ratio, i.e. the ratio between the quantity of herbal drug used in the manufacture of a herbal drug preparation and the quantity of herbal drug preparation obtained;

MDD_{HP} = daily dose of the herbal drug preparation, in kilograms.

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 according to good agricultural and collection practice (GACP).

Table 2.8.13.-1

Substance	Limit (mg/kg)
Acephate	0.1
Alachlor	0.05
Aldrin and dieldrin (sum of)	0.05
Azinphos-ethyl	0.1
Azinphos-methyl	1
Bromophos-ethyl	0.05
Bromophos-methyl	0.05
Brompropylate	3
Chlordane (sum of *cis*-, *trans* - and oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyriphos-ethyl	0.2
Chlorpyriphos-methyl	0.1
Chlorthal-dimethyl	0.01
Cyfluthrin (sum of)	0.1
λ-Cyhalothrin	1
Cypermethrin and isomers (sum of)	1
DDT (sum of *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDT, *o,p′*-TDE and *p,p′*-TDE)	1
Deltamethrin	0.5
Diazinon	0.5
Dichlofluanid	0.1
Dichlorvos	1
Dicofol	0.5
Dimethoate and omethoate (sum of)	0.1
Dithiocarbamates (expressed as CS2)	2
Endosulfan (sum of isomers and endosulfan sulfate)	3
Endrin	0.05
Ethion	2
Etrimphos	0.05
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)	0.1
Fenitrothion	0.5
Fenpropathrin	0.03
Fensulfothion (sum of fensulfothion, fensulfothion-oxon, fensulfothion-oxonsulfon and fensulfothion-sulfon)	0.05
Fenthion (sum of fenthion, fenthion-oxon, fenthion-oxon-sulfon, fenthion-oxon-sulfoxid, fenthion-sulfon and fenthion-sulfoxid)	0.05
Fenvalerate	1.5
Flucytrinate	0.05
τ-Fluvalinate	0.05
Fonophos	0.05
Heptachlor (sum of heptachlor, *cis*-heptachlorepoxide and *trans*-heptachlorepoxide)	0.05
Hexachlorbenzene	0.1
Hexachlorocyclohexane (sum of isomers α-, β-, δ- and ε)	0.3
Lindan (γ-hexachlorocyclohexane)	0.6
Malathion and malaoxon (sum of)	1
Mecarbam	0.05
Methacriphos	0.05
Methamidophos	0.05
Methidathion	0.2
Methoxychlor	0.05
Mirex	0.01
Monocrotophos	0.1
Parathion-ethyl and paraoxon-ethyl (sum of)	0.5
Parathion-methyl and paraoxon-methyl (sum of)	0.2
Pendimethalin	0.5
Pentachloranisol	0.01
Permethrin and isomers (sum of)	1
Phosalone	0.1
Phosmet	0.05
Piperonyl butoxide	3
Pirimiphos-ethyl	0.05
Pirimiphos-methyl (sum of pirimiphos-methyl and *N*-desethyl-pirimiphos-methyl)	4
Procymidone	0.1
Profenophos	0.1
Prothiophos	0.05

Substance	Limit (mg/kg)
Pyrethrum (sum of cinerin I, cinerin II, jasmolin I, jasmolin II, pyrethrin I and pyrethrin II)	3
Quinalphos	0.05
Quintozene (sum of quintozene, pentachloraniline and methyl penthachlorphenyl sulfide)	1
S-421	0.02
Tecnazene	0.05
Tetradifon	0.3
Vinclozolin	0.4

Sampling of herbal drugs. Sampling is done according to general chapter *2.8.20. Herbal drugs: sampling and sample preparation*.

