1. Introduction

This manual has been designed to be used in conjunction with two earlier World Health Organization (WHO) publications, *Basic tests for pharmaceutical substances*[1] and *Basic tests for pharmaceutical dosage forms*.[2] Most of the pharmaceutical substances and dosage forms covered are included in the WHO Model List of Essential Drugs.[3] The present volume describes procedures for testing a further 23 pharmaceutical substances and 58 pharmaceutical dosage forms and also for testing four medicinal plant materials (sections 3–5).

These basic tests represent one of the many elements of quality assurance in the pharmaceutical supply system. They have been devised with the following objectives:

(a) to provide a simple and readily applicable method for verifying the identity of a substance, using a limited range of easily available reagents, when the labelling and physical attributes give rise to doubt;

(b) to provide a practicable means of confirming the identity of a substance when a fully equipped laboratory is not available;

(c) to indicate whether gross degradation has occurred in certain substances that are known to decompose readily under adverse conditions.

Basic tests are not, in any circumstances, intended to replace the requirements of *The International Pharmacopoeia*[4] or other pharmacopoeial monographs. These give an assurance of quality whereas basic tests merely confirm identity.

In 1994, the WHO Expert Committee on Specifications for Pharmaceutical Preparations[5] agreed that the scope of these tests should be extended to include additional information and references to other simple test methodologies.

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[1] *Basic tests for pharmaceutical substances.* Geneva, World Health Organization, 1986.

[2] *Basic tests for pharmaceutical dosage forms.* Geneva, World Health Organization, 1991.

[3] *The use of essential drugs. Seventh report of the WHO Expert Committee.* Geneva, World Health Organization, 1997 (WHO Technical Report Series, No. 867).

[4] *The International Pharmacopoeia*, 3rd ed. Geneva, World Health Organization. Volume 1: *General methods of analysis*, 1979. Volume 2: *Quality specifications*, 1981. Volume 3: *Quality specifications*, 1988. Volume 4: *Tests, methods, and general requirements. Quality specifications for pharmaceutical substances, excipients, and dosage forms*, 1994.

[5] *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-fourth Report.* Geneva, World Health Organization, 1996 (WHO Technical Report Series, No. 863).

The usefulness of simplified analytical technology and supporting elements, such as thin-layer chromatography (TLC) kits, reference tablets and associated training materials, was fully endorsed by the Committee. They are considered to be valuable tools for primary screening and could play an important part in identifying counterfeit and spurious products. Several collections of simplified tests are therefore reviewed in this manual (see section 2).

Degradation during storage and transportation is of particular importance in tropical countries. Indeed, an expiry date determined for a temperate climate may be inappropriate in a tropical region even when high standards of packaging are met. For this reason, particular importance is accorded to visual inspection of dosage forms, since this frequently provides a first vital indication of degradation. This also applies in cases where there are reasons to suspect quality defects due to poor manufacture, tampering, or counterfeiting. Visual inspection should precede any testing. Inspection procedures are outlined in *Basic tests for pharmaceutical dosage forms*.

Basic tests need not be carried out by fully qualified pharmacists or chemists, but they should be performed by persons with some understanding of analytical chemistry such as that acquired in courses for pharmaceutical assistants.

The facilities needed for carrying out basic tests, the equipment required and methods for the determination of melting characteristics are described in detail in the two earlier manuals of basic tests. Reagents additional to the ones described in those two manuals are listed in section 6.

Several tests are described for most preparations. Not all of these need to be applied to any one sample. If, however, there is any reason to suspect that the product is mislabelled or substandard, all tests described should be performed. By their nature, simplified tests cannot be totally reliable. An adverse result, even in one test, should be taken as a warning of potential unsuitability of a drug. In these circumstances, a final conclusion should not be drawn until a full analytical examination has been carried out in a properly equipped quality control laboratory.

For easy reference, section 7 provides a cumulative index of WHO basic tests.

Comments on the tests described are invited and should be addressed to: Quality Assurance, Division of Drug Management and Policies, World Health Organization, 1211 Geneva 27, Switzerland.

3. Test procedures for pharmaceutical substances

AMIKACIN SULFATE

Identity tests

Description. A white to yellowish white, crystalline powder; almost odourless.

Colour and other reactions

1. Dissolve 10 mg in 1 ml of water, add 1 ml of sodium hydroxide (~80 g/l) TS and mix, then add 2 ml of cobalt(II) nitrate (10 g/l) TS; a violet colour is produced.
2. Dissolve 0.05 g in 3 ml of water and add slowly 4 ml of anthrone TS; a bluish violet colour is produced.
3. Dissolve 20 mg in 1 ml of water and add 1 ml of barium chloride (50 g/l) TS; a white precipitate is produced which is practically insoluble in hydrochloric acid (~250 g/l) TS.

BACITRACIN ZINC

Composition. Bacitracin zinc is a zinc complex of bacitracins, polypeptides produced by the growth of an organism of the licheniformis group of *Bacillus subtilis*. The main components are the bacitracins A, B1 and B2.

Identity tests

Description. A white or pale brownish yellow powder; odourless or with a faint, characteristic odour; hygroscopic.

Colour and other reactions

1. Shake 5 mg with 1 ml of water, add 1 ml of triketohydrindene/butanol TS and 0.5 ml of pyridine R and heat to 100 °C for 5 minutes; a violet colour is produced.
2. Transfer about 0.5 g to a silica crucible and ignite. Dissolve the residue in 5 ml of sulfuric acid (~5 g/l) TS and filter. Divide the filtrate into 2 equal volumes.

(*a*) To 1 volume add 1 ml of potassium ferrocyanide (45 g/l) TS; a white precipitate is produced which is insoluble in hydrochloric acid (~250 g/l) TS.

(*b*) To 1 volume add 1 drop of copper(II) sulfate (1 g/l) TS and 1 ml of ammonium mercurithiocyanate TS; a violet precipitate is produced.

Degradation tests

Discoloration of the test substance and non-compliance with the following test usually indicate gross degradation:

Dissolve 0.10 g in 100 ml of water; a clear, colourless or slightly yellowish solution is produced.

CAPTOPRIL

Identity tests

Description. A white or almost white, crystalline powder; odour, characteristic but faint.

Colour and other reactions

1. Dissolve 10 mg in 2 ml of hydrochloric acid (0.1 mol/l) VS and add about 1 ml of iodine TS; the colour of the iodine disappears immediately and a white, turbid solution is produced.
2. Dissolve 10 mg in 2 ml of water and add 0.5 ml of lead acetate (80 g/l) TS; a white precipitate is produced.
3. Dissolve 10 mg in 5 ml of ethanol (~750 g/l) TS, add 0.5 ml of tetramethylammonium hydroxide/ethanol TS and shake. Then add 0.5 ml of triphenyltetrazolium chloride/ethanol TS and shake again; a red colour is produced.

CHLORAMPHENICOL SODIUM SUCCINATE

Identity tests

Description. A white or almost white powder; hygroscopic.

Colour and other reactions

Dissolve about 1.4 g in 5 ml of water and use as the test solution for the following tests:

1. To 1 drop of the test solution add 5 ml of ethanol (~750 g/l) TS, 0.2 g of zinc R powder and 1 ml of sulfuric acid (~100 g/l) TS and allow to stand for 10 minutes. Filter; to the filtrate add 0.5 ml of sodium nitrite (10 g/l)

TS and allow to stand for 2 minutes. Then add about 1 g of urea R and a solution containing 10 mg of 2-naphthol R in 2 ml of sodium hydroxide (~80 g/l) TS; a red colour is produced.

2. Repeat test 1 omitting the zinc R powder; no red colour is produced.
3. Carefully heat 1 drop of the test solution with 10 mg of resorcinol R and 3 drops of sulfuric acid (~1760 g/l) TS, cool and add 2 ml of water. Cool again and pour the solution into a mixture of 100 ml of water and about 1 ml of sodium hydroxide (~400 g/l) TS; a yellow-green fluorescence appears which disappears on the addition of 1 ml of hydrochloric acid (~250 g/l) TS.
4. Introduce the test solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.

Degradation tests

Discoloration of the test substance and non-compliance with the following test usually indicate gross degradation:

Dissolve 0.2 g in 10 ml of water; a clear solution is produced.

CISPLATIN

Identity tests

Description. White to yellowish crystals or a yellow powder.

***Note.* This substance is very toxic and should be handled with care.**

Colour and other reactions

1. Dissolve 5 mg in 5 ml of hydrochloric acid (~420 g/l) TS and heat to boiling. To half of the solution (keep the unused portion for test 2) add a few crystals of potassium iodide R; a brownish yellow colour is produced which changes to reddish brown on standing.
2. To the remaining solution from test 1 add a few crystals of tin(II) chloride R; a reddish orange colour is produced which changes to reddish brown on standing.
3. Transfer 0.05 g to a glass dish and add 2 ml of sodium hydroxide (~80 g/l) TS. Evaporate to dryness and dissolve the residue in a mixture of 0.5 ml of nitric acid (~1000 g/l) TS and 1.5 ml of hydrochloric acid (~420 g/l) TS. Again evaporate to dryness; an orange-coloured residue is produced. Dissolve the residue in 0.5 ml of water and add 0.5 ml of ammonium chloride (100 g/l) TS; a yellow, crystalline precipitate is produced.

COAL TAR

Composition. Coal tar is a by-product usually obtained during the destructive distillation of coal. It is a complex and undefined mixture of a great number of chemical compounds. The product is available in various compositions.

Identity tests

Description. Brown-black or black, viscous liquid; odour, characteristic and strong, resembling naphthalene. On exposure to air the liquid solidifies.

Heating behaviour. When ignited, the liquid burns in air with a luminous sooty flame and almost no residue remains.

Colour and other reactions

1. Shake 1 drop vigorously with 5 ml of ethanol (~750 g/l) TS and filter; the filtrate is yellow with a bluish green fluorescence.
2. Shake about 1 g vigorously with 9 ml of water for 10 minutes and filter; the filtrate gives a neutral or only slightly acid reaction when tested with litmus paper R (unlike wood tar) and an odour of naphthalene is discernible (keep the filtrate for test 3).
3. To 5 ml of the filtrate from test 2 add a few drops of bromine TS; a yellow turbidity develops (phenols).

Degradation test

If the substance does not pass the following test, this usually indicates that gross degradation has occurred:

Dissolve 0.10 g in 10 ml of nitrobenzene R; a clear or almost clear solution is produced.

DOXORUBICIN HYDROCHLORIDE

Identity tests

Description. A red-orange, crystalline powder; hygroscopic.

Note. **This substance is very toxic and should be handled with care.**

Colour and other reactions

1. Place a small quantity of the test substance on a white test plate and add 1 drop of formaldehyde/sulfuric acid TS; the orange-red colour of the substance changes to violet.

2. Dissolve about 2 mg in 2 ml of methanol R and add 2 ml of water and 1 drop of sodium hydroxide (~80 g/l) TS; the orange-red colour of the solution changes to blue-violet.
3. Dissolve 0.05 g in 1 ml of water, add 5 drops of ammonia (~100 g/l) TS and filter. Acidify the filtrate with nitric acid (~130 g/l) TS and add 1 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced which is soluble in ammonia (~100 g/l) TS but practically insoluble in nitric acid (~1000 g/l) TS.

FLUPHENAZINE DECANOATE

Identity tests

Description. A pale yellow, viscous liquid or a yellow, crystalline solid with an oily appearance; odour, faint and ester-like.

Colour and other reactions

1. Dissolve 5 mg in 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a reddish brown colour is produced.
2. Dissolve 5 mg in about 2 ml of formaldehyde/sulfuric acid TS; an orange colour is produced. Heat in a water-bath for 2 minutes; the colour changes to dark brown.
3. Dissolve 5 mg in 2 ml of water and add 3 drops of potassium dichromate (100 g/l) TS; a yellow precipitate is produced.
4. Dissolve 5 mg in about 1 ml of sucrose/hydrochloric acid TS and allow to stand for 5 minutes; a red colour is produced.

Degradation tests

Discoloration and a change in the physical state of the test substance usually indicate gross degradation.

GALLAMINE TRIETHIODIDE

Identity tests

Description. A white or almost white powder; odourless; hygroscopic.

Colour and other reactions

1. Dissolve 0.05 g in 5 ml of water and add 1 ml of potassio-mercuric iodide TS; a yellow precipitate is produced.
2. Dissolve 0.05 g in 5 ml of water and add 1 ml of sulfuric acid (~100 g/l) TS and 1 ml of potassium nitrite (100 g/l) TS; a brownish coloration is produced.

3. Dissolve 0.05 g in 5 ml of water and add 1 drop of nitric acid (~1000 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; a yellow precipitate is produced which is insoluble in ammonia (~100 g/l) TS and nitric acid (~1000 g/l) TS.

HYDROCORTISONE SODIUM SUCCINATE

Identity tests

Description. A white or almost white, crystalline powder or amorphous solid; odourless; hygroscopic.

Colour and other reactions

1. Dissolve about 2 mg in 1 ml of alkaline potassio-mercuric iodide TS; a dark precipitate is produced.
2. Dissolve a small quantity in about 2 ml of sulfuric acid (~1760 g/l) TS; a yellow solution with a greenish fluorescence is produced. Very cautiously pour the solution into 10 ml of water; the colour of the solution changes to brownish yellow but the fluorescence remains.
3. Dissolve a small quantity in about 1 ml of phosphoric acid (~1440 g/l) TS and heat cautiously; a yellow solution is produced with a pale greenish fluorescence.
4. Dissolve about 2 mg in 1 ml of water and introduce the solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.
5. Heat carefully 10 mg with 1 drop of water, 10 mg of resorcinol R and 3 drops of sulfuric acid (~1760 g/l) TS, cool and add 2 ml of water. Cool again and pour the solution into a mixture of 100 ml of water and 1 ml of sodium hydroxide (~400 g/l) TS; a yellowish green fluorescence appears.

Degradation tests

Discoloration of the substance and non-compliance with the following test usually indicate gross degradation:

Dissolve 0.20 g in 1.0 ml of water; a clear and colourless solution is produced.

KETAMINE HYDROCHLORIDE

Identity tests

Description. A white, crystalline powder; odour, characteristic.

Melting behaviour. About 260 °C.

Colour and other reactions

1. Dissolve about 0.2 g in 4 ml of water and chill the solution in ice. Add potassium carbonate (100 g/l) TS, drop by drop, until the solution is slightly alkaline when tested with pH-indicator paper R and allow to stand to crystallize. Filter and dry the crystals in a vacuum over phosphorus pentoxide R; melting temperature, about 92 °C.
2. Dissolve 10 mg in 4 ml of water and add 0.5 ml of nitric acid (~130 g/l) TS and 0.5 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.
3. Dissolve 10 mg in 4 ml of sulfuric acid (~5 g/l) TS and add 1 drop of potassium iodobismuthate/acetic acid TS; a reddish brown precipitate is produced.

LEVAMISOLE

Identity tests

Description. A white, crystalline powder.

Melting point. About 59 °C.

Colour reaction

Dissolve 0.05 g in 20 ml of water. Add 1 ml of sodium hydroxide (~80 g/l) TS, boil for 10 minutes and cool. Add a few drops of sodium nitroprusside (45 g/l) TS; a red colour is produced which fades with time.

LEVAMISOLE HYDROCHLORIDE

Identity tests

Description. A white to pale cream-coloured, crystalline powder; odourless or almost odourless.

Melting point. About 228 °C.

Colour and other reactions

1. Dissolve 0.25 g in 20 ml of water and add 1.5 ml of sodium hydroxide (~80 g/l) TS. Extract with 20 ml of dichloromethane R, discard the aqueous layer and wash the dichloromethane layer with 10 ml of water. Discard the aqueous layer, shake the dichloromethane layer with about 1 g of anhydrous sodium sulfate R and filter. Evaporate the filtrate at room temperature and dry in a vacuum at a temperature not exceeding 40 °C; the residue melts at about 59 °C.
2. Dissolve 0.10 g in 40 ml of water. To 20 ml of this solution (keep the unused portion for test 3) add 1 ml of sodium hydroxide (~80 g/l) TS, boil for 10 minutes and cool. Add a few drops of sodium nitroprusside (45 g/l) TS; a red colour is produced which fades with time.
3. To 20 ml of the solution prepared for test 2 add 1 ml of nitric acid (~130 g/l) TS and a few drops of silver nitrate (40 g/l) TS; a white precipitate is produced. Separate the precipitate, wash with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

MAGNESIUM SULFATE

Identity tests

Description. Brilliant, colourless crystals or a white, crystalline powder; odourless; effloresces in warm and dry air.

Colour and other reactions

1. Dissolve 10 mg in 2 ml of water and add 1 ml of ammonia (~100 g/l) TS; a white precipitate is produced. Add 1 ml of ammonium chloride (100 g/l) TS; the precipitate dissolves. Add 1 ml of disodium hydrogen phosphate (100 g/l) TS; a white precipitate is produced.
2. Dissolve 10 mg in 2 ml of water and add 3 drops of titan yellow TS and 2 ml of sodium hydroxide (~80 g/l) TS; a distinct pink colour is produced.
3. Dissolve 0.05 g in 5 ml of water. Add 1 ml of hydrochloric acid (~70 g/l) TS and 1 ml of barium chloride (50 g/l) TS; a white precipitate is produced.

MEDROXYPROGESTERONE ACETATE

Identity tests

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Melting point. About 204 °C.

Colour and other reactions

1. Dissolve 5 mg in 5 ml of sulfuric acid (~1760 g/l) TS. Incline the tube and carefully add, without mixing, 5 ml of ethanol (~750 g/l) TS; a blue-violet colour is produced at the interface of the two liquids.

2. Heat 0.05 g with 2 ml of potassium hydroxide/ethanol TS in a water-bath for 5 minutes. Cool and add 1 ml of water and about 1 ml of sulfuric acid (~1760 g/l) TS. Boil gently for 1 minute; ethyl acetate, perceptible by its odour (proceed with caution), is produced.