Qualitative and quantitative analysis of pesticide residues. The analytical procedures used are validated (e.g. according to Document No. SANCO/10232/2006 or any subsequent revisions of this document). In particular, they satisfy the following criteria:

- the chosen method, especially the purification steps, is suitable for the combination pesticide residue/substance to be examined, and not susceptible to interference from co-extractives;
- natural occurrence of some constituents is considered in the interpretation of results (e.g. disulfide from crucifers);
- the concentration of test and reference solutions and the setting of the apparatus are such that the responses used for quantification of the pesticide residues are within the dynamic range of the detector; test solutions containing pesticide residues at a level outside the dynamic range, may be diluted within the calibration range, provided that the concentration of the matrix in the solution is adjusted in the case where the calibration solutions must be matrix-matched;
- between 70 per cent to 110 per cent of each pesticide is recovered;
- repeatability of the method: RSD is not greater than the values indicated in Table 2.8.13.-2;
- reproducibility of the method: RSD is not greater than the values indicated in Table 2.8.13.-2.

Table 2.8.13.-2

Concentration range of the pesticide (mg/kg)	Repeatability (RSD) (per cent)	Reproducibility (RSD) (per cent)
0.001 - 0.01	30	60
> 0.01 - 0.1	20	40
> 0.1 - 1	15	30
> 1	10	20

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2.8.14. TANNINS IN HERBAL DRUGS

Carry out all the extraction and dilution operations protected from light.

In the case of a herbal drug or a dry extract, to the stated amount of the powdered drug (180) (*2.9.12*) or the extract in a 250 mL round-bottomed flask add 150 mL of *water R*. Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 mL volumetric flask. Rinse the round-bottomed flask and collect the washings in the volumetric flask, then dilute to 250.0 mL with *water R*. Allow the solids to settle and filter the liquid through a filter paper 125 mm in diameter. Discard the first 50 mL of the filtrate.

In the case of a liquid extract or a tincture, dilute the stated amount of the liquid extract or tincture to 250.0 mL with *water R*. Filter the mixture through a filter paper 125 mm in diameter. Discard the first 50 mL of the filtrate.

Total polyphenols. Dilute 5.0 mL of the filtrate to 25.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_1), using *water R* as the compensation liquid.

Polyphenols not adsorbed by hide powder. To 10.0 mL of the filtrate, add 0.10 g of *hide powder CRS* and shake vigorously for 60 min. Filter and dilute 5.0 mL of the filtrate to 25.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_2), using *water R* as the compensation liquid.

Standard. Dissolve immediately before use 50.0 mg of *pyrogallol R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 5.0 mL of the solution to 100.0 mL with *water R*. Mix 2.0 mL of this solution with 1.0 mL of *phosphomolybdotungstic reagent R* and 10.0 mL of *water R* and dilute to 25.0 mL with a 290 g/L solution of *sodium carbonate R*. After 30 min measure the absorbance (*2.2.25*) at 760 nm (A_3), using *water R* as the compensation liquid.

Calculate the percentage content of tannins expressed as pyrogallol from the expression:

$$\frac{62.5(A_1 - A_2)m_2}{A_3 \times m_1}$$

m_1 = mass of the sample to be examined, in grams;

m_2 = mass of pyrogallol, in grams.

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2.8.15. BITTERNESS VALUE

The bitterness value is the reciprocal of the dilution of a compound, a liquid or an extract that still has a bitter taste. It is determined by comparison with quinine hydrochloride, the bitterness value of which is set at 200 000.

Determination of the correction factor

A taste panel comprising at least 6 persons is recommended. The mouth must be rinsed with *water R* before tasting.

To correct for individual differences in tasting bitterness amongst the panel members it is necessary to determine a correction factor for each panel member.

Stock solution. Dissolve 0.100 g of *quinine hydrochloride R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 1.0 mL of this solution to 100.0 mL with *water R*.

Reference solutions. Prepare a series of dilutions by placing in a first tube 3.6 mL of the stock solution and increasing the volume by 0.2 mL in each subsequent tube to a total of 5.8 mL; dilute the contents of each tube to 10.0 mL with *water R*.

Determine as follows the dilution with the lowest concentration that still has a bitter taste. Take 10.0 mL of the weakest solution into the mouth and pass it from side to side over the back of the tongue for 30 s. If the solution is not found to be bitter, spit it out and wait for 1 min. Rinse the mouth with *water R*. After 10 min, use the next dilution in order of increasing concentration.