3. Dissolve 5 mg in 0.5 ml of methanol R in a small test-tube, add about 3 mg of sodium nitroprusside R, 0.05 g of sodium carbonate R and 0.5 g of ammonium acetate R and shake. Allow to stand for 10–30 minutes; a violet-red colour is produced (distinction from progesterone).

4. Dissolve a few crystals in about 1 ml of sulfuric acid (~1760 g/l) TS. Incline the tube and carefully add, without mixing, 1 ml of water; a green colour is produced at the interface of the two liquids. Allow to stand; the colour changes to bluish violet (distinction from hydroxyprogesterone caproate).

METHIONINE

Identity tests

Description. White crystals or a white, crystalline powder; odour, characteristic.

Colour and other reactions

1. Dissolve about 0.1 g in 5 ml of hydrochloric acid (0.1 mol/l) VS, add 0.2 ml of triketohydrindene/ethanol TS and heat; a violet colour is produced.

2. Dissolve 10 mg in 1 ml of water and add 1–2 drops of hydrochloric acid (~250 g/l) TS, 0.5 ml of copper(II) sulfate (1 g/l) TS and 1–2 ml of sodium hydroxide (~150 g/l) TS; a blue-violet colour is produced.

3. Dissolve about 0.1 g in 5 ml of potassium hydroxide (~110 g/l) TS and add about 0.3 ml of sodium nitroprusside (45 g/l) TS with shaking. Heat the solution in a water-bath at a temperature of 35–40°C for 10 minutes. Cool in ice for 2 minutes, add about 2 ml of hydrochloric acid (~250 g/l) TS and shake well; a red colour is produced.

METHYLROSANILINIUM CHLORIDE

Identity tests

Description. A dark green powder or greenish, glistening pieces with a metallic lustre; odourless or almost odourless.

Colour and other reactions

1. Add a few crystals to about 1 ml of sulfuric acid (~1760 g/l) TS and shake; an orange or brown-red coloured solution is produced. Cautiously dilute with water; the colour changes to brown, then to green and finally to blue.
2. Dissolve 20 mg in 10 ml of water and add 5 drops of hydrochloric acid (~420 g/l) TS. To 5 ml of this solution (keep the unused portion for test 3) add, drop by drop, tannic acid (100 g/l) TS; a blue precipitate is produced.
3. To the remaining solution from test 2 add 0.5 g of zinc R powder and warm the mixture; the solution discolours rapidly. On a filter-paper, place 1 drop of this solution adjacent to 1 drop of ammonia (~100 g/l) TS; a blue colour is produced at the zone of contact.

PENTAMIDINE ISETIONATE

Identity tests

Description. A white or almost white, crystalline powder; odourless; hygroscopic.

Melting point. About 190 °C.

Colour and other reactions

1. To about 1 g add 10 ml of water and heat to 80 °C to dissolve. Add 15 ml of sodium hydroxide (~80 g/l) TS, cool in ice and filter. To 2 ml of the filtrate (keep the unused portion for tests 2 and 3) add about 0.2 ml of nitric acid (~1000 g/l) TS and about 0.2 ml of ceric ammonium nitrate TS; a red-orange colour is produced.
2. Neutralize 5 ml of the remaining filtrate from test 1 with hydrochloric acid (~70 g/l) TS, testing with pH-indicator paper R, then add 3 ml of the same acid and a few drops of barium chloride (50 g/l) TS; no precipitate is produced.
3. Transfer a further 10 ml of the remaining filtrate from test 1 to a crucible, add 2.5 ml of hydrogen peroxide (~60 g/l) TS, mix and evaporate to dryness over a water-bath. Dissolve the residue in 1 ml of water, add about 1 ml of glacial acetic acid R, evaporate again and ignite until free

from carbon. After cooling, add 5 ml of water and filter. If necessary, neutralize the filtrate with hydrochloric acid (~70 g/l) TS, testing with pH-indicator paper R, add 3 ml of the same acid, heat to boiling for 30 seconds, cool and add a few drops of barium chloride (50 g/l) TS; a white precipitate is produced which is practically insoluble in hydrochloric acid (~250 g/l) TS.

PENTAMIDINE MESILATE

Identity tests

Description. A white or light pink, granular powder; almost odourless.

Melting behaviour. About 265 °C.

Colour and other reactions

1. To about 1 g add 10 ml of water and heat to 80 °C to dissolve. Add 15 ml of sodium hydroxide (~80 g/l) TS, cool in ice and filter. To 2 ml of the filtrate (keep the unused portion for tests 2 and 3) add about 0.2 ml of nitric acid (~1000 g/l) TS and about 0.2 ml of ceric ammonium nitrate TS; a yellow colour is produced.
2. Neutralize 5 ml of the remaining filtrate from test 1 with hydrochloric acid (~70 g/l) TS, testing with pH-indicator paper R, and add 3 ml of the same acid and a few drops of barium chloride (50 g/l) TS; no precipitate is produced.
3. Transfer a further 10 ml of the remaining filtrate from test 1 to a crucible, add 2.5 ml of hydrogen peroxide (~60 g/l) TS, mix and evaporate to dryness over a water-bath. Dissolve the residue in 1 ml of water, add about 1 ml of glacial acetic acid R, evaporate again and ignite until free from carbon. After cooling, add 5 ml of water and filter. If necessary, neutralize the filtrate with hydrochloric acid (~70 g/l) TS, testing with pH-indicator paper R, add 3 ml of the same acid, heat to boiling for 30 seconds, cool and add a few drops of barium chloride (~50 g/l) TS; a white precipitate is produced which is practically insoluble in hydrochloric acid (~250 g/l) TS.

PREDNISOLONE SODIUM PHOSPHATE

Identity tests

Description. A white or almost white powder; odourless; hygroscopic.

Colour and other reactions

1. Dissolve about 2 mg in about 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a wine-red solution is produced. Dilute the

solution very cautiously with 10 ml of water; the colour fades and a greyish brown, flocculent precipitate is produced.

2. Dissolve 5 mg in about 1 ml of phosphoric acid (~1440 g/l) TS and heat cautiously; the solution changes from colourless to yellow, then to orange and later to reddish brown.

3. Dissolve 10 mg in 1 ml of methanol R, add 1 ml of potassio-cupric tartrate TS and heat in a water-bath; an orange precipitate is gradually produced.

4. Carefully heat 0.04 g with about 2 ml of sulfuric acid (~1760 g/l) TS until white fumes are evolved. Add, drop by drop, nitric acid (~1000 g/l) TS until oxidation is complete. Allow to cool, add 10 ml of water and heat again until white fumes are evolved. Cool, add 10 ml of water and neutralize with ammonia (~100 g/l) TS, using pH-indicator paper R. Introduce an aliquot of this solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour. To the remaining solution add 5 ml of ammonium molybdate (95 g/l) TS, acidify with nitric acid (~130 g/l) TS and heat; a bright yellow precipitate is produced.

PYRIMETHAMINE

Identity tests

Description. A white, crystalline powder; odourless.

Melting point. About 240 °C.

Colour and other reactions

1. Dissolve 0.05 g in 5 ml of sulfuric acid (~100 g/l) TS and add about 0.2 ml of freshly prepared potassio-mercuric iodide TS; a creamy white precipitate is produced.

2. To 1 ml of methyl orange/ethanol TS add 5 ml of water and 2 ml of ethyl acetate R and shake; the ethyl acetate layer remains colourless. Add a solution of 5 mg of the test substance dissolved in 5 ml of sulfuric acid (~5 g/l) TS, shake and allow to separate (about 30 minutes); a yellow colour is produced in the ethyl acetate layer.

3. Ignite about 0.1 g with 0.5 g of anhydrous sodium carbonate R, extract the residue with water and filter. Neutralize the filtrate with nitric acid (~130 g/l) TS and add 0.5 ml of silver nitrate (~40 g/l) TS; a white precipitate is produced. Add ammonia (~100 g/l) TS; the precipitate dissolves.

TAMOXIFEN CITRATE

Identity tests

Description. A white or almost white, crystalline powder.

Melting behaviour. About 142 °C with decomposition.

Colour and other reactions

1. Shake 10 mg with 5 ml of dehydrated ethanol R. Add 3 ml of water, 0.5 ml of ammonia (~100 g/l) TS and 2 ml of phosphomolybdic acid/ethanol TS; within a few minutes the colour of the solution changes to light blue.
2. To 10 mg add 4 ml of pyridine R and about 2 ml of acetic anhydride R and shake; a yellow colour is immediately produced. Heat in a water-bath for 2 minutes; a light pink to red colour is produced.

VINBLASTINE SULFATE

Identity tests

Description. A white to slightly yellow, amorphous or crystalline powder; hygroscopic.

Note. **This substance is very toxic and should be handled with care.**

Colour and other reactions

1. To about 0.5 mg add 2 drops of a 10 mg/ml solution of ceric ammonium sulfate R in phosphoric acid (~1440 g/l) TS; a purplish red colour is produced which darkens with time.
2. To about 1 mg add 0.2 ml of a freshly prepared 10 mg/ml solution of vanillin R in hydrochloric acid (~420 g/l) TS; after about 1 minute a pink colour is produced (distinction from vincristine sulfate).
3. Mix about 0.5 mg with 5 mg of 4-dimethylaminobenzaldehyde R, about 0.2 ml of glacial acetic acid R and about 0.2 ml of sulfuric acid (~1760 g/l) TS; a reddish brown colour is produced which changes to pink after addition of about 1 ml of glacial acetic acid R.
4. Dissolve 10 mg in 2 ml of water. Add 1 ml of hydrochloric acid (~70 g/l) TS and 1 ml of barium chloride (50 g/l) TS; a white precipitate is produced which is practically insoluble in hydrochloric acid (~250 g/l) TS.

4. Test procedures for medicinal plant materials

IPECACUANHA ROOT

Composition. Ipecacuanha root is the dried rhizome and roots of the shrub *Cephaëlis ipecacuanha* (Brotero) A. Richard (family Rubiaceae) or of *C. acuminata* Karsten, or of a mixture of both species. The principal alkaloids are emetine and cephaëline.

Identity tests

Description. Odour, slight; taste, bitter, nauseous and acrid.

Macroscopic characteristics

C. ipecacuanha. Dark brick-red to very dark brown. A somewhat tortuous root, seldom more than 15 cm in length or 6 mm in diameter; the root is closely annulated externally, with rounded ridges that completely encircle it; the fracture is short in the bark and splintery in the wood; a transversely cut surface shows a wide greyish bark and a small uniformly dense wood. The rhizomes are short lengths attached to the roots; they are cylindrical, up to 2 mm in diameter, finely wrinkled longitudinally, and with pith occupying approximately one-sixth of the whole diameter.

C. acuminata. In general, resembles the root of *C. ipecacuanha*, but differs in the following particulars: often up to 9 mm in diameter; external surface greyish brown or reddish brown with transverse ridges at intervals of about 1–3 mm; the ridges are about 0.5–1 mm wide, extending about half-way round the circumference and fading at the extremities into the general surface level.

Colour and other reactions

1. Coarsely powder the root, mix 0.05 g with about 2 ml of hydrochloric acid (~420 g/l) TS and 1 drop of hydrogen peroxide (~330 g/l) TS and warm the mixture; an orange colour is produced (rubremetine).
2. Coarsely powder the root, mix about 0.2 g with 2 drops of ammonia (~260 g/l) TS and 2 ml of dichloromethane R, shake and filter. Evaporate to dryness about 1 ml of the filtrate (keep the unused portion for test 3), dissolve the residue in about 0.2 ml of water and add 3 drops of potassium iodobismuthate/acetic acid TS; an orange precipitate is produced.

3. To the remaining filtrate from test 2 add 0.5 ml of ethanol (~750 g/l) TS and transfer to a small test-tube, 100 × 10 mm. Dip vertically into the tube a strip of filter-paper, 100 × 6 mm, and allow the solution to ascend 70 mm. Dry the paper strip in air and expose it to iodine vapours for 30 seconds. Observe under ultraviolet light at 365 nm; a blue fluorescence appears.

Degradation test

Discoloration of the test material usually indicates gross degradation.

PODOPHYLLUM RESIN

Composition. Podophyllum resin is a mixture of resins obtained from the rhizomes and roots of the herbaceous plant *Podophyllum hexandrum* Royle (*P. emodi* Wall.) or *P. peltatum* L. after percolation with ethanol and precipitation from water or very dilute acids.

Identity tests

Description. Light brown to greenish yellow or brownish grey masses or an amorphous powder. Darkens when exposed to light or stored at temperatures above 25 °C.

Note. **This material is very toxic and should be handled with care.**

Colour and other reactions

1. Finely powder the resin, dissolve about 0.2 g in 10 ml of potassium hydroxide (~55 g/l) TS; a clear, yellow solution is formed which darkens on standing. Acidify with hydrochloric acid (~70 g/l) TS; the resin precipitates.

2. Finely powder the resin, add 0.4 g to 2 ml of ethanol (~750 g/l) TS, then add 0.5 ml of potassium hydroxide (~55 g/l) TS, shake gently and allow to stand; the resin of *P. hexandrum* produces a stiff jelly whereas that of *P. peltatum* does not gelatinize.

3. Dissolve 10 mg in 2 ml of ethanol (~750 g/l) TS and add 1 drop of ferric chloride (25 g/l) TS; a deep, dark green colour is produced and the solution appears black in reflected light.

4. Dissolve 10 mg in 1 ml of ethanol (~750 g/l) TS, add 4 ml of water and about 1 ml of sulfuric acid (~1760 g/l) TS and cool; the resin of *P. hexandrum* forms an orange to brownish red solution whereas that of *P. peltatum* forms a yellowish green solution.

SENNA FRUIT

Composition. Alexandrian or Khartoum senna fruit is the dried ripe fruit of *Cassia senna* L. (*C. acutifolia* Delile) and Tinnevelly senna fruit is the dried ripe fruit of *C. angustifolia* Vahl.

Identity tests

Description. Odour, slight; taste, first mucilaginous and sweet, then slightly bitter.

Macroscopic characteristics

Leaflike, flat and thin pods, yellowish green to yellowish brown with a dark brown central area, oblong or reniform.

Alexandrian senna fruit. Pale to greyish green; length, about 40–50 mm; width, 20–25 mm; stylar point at one end; containing 6–7 obovate green to pale brown seeds, with prominent longitudinal ridges on the testa.

Tinnevelly senna fruit. Brown to greyish black; length, about 35–60 mm; width, 14–18 mm; stylar point at one end; containing up to 10 obovate green to pale brown seeds, with indefinite transverse ridges on the testa.

Colour and other reactions

Before carrying out any tests, crush the fruit to a fine powder.

1. Mix about 0.2 g of the powdered fruit with 5 ml of hydrochloric acid (~250 g/l) TS and warm for 2 minutes. Cool and filter, shake the filtrate with 5 ml of toluene R and evaporate 1 ml of the yellowish coloured toluene extract to dryness. Dissolve the residue in 0.5 ml of ammonia (~100 g/l) TS and warm the solution; a pink to red-violet colour is produced.
2. Sprinkle 10 mg of the powdered fruit on the surface of about 1 ml of sulfuric acid (~1760 g/l) TS without stirring; within 5 minutes a greenish to brownish colour appears (other colours such as red indicate the presence of other species, e.g. *C. auriculata* L., *C. goratensis* Fres.).

Degradation test

Discoloration of the test material usually indicates gross degradation.

SENNA LEAF

Composition. Senna leaf consists of the dried leaflets of *Cassia senna* L., known as Alexandrian or Khartoum senna (*C. acutifolia* Delile), and Tinnevelly senna (*C. angustifolia* Vahl), or a mixture of both species.

Identity tests

Description. Odour, slight; taste, first mucilaginous and sweet, then slightly bitter.

Macroscopic characteristics

Alexandrian senna leaf. Pale greyish green, thin, fragile leaflets; lanceolate, mucronate; length, 20–40 mm; width, 5–15 mm, the maximum width being at a point slightly below the centre; lamina, slightly undulant; both surfaces covered with fine, short trichomes; pinnate venation, slightly prominent midrib with lateral veins leaving the midrib at an angle of about 60° and anastomosing to form a ridge parallel to the margin.

Tinnevelly senna leaf. Yellowish green leaflets; elongated and lanceolate; length, 25–50 mm; width at the centre, 7–20 mm; lamina, flat; both surfaces are smooth, with a very small number of trichomes, and marked with impressed transverse or oblique lines.

Colour and other reactions

Before carrying out any tests, powder the leaves to a particle size that allows them to pass through a sieve no. 45 (nominal aperture size, 0.045 mm).

1. To 0.5 g of powdered leaves add 10 ml of ethanol (~375 g/l) TS, warm in a water-bath for 5 minutes and filter while hot. To the filtrate add about 1 ml of hydrochloric acid (~420 g/l) TS, heat in a water-bath for 10 minutes and cool. Mix with 5 ml of ethyl acetate R, shake and allow to stand. Separate the ethyl acetate layer, add 2 ml of sodium hydrogen carbonate (40 g/l) TS and shake; a reddish yellow colour is produced in the aqueous layer. Remove the ethyl acetate layer, add 1 drop of hydrogen peroxide (~330 g/l) TS and heat in a water-bath; the colour of the solution changes to red.
2. Heat 0.10 g of powdered leaves with 10 ml of water in a water-bath for 30 minutes and filter. To the filtrate add 1 drop of hydrochloric acid (~420 g/l) TS, shake with 2 quantities, each of 5 ml, of dichloromethane R and discard the dichloromethane layer. Adjust the pH of the aqueous layer to 7–8, adding sodium carbonate (50 g/l) TS and testing with pH-indicator paper R. Add 10 ml of a solution composed of 4 ml of ferric chloride (25 g/l) TS and 6 ml of water, mix and heat in a water-bath for 20 minutes. Add about 1 ml of hydrochloric acid (~420 g/l) TS and continue to heat for a further 20 minutes, shaking the flask frequently. Filter, extract the filtrate with 10 ml of dichloromethane R, evaporate the dichloromethane extract to dryness over a water-bath and dissolve the residue in 2 ml of potassium hydroxide (~55 g/l) TS; a red-orange colour is produced.
3. Sprinkle 10 mg of the powdered leaves on the surface of about 1 ml of sulfuric acid (~1760 g/l) TS without stirring; within 5 minutes a greenish

to brownish colour appears (other colours such as red indicate the presence of other species, e.g. *C. auriculata* L., *C. goratensis* Fres.).

Degradation test

Discoloration of the test material usually indicates gross degradation.

5. Test procedures for pharmaceutical dosage forms

ALLOPURINOL TABLETS

Description. Each tablet usually contains 100 mg of allopurinol.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.30 g of allopurinol.
2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material, triturate it with 10 ml of sodium hydroxide (0.1 mol/l) VS and filter. Acidify the filtrate with acetic acid (~60 g/l) TS, filter, wash the precipitate with 3 ml of dehydrated ethanol R and allow to dry in air for 5 minutes. Then dry at 105 °C for 3 hours and use the dried material as the test substance, dividing it into 6 equal parts.

Identity tests

Colour and other reactions

1. Dissolve 1 part of the test substance in 5 ml of sodium hydroxide (~80 g/l) TS, add 1 ml of alkaline potassio-mercuric iodide TS, heat to boiling and allow to stand; a yellow, flocculent precipitate is produced.
2. Dissolve 4 parts of the test substance in a mixture of 2 ml of sodium hydroxide (~80 g/l) TS and 2 ml of water. Add 3 ml of citric acid (90 g/l) TS and shake vigorously; a white precipitate is produced.
3. Dissolve 1 part of the test substance in 25 ml of water by warming, cool and filter. To 5 ml of the filtrate (keep the unused portion for test 4) add 1 ml of ammonia (~100 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; a white precipitate is produced.
4. To 5 ml of the filtrate from test 3 add 0.5 ml of copper(II) sulfate (160 g/l) TS; a blue precipitate is produced.

AMIKACIN SULFATE INJECTION

Description. The injection is a sterile solution usually containing 250 mg of amikacin sulfate in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the contents of the ampoules equivalent to 1.0 g of amikacin sulfate and use directly as test solution 1, dividing it into 2 equal volumes.
2. Dilute 1 volume of test solution 1 to 25 ml with water and use it as test solution 2.

Identity tests

Colour and other reactions

1. To 3 ml of test solution 2 add 1 ml of sodium hydroxide (~80 g/l) TS, mix and add 2 ml of cobalt(II) nitrate (10 g/l) TS; a violet colour is produced.
2. To 1 volume of test solution 1 add slowly 2 ml of anthrone TS; a bluish violet colour is produced.
3. To 2 ml of test solution 2 add a few drops of barium chloride (50 g/l) TS; a white precipitate is produced which is practically insoluble in hydrochloric acid (~250 g/l) TS.

Degradation tests

Discoloration and a change in the physical state of test solution 1 usually indicate gross degradation.

AZATHIOPRINE SODIUM POWDER FOR INJECTION

Description. Each vial contains a sterile powder of azathioprine sodium usually equivalent to 50–100 mg of azathioprine.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amount equivalent to 0.05 g of azathioprine.
2. Empty the vials, weigh out the above-calculated equivalent amount and use directly as the test substance, dividing it into 3 equal parts.
3. Dissolve 1 part of the test substance in 100 ml of water and use it as the test solution.

Identity tests

Colour and other reactions

1. To 5 ml of the test solution add 1 ml of hydrochloric acid (~70 g/l) TS and 10 mg of zinc R powder and allow to stand for 5 minutes; the colour of the solution changes to yellow. Filter, cool in ice and add 3–4 drops of sodium nitrite (10 g/l) TS and 5–6 drops of hydrochloric acid (~70 g/l) TS. Shake and allow to stand for 2 minutes. Then add about 0.25 g of

urea R, shake and again allow to stand for 2 minutes. Add 0.5 ml of *N*-(1-naphthyl)ethylenediamine/ethanol TS; a red-violet solution is produced.

2. Transfer 2 parts of the test substance to a test-tube, add 0.05 g of potassium nitrate R and about 0.1 g of potassium hydroxide R and heat carefully until fused. Cool, dissolve the residue in 20 ml of water and filter. To 5 ml of the filtrate add 1.5 ml of hydrochloric acid (~70 g/l) TS and 5–6 drops of barium chloride (50 g/l) TS; a white turbidity appears.
3. To about 1 ml of the test solution add 0.5 ml of phosphotungstic acid (10 g/l) TS and 0.5 ml of hydrochloric acid (~70 g/l) TS; a white precipitate is produced.

BARIUM SULFATE POWDER FOR SUSPENSION

Description. A white or creamy white powder; bulky or granular.

Preparation of the sample. Use the powder directly as the test substance.

Identity tests

Colour and other reactions

1. Suspend 20 mg of the test substance in about 1 ml of hydrochloric acid (~420 g/l) TS and introduce it into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; a pale green colour appears in the flame.
2. To 0.30 g of the test substance add 10 ml of water and about 1 g of anhydrous sodium carbonate R, boil for 5 minutes and filter. Acidify the filtrate with hydrochloric acid (~420 g/l) TS and add 1 ml of barium chloride (50 g/l) TS; a white precipitate is produced.
3. To about 1 g of the test substance add 1 ml of acetic acid (~300 g/l) TS and 25 ml of water and boil. While shaking, cool and filter; if the filtrate is turbid, repeat the filtration until a clear solution is obtained and then add 1 ml of sulfuric acid (~100 g/l) TS; no opalescence is produced within 30 minutes (absence of soluble barium salts).

BECLOMETASONE DIPROPIONATE INHALATION (AEROSOL)

Description. The inhalation, supplied in a pressurized canister, contains a fine suspension of beclometasone dipropionate in a suitable propellant, usually equivalent to 50 µg per spray dose.

Preparation of the sample

1. Place 25 ml of ethanol (~750 g/l) TS in a small beaker and expel under the surface of the solvent 60 spray doses equivalent to about 3 mg of beclometasone dipropionate. Use this solution as the test solution.
2. Evaporate 10 ml of the test solution and use the residue as test substance 1.
3. Evaporate 15 ml of the test solution and use the residue as test substance 2.

Identity tests

Colour and other reactions

1. Dissolve test substance 1 in about 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a dark reddish brown solution is produced. Very cautiously pour the solution into 10 ml of water; a very light bluish grey precipitate is produced.
2. Dissolve test substance 2 in 2.0 ml of ethanol (~750 g/l) TS and add 1.0 ml of tetramethylammonium hydroxide/ethanol TS and 1.0 ml of tri-phenyltetrazolium chloride/ethanol TS, shaking thoroughly after each addition. Allow to stand in the dark for 20 minutes; a red colour is produced.

BENZATHINE BENZYLPENICILLIN POWDER FOR INJECTION

Description. Each vial contains a sterile powder of benzathine benzylpenicillin, usually equivalent to 1.44 g (2.4 million IU) of benzylpenicillin.

Preparation of the sample. Empty the vials and use directly as the test substance.

Identity tests

Colour and other reactions

1. To 5 mg of the test substance add 3 ml of water, 0.1 g of hydroxylamine hydrochloride R and 1 ml of sodium hydroxide (~80 g/l) TS, mix and allow to stand for 5 minutes. Then add 2 ml of hydrochloric acid (~70 g/l) TS and 0.5 ml of ferric chloride (25 g/l) TS; a violet-brown colour is produced.
2. To 2 mg of the test substance add carefully 1 drop of water and 2 ml of sulfuric acid (~1760 g/l) TS and mix; the solution is almost colourless. Heat in a water-bath for 1 minute; the solution remains almost colourless or changes to slightly yellow.
3. To 2 mg of the test substance add 1 drop of water and 2 ml of sulfuric acid (~1760 g/l) TS. After cooling, add 2 drops of formaldehyde TS; the

solution remains almost colourless. Heat in a water-bath for 1 minute; a reddish brown colour is produced.

4. Dissolve 10 mg of the test substance in 1 ml of sodium hydroxide (~80 g/l) TS and add 3 ml of water and 1 ml of potassium permanganate (10 g/l) TS; a green colour is produced. Heat the solution; an odour of benzaldehyde is perceptible.

CALCIUM GLUCONATE INJECTION

Description. The injection is a sterile solution usually containing 100 mg of calcium gluconate in 1.0 ml of a suitable vehicle.

Preparation of the sample. Dilute the contents of 1 ampoule to obtain a concentration of 10 mg of calcium gluconate in 1 ml of water and use as the test solution.

Identity tests

Colour and other reactions

1. Evaporate 1 ml of the test solution to dryness over a water-bath, add 5 mg of 2-naphthol R dissolved in about 1 ml of sulfuric acid (~1760 g/l) TS and heat in a water-bath for 1 minute; a dark blue-green colour is produced.

2. To 2 ml of silver nitrate (40 g/l) TS add ammonia (~100 g/l) TS, drop by drop, until the initially formed brown precipitate just dissolves. Add 1 ml of the test solution and heat to boiling for 1–2 minutes; a silver mirror is produced.

3. To 2 ml of the test solution add 5 drops of ammonium oxalate (25 g/l) TS; a white precipitate is produced. To a portion of the precipitate add a few drops of hydrochloric acid (~70 g/l) TS; the precipitate dissolves. To the remaining precipitate add a few drops of acetic acid (~300 g/l) TS; the precipitate is practically insoluble.

Degradation test

Discoloration of the test solution usually indicates gross degradation.

CETRIMIDE CREAM

Description. The cream usually contains 5 mg of cetrimide in 1.0 g of a suitable cream base.

Preparation of the sample

1. Withdraw and weigh an amount equivalent to 10 mg of cetrimide and use directly as the test substance.
2. Withdraw and weigh an amount equivalent to 20 mg of cetrimide, transfer to a test-tube, add 10 ml of sodium chloride (100 g/l) TS and shake thoroughly. While warming in a water-bath, keep shaking to effect separation of the emulsion, remove the test-tube from the bath, cool, filter the aqueous phase and use the filtrate as the test solution.

Identity tests

Colour and other reactions

1. Transfer the test substance to a stoppered test-tube and shake with 10 ml of water; a large amount of foam is produced (keep the solution for test 2).
2. To 5 ml of the solution from test 1 add 5 ml of nitric acid (~130 g/l) TS, shake, filter and add 5 ml of silver nitrate (40 g/l) TS; a faint opalescence is produced. Allow to stand in the dark for 30 minutes; a faint yellow-grey precipitate is produced.
3. To 5 ml of water add 1 ml of sulfamic acid (100 g/l) TS, 1 drop of methyl orange/ethanol TS and 2 ml of dichloromethane R and shake; the dichloromethane layer remains colourless. Add 3 ml of the test solution (keep the unused portion for test 4) to the tube, shake and allow the layers to separate; a yellow colour is produced in the dichloromethane layer.
4. Place the remaining test solution from test 3 over a water-bath, reduce the volume to about 2 ml and add 2 ml of potassium ferricyanide (50 g/l) TS; a yellow precipitate is produced.

CHLORPROMAZINE HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 25 mg of chlorpromazine hydrochloride in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.05 g of chlorpromazine hydrochloride, evaporate to dryness over a water-bath and use the residue as the test substance.

Identity tests

Colour and other reactions

1. Dissolve 10 mg of the test substance in 1.5 ml of water and add 1 ml of tosylchloramide sodium (40 g/l) TS; a cloudy, blue-violet solution is produced. Shake; a dark resinous deposit separates on the walls of the

test-tube. Add 4 ml of ethyl acetate R and shake; a red-violet colour is produced in the ethyl acetate layer.

2. Dissolve about 10 mg of the test substance in 3 ml of water and add 1 ml of ethyl acetate R, 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of chloramine B (50 g/l) TS; a red colour is produced in the aqueous layer and the ethyl acetate layer remains colourless (distinction from promethazine).

3. Dissolve the remaining test substance in 5 ml of water, add 5 drops of ammonia (~100 g/l) TS, warm until oily drops separate and filter. To the filtrate add 1 ml of nitric acid (~130 g/l) TS and a few drops of silver nitrate (40 g/l) TS; a white precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

CIMETIDINE INJECTION

Description. The injection is a sterile solution usually containing 200 mg of cimetidine in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.4 g and use directly as the test solution, dividing it into 2 equal volumes.

Identity tests

Colour and other reactions

1. Place 1 volume of the test solution in a test-tube and evaporate to dryness over a water-bath. Ignite the residue; the vapours produced darken moistened lead nitrate paper R.

2. Dilute 1 volume of the test solution with 10 ml of water. To 1 ml (keep the unused portion for test 3) add 1 drop of ammonia (~100 g/l) TS and 1 drop of copper(II) sulfate (160 g/l) TS and heat in a water-bath; a greyish green precipitate is produced which is soluble in an excess of ammonia (~100 g/l) TS.

3. To 1 ml of the diluted solution from test 2 add a few drops of potassium iodobismuthate/acetic acid TS; an orange precipitate is produced.

CLOXACILLIN SODIUM POWDER FOR INJECTION

Description. Each vial contains a sterile powder of cloxacillin sodium, usually equivalent to 500 mg of cloxacillin.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amount equivalent to 30 mg of cloxacillin sodium.
2. Empty the vial, weigh out the above-calculated equivalent amount and use directly as the test substance, dividing it into 6 equal parts.

Identity tests

Colour and other reactions

1. Dissolve 1 part of the test substance in 3 ml of water and filter. To the filtrate add 0.10 g of hydroxylamine hydrochloride R and 1 ml of sodium hydroxide (~80 g/l) TS and allow to stand for 5 minutes. Then add 1.3 ml of hydrochloric acid (~70 g/l) TS and 0.5 ml of ferric chloride (25 g/l) TS; a dark violet-red colour is produced.
2. Dissolve 2 parts of the test substance in 2 ml of water and add 2 ml of a solution composed of 2 ml of potassio-cupric tartrate TS and 6 ml of water; a light blue solution is immediately produced.
3. Dissolve 1 part of the test substance in 1 ml of water, shake and filter. To the filtrate add 1 drop of ferric chloride (25 g/l) TS; a greenish yellow precipitate is produced.
4. To 10 mg of paraformaldehyde R dissolved in about 1 ml of sulfuric acid (~1760 g/l) TS add a small quantity of the test substance; a light yellow colour is produced. Heat the mixture in a water-bath for 2 minutes and cool; the colour of the solution changes to brownish.
5. Dissolve 1 part of the test substance in 5 ml of water and filter. Acidify with about 0.5 ml of glacial acetic acid R and filter using a coarse-porosity filter-paper. To 2 ml of the filtrate add 1 ml of magnesium uranyl acetate TS and scratch the inside of the tube with a glass rod to induce crystallization; a yellow, crystalline precipitate is produced.

DEXTRAN 70 INJECTION

Description. The injection is a sterile solution usually containing 6 g of dextran 70 in 100 ml of a suitable vehicle (normally dextrose or sodium chloride solution).

Preparation of the sample. Pool a volume equivalent to 0.12 g of dextran 70 and use directly as the test solution, dividing it into 4 equal volumes.

Identity tests

Colour and other reactions

For dextran 70 in dextrose solution:

To 1 volume of the test solution add 5 ml of potassio-cupric tartrate TS and boil; a brick-red precipitate is formed. Filter and to the filtrate add about 1.5 ml of hydrochloric acid (~420 g/l) TS. Boil for 5 minutes, cool and neutralize by adding sodium carbonate R until the effervescence ceases. Add 2 ml of potassio-cupric tartrate TS and boil again; a brick-red precipitate is again produced.

For dextran 70 in sodium chloride solution:

1. To 1 volume of the test solution add 5 ml of potassio-cupric tartrate TS and boil; the solution remains greenish and no precipitate is formed. Dilute a further 0.5 ml of the test solution to 5 ml with water and add about 0.5 ml of hydrochloric acid (~420 g/l) TS. Boil for 5 minutes, cool and neutralize by adding sodium carbonate R until the effervescence ceases. Add 2 ml of potassio-cupric tartrate TS and boil again; a brick-red precipitate is produced.

2. Introduce the test solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.

3. Acidify 1 volume of the test solution with a small volume of nitric acid (~130 g/l) TS and add 2 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add a few drops of ammonia (~100 g/l) TS; the precipitate dissolves.

DIPHENHYDRAMINE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 25–50 mg of diphenhydramine hydrochloride.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.20 g of diphenhydramine hydrochloride.

2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material and use directly as the test substance, dividing it into 4 equal parts.

Identity tests

Colour and other reactions

1. Place a small quantity of the test substance on a white test plate and add 1 drop of a mixture of about 0.5 ml of nitric acid (~1000 g/l) TS and about 0.5 ml of sulfuric acid (~1760 g/l) TS; a momentary violet colour is produced which changes to red and finally to yellow.

2. To 2 parts of the test substance add 5 ml of water, shake and filter. To the filtrate add 3 ml of hydrochloric acid (~250 g/l) TS and boil for 3 minutes.

Cool the test-tube in ice and scratch the inside of the tube with a glass rod to induce crystallization. Separate the crystals and dry over silica gel, desiccant, R; melting point, about 64 °C. If the melting point is lower than 64 °C, recrystallize from 1–2 ml of water.

3. To 1 part of the test substance add 5 ml of water, shake and filter. To the filtrate add 0.5 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

DOXORUBICIN HYDROCHLORIDE POWDER FOR INJECTION

Description. Each vial contains a sterile powder usually equivalent to 10–50 mg of doxorubicin hydrochloride.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amount equivalent to 0.07 g of doxorubicin hydrochloride.
2. Empty the vials, weigh out the above-calculated equivalent amount and use directly as the test substance.

Identity tests

Colour and other reactions

1. Place a small quantity of the test substance on a white test plate and add 1 drop of formaldehyde/sulfuric acid TS; the orange-red colour of the substance changes to violet.
2. Dissolve about 2 mg of the test substance in 2 ml of methanol R and add 2 ml of water and 1 drop of sodium hydroxide (~80 g/l) TS; the orange-red colour of the solution changes to blue-violet.
3. Dissolve 0.05 g of the test substance in 1 ml of water, add 5 drops of ammonia (~100 g/l) TS and filter. Acidify the filtrate with nitric acid (~130 g/l) TS and add 1 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced which is soluble in ammonia (~100 g/l) TS but practically insoluble in nitric acid (~1000 g/l) TS.

DOXYCYCLINE HYCLATE INJECTION

Description. The injection is a sterile solution usually containing 20 mg of doxycycline hyclate[1] in 1.0 ml of a suitable vehicle.

[1] Referred to as doxycycline hydrochloride in *Basic tests for pharmaceutical substances*, 1986.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.16 g of doxycycline hyclate and use directly as the test solution, dividing it into 4 equal volumes.

Identity tests

Colour and other reactions

1. To 1 volume of the test solution add about 2 ml of sulfuric acid (~1760 g/l) TS; an intense yellow colour is produced.
2. Place 2 ml of zinc chloride (500 g/l) TS in a porcelain dish and warm on a hotplate or over a small flame until a skin forms on the surface of the solution. Then add 2 drops of the test solution and continue to warm for 1 minute; a yellow-orange colour is produced.
3. To 1 volume of the test solution add 1 drop of ferric chloride (25 g/l) TS; a dark red-brown colour is produced.
4. To 1 volume of test solution add 0.5 ml of nitric acid (~130 g/l) TS and 0.5 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

Degradation test

Discoloration of the test solution usually indicates gross degradation.

DOXYCYCLINE HYCLATE TABLETS

Description. Each tablet usually contains 100 mg of doxycycline hyclate.[1]

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 0.10 g and 25 mg of doxycycline hyclate.
2. Grind the tablets, weigh out the above-calculated equivalent amounts as powdered material and use directly, 0.10 g as test substance 1 and 25 mg as test substance 2.
3. Shake test substance 1 with 10 ml of water and filter. Use the filtrate as the test solution.

Identity tests

Colour and other reactions

1. To test substance 2 add about 2 ml of sulfuric acid (~1760 g/l) TS; an intense yellow colour is produced.

[1] Referred to as doxycycline hydrochloride in *Basic tests for pharmaceutical substances*, 1986.

2. Place 2 ml of zinc chloride (500 g/l) TS in a porcelain dish and warm on a hotplate or over a small flame until a skin forms on the surface of the solution. Then add 2 drops of the test solution and continue to warm for 1 minute; a yellow-orange colour is produced.
3. To 2 ml of the test solution add 1 drop of ferric chloride (25 g/l) TS; a dark red-brown colour is produced.
4. To 1 ml of the test solution add 0.5 ml of nitric acid (~130 g/l) TS and 0.5 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

EPINEPHRINE HYDROGEN TARTRATE INJECTION

Description. The injection is a sterile solution of epinephrine hydrogen tartrate, usually containing the equivalent of 1.0 mg of epinephrine in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 10 mg of epinephrine hydrogen tartrate; if necessary, reduce the volume by evaporation to 10 ml or dilute to 10 ml with water. Use directly as the test solution.

Identity tests

Colour and other reactions

1. To 5 ml of the test solution add 1–2 drops of ferric chloride (25 g/l) TS; a green solution is produced. Add 1 drop of ammonia (~260 g/l) TS; the colour changes to dark red.
2. To 2 ml of the test solution add 1 ml of sulfuric acid (~5 g/l) TS and 3 ml of ammonium molybdate (95 g/l) TS and mix; an orange colour is produced. Then add slowly, while mixing, 2 ml of sodium hydroxide (~80 g/l) TS; a yellow-green solution is produced.
3. To 2 ml of the test solution add 2 ml of potassium bromide (100 g/l) TS, 2 ml of resorcinol (20 g/l) TS and 3 ml of sulfuric acid (~1760 g/l) TS. Heat in a water-bath for 5–10 minutes; a dark blue colour is produced which changes to red when the solution is cooled and poured into water.

FERROUS FUMARATE TABLETS

Description. Each tablet contains ferrous fumarate, usually equivalent to 60 mg of iron.

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 0.5 g and 0.05 g of ferrous fumarate.

2. Grind the tablets, weigh out the above-calculated equivalent amounts as powdered material and use directly, 0.5 g as test substance 1 and 0.05 g as test substance 2.

Identity tests

Colour and other reactions

1. To test substance 1 add 20 ml of sulfuric acid (~100 g/l) TS and heat in a water-bath for 15 minutes. Filter while hot and cool the filtrate in ice; a white precipitate is produced (keep the filtrate for test 2). Wash the precipitate with acidified water and dry at 105 °C. Dissolve the precipitate in 5 ml of sodium carbonate (50 g/l) TS and add 0.5 ml of potassium permanganate (10 g/l) TS; the colour of the permanganate fades and a brownish solution is produced.

2. To 2 ml of the filtrate obtained in test 1 add 2 ml of potassium ferricyanide (50 g/l) TS; an intense dark blue precipitate is produced which is insoluble in hydrochloric acid (~70 g/l) TS.

3. Mix test substance 2 with 0.10 g of resorcinol R, add 5–10 drops of sulfuric acid (~1760 g/l) TS and heat gently; a deep red, semi-solid mass is produced. Cool, add 25 ml of water, swirl to dissolve and filter. To 1 ml of the filtrate add 10 ml of water and mix; an orange-yellow solution is produced which exhibits a green fluorescence. Make the solution alkaline by adding a few drops of sodium hydroxide (~80 g/l) TS; a red solution is produced which exhibits a green fluorescence.

FLUCYTOSINE TABLETS

Description. Each tablet usually contains 500 mg of flucytosine.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.30 g of flucytosine.

2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material, shake with 30 ml of water, filter and use the filtrate as the test solution, dividing it into 6 equal volumes.

3. Evaporate 2 volumes of the test solution to dryness over a water-bath and use the residue as the test substance.

Identity tests

Melting behaviour. The test substance melts at about 295 °C with decomposition.

Colour and other reactions

1. To 1 volume of the test solution add 5 ml of water, 1.5 ml of hydrochloric acid (~250 g/l) TS and 0.5 ml of sodium nitrite (10 g/l) TS and shake for 2 minutes. Add 0.5 ml of 2-naphthol TS; a yellowish brown precipitate is produced.
2. To 1 volume of the test solution add 5 ml of water and 2 ml of silver nitrate (40 g/l) TS, shake and allow to stand for 2–3 minutes; a white precipitate is produced.
3. To 0.5 volume of the test solution add 2 ml of bromine TS; the colour of bromine is discharged. Add 4 ml of freshly prepared barium hydroxide (40 g/l) TS; a purple precipitate is produced.
4. Mix 3 drops of potassium dichromate (100 g/l) TS with about 0.5 ml of sulfuric acid (~1760 g/l) TS and heat in a water-bath for 5 minutes; the solution wets the sides of the tube. Add 10 mg of the test substance, shake well and heat again for 5 minutes in a water-bath; the colour changes to green and the solution no longer wets the sides of the tube.

GENTAMICIN SULFATE INJECTION

Description. The injection is a sterile solution usually containing 10–40 mg of gentamicin sulfate in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.20 g of gentamicin sulfate; if necessary, reduce the volume by evaporation to about 5 ml or dilute to 5 ml with water. Use directly as the test solution.

Identity tests

Colour and other reactions

1. To 1 ml of the test solution add a solution of 5 mg of 1-naphthol R dissolved in 1 drop of ethanol (~750 g/l) TS, 4 drops of water and about 0.5 ml of sulfuric acid (~1760 g/l) TS; a yellow colour is produced.
2. To 1 ml of the test solution add a solution of 20 mg of triketohydrindene hydrate R dissolved in 2 ml of water and 0.10 g of sodium acetate R. Mix and heat in a water-bath for 5 minutes; a dark violet colour is produced.
3. Dilute 1 ml of the test solution with 1 ml of water and add 5 drops of alkaline potassio-mercuric iodide TS; a yellowish white, turbid solution is produced.

4. Dilute 1 ml of the test solution with 2 ml of water and add 5 drops of hydrochloric acid (~70 g/l) TS and 5 drops of barium chloride (50 g/l) TS; a white precipitate is produced.

HYDRALAZINE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 25–50 mg of hydralazine hydrochloride.

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 5 mg and 0.05 g of hydralazine hydrochloride.
2. Grind the tablets, weigh out the above-calculated equivalent amounts as powdered material and use directly, 5 mg as test substance 1 and 0.05 g as test substance 2.
3. Shake test substance 2 with 5 ml of water, filter and use the filtrate as the test solution.

Identity tests

Colour and other reactions

1. Shake test substance 1 with 2 ml of water, add 3 drops of freshly prepared ferrous sulfate (15 g/l) TS and heat to boiling for 3 minutes; a brown-red colour is produced. Add 5–10 drops of iodine TS; the colour changes to dark violet.
2. To about 1 ml of the test solution add 0.5 ml of nitric acid (~130 g/l) TS and 5 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.
3. To 4 ml of the test solution add a mixture of 100 ml of water and 8 ml of hydrochloric acid (~70 g/l) TS, then add 20 ml of sodium nitrite (10 g/l) TS and allow to stand for 10 minutes; a white to ivory-coloured precipitate is produced.

HYDROCORTISONE OINTMENT

Description. The ointment usually contains 10–25 mg of hydrocortisone in 1.0 g of a suitable ointment base.

Preparation of the sample. Withdraw and weigh an amount equivalent to 20 mg of hydrocortisone, add 10 ml of ethanol (~750 g/l) TS and heat in a water-bath for 5 minutes with frequent shaking. Cool to solidify the

ointment base, filter and use the filtrate as the test solution, dividing it into 4 equal volumes.

Identity tests

Colour and other reactions

1. Evaporate 1 volume of the test solution to dryness over a water-bath. Add about 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a yellow solution is produced with a greenish fluorescence. Very cautiously pour the solution into 10 ml of water; the colour of the solution changes to brownish yellow, but the fluorescence remains (certain excipients may interfere with the reaction and the colour of the solution may fade to nearly colourless).
2. Evaporate 1 volume of the test solution to dryness over a water-bath. Add about 1 ml of phosphoric acid (~1440 g/l) TS and heat cautiously; the solution, initially colourless, changes to yellow and shows a slightly greenish fluorescence.
3. Evaporate 2 volumes of the test solution to about 2 ml over a water-bath and add an equal volume of potassio-cupric tartrate TS; an orange-red precipitate is produced.

HYDROCORTISONE ACETATE CREAM

Description. The cream usually contains 10 mg of hydrocortisone acetate in 1.0 g of a suitable cream base.

Preparation of the sample. Withdraw and weigh an amount equivalent to 0.10 g of hydrocortisone acetate, add 30 ml of methanol R, boil and cool in ice for 30 minutes. Filter, evaporate the filtrate to dryness and use the residue as the test substance.

Identity tests

Colour and other reactions

1. Dissolve about 2 mg of the test substance in about 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a yellow solution with a greenish fluorescence is produced. Very cautiously pour the solution into 10 ml of water; the colour of the solution changes to brownish yellow, but the fluorescence remains (certain excipients may interfere with the reaction and the colour of the solution may fade to nearly colourless within a very short period of time).
2. Dissolve about 2 mg of the test substance in about 1 ml of phosphoric acid (~1440 g/l) TS. Heat cautiously; the colourless solution changes to yellow and shows a slightly greenish fluorescence.

3. Dissolve 20 mg of the test substance in 2 ml of methanol R. Add 1 ml of tetramethylammonium hydroxide/ethanol TS and 1 ml of triphenyltetrazolium chloride/ethanol TS, mixing thoroughly after each addition, and allow to stand in the dark for 20 minutes; a red colour is produced.
4. Place 15 mg of the test substance in a test-tube and add 3 drops of phosphoric acid (~1440 g/l) TS. Close the tube with a stopper through which passes a smaller test-tube filled with water to act as a condenser. Allow a drop of lanthanum nitrate (30 g/l) TS to hang on the outside of this smaller tube. Heat the apparatus in a water-bath for 5 minutes. Transfer the drop of lanthanum nitrate to a white test plate, mix it with 1 drop of iodine (0.05 mol/l) VS and add, at the edge of the two liquids, 1 drop of ammonia (~100 g/l) TS; after 1–2 minutes a blue colour is produced at the interface of the two liquids which persists for a short time.

HYDROCORTISONE SODIUM SUCCINATE POWDER FOR INJECTION

Description. Each vial contains a sterile powder usually equivalent to 100 mg of hydrocortisone sodium succinate.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amount equivalent to 0.10 g of hydrocortisone sodium succinate.
2. Empty the vial, weigh out the above-calculated equivalent amount and use directly as the test substance.

Identity tests

Colour and other reactions

1. Dissolve about 2 mg of the test substance in 1 ml of alkaline potassio-mercuric iodide TS; a dark precipitate is produced.
2. Dissolve a small quantity of the test substance in about 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; a yellow solution with a greenish fluorescence is produced. Very cautiously pour the solution into 10 ml of water; the colour of the solution changes to brownish yellow, but the fluorescence remains (certain excipients may interfere with the reaction and the colour may fade to nearly colourless within a very short period of time).
3. Dissolve a small quantity of the test substance in about 1 ml of phosphoric acid (~1440 g/l) TS and heat cautiously; a yellow solution with a pale greenish fluorescence is produced.

4. Dissolve about 2 mg of the test substance in 1 ml of water and introduce the solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.
5. Heat carefully 10 mg of the test substance with 1 drop of water, 10 mg of resorcinol R and 3 drops of sulfuric acid (~1760 g/l) TS, cool and add 2 ml of water. Cool again and pour the solution into a mixture of 100 ml of water and 1 ml of sodium hydroxide (~400 g/l) TS; a yellowish green fluorescence appears.

INDOMETACIN TABLETS

Description. Each tablet usually contains 25 mg of indometacin.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.05 g of indometacin.
2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material and use directly as the test substance, dividing it into 2 equal parts.

Identity tests

Colour and other reactions

1. To 1 part of the test substance add 1 ml of water and 1 drop of sodium hydroxide (~80 g/l) TS, shake and filter. To the filtrate add 1 ml of sodium nitrite (10 g/l) TS, allow to stand for 5 minutes and cautiously add 0.5 ml of hydrochloric acid (~250 g/l) TS; a green colour is produced.
2. Mix 1 part of the test substance with 2 ml of water and 2 ml of sodium hydroxide (~80 g/l) TS; a strong yellow colour is produced which fades rapidly.

IRON DEXTRAN INJECTION

Description. The injection is a sterile solution of iron dextran, usually containing the equivalent of 50 mg of iron in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.15 g of iron; if necessary, reduce the volume to about 3 ml or dilute with water to 3 ml. Use directly as the test solution.

Identity tests

Colour and other reactions

1. Place 1 ml of the test solution on a watch-glass and add 2 drops of ammonia (~260 g/l) TS; no precipitate is observed. Add about 2 ml of hydrochloric acid (~420 g/l) TS, mix and add about 2 ml of ammonia (~260 g/l) TS; a reddish brown precipitate is produced.
2. Acidify 1 ml of the test solution with hydrochloric acid (~70 g/l) TS and add 1 ml of ammonium thiocyanate (75 g/l) TS; a blood-red colour is produced. Extract with 5 ml of amyl alcohol R and add a few drops of mercuric chloride (65 g/l) TS or phosphoric acid (~1440 g/l) TS; the colour is discharged.
3. Dilute about 0.2 ml of the test solution with 20 ml of water. To 10 ml of this solution add 4 drops of hydrochloric acid (~420 g/l) TS and boil for 30 seconds. Cool rapidly and add 4 ml of ammonia (~260 g/l) TS and 10 ml of hydrogen sulfide TS. Boil to remove the excess hydrogen sulfide, cool and filter. Boil 5 ml of the filtrate with 5 ml of potassio-cupric tartrate TS; the colour of the solution remains greenish and no precipitate is produced. Boil a further 5 ml of the filtrate with about 0.5 ml of hydrochloric acid (~420 g/l) TS for 5 minutes, cool, add 2.5 ml of sodium hydroxide (~80 g/l) TS and 5 ml of potassio-cupric tartrate TS and boil again; a reddish precipitate is produced.

KETAMINE HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution of ketamine hydrochloride, usually containing the equivalent of 50 mg of ketamine in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.15 g of ketamine hydrochloride and use directly as the test solution, dividing it into 3 equal volumes.

Identity tests

Colour and other reactions

1. To one-third of 1 volume of the test solution add a few drops of sodium hydroxide (~80 g/l) TS; a white turbidity is produced. Add a few drops of methanol R; the turbidity disappears.
2. To one-third of 1 volume of the test solution add 0.5 ml of trinitrophenol/ethanol TS; a yellow precipitate is produced.
3. Acidify one-third of 1 volume of the test solution with a small volume of nitric acid (~130 g/l) TS and add 2 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it

with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

4. Evaporate 2 volumes of the test solution to dryness. Recrystallize the residue from ethanol (~750 g/l) TS and dry at 105 °C for 2 hours; melting point, about 261 °C.

LEVOTHYROXINE SODIUM TABLETS

Description. Each tablet usually contains 50–100 µg of levothyroxine sodium.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 5 mg of levothyroxine sodium.
2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material and use directly as test substance 1, dividing it into 2 equal parts.
3. Divide 1 part of test substance 1 into 5 equal parts and use as test substance 2.
4. Shake 2 parts of test substance 2 with 3 ml of dehydrated ethanol R for 5 minutes, then allow to stand for 5 minutes. Place a strip of filter-paper into the suspension and allow the liquid to ascend about 4 cm. Take out the strip, cut away the lower dipped portion as well as the part that has not been wetted by the solution, dry the remaining part of the strip in air at room temperature and use as the test paper.

Identity tests

Colour and other reactions

1. To 1 part of test substance 2 add 1.5 ml of ethanol (~750 g/l) TS, 1.5 ml of water and 0.5 ml of sodium hydroxide (~80 g/l) TS, shake well and filter. To the filtrate add about 0.5 ml of hydrochloric acid (~250 g/l) TS and 0.2 ml of sodium nitrite (100 g/l) TS; a yellow colour is produced. Allow to stand for 5 minutes and add 1 drop of ammonia (~260 g/l) TS; the colour of the solution changes to orange-red.
2. Place 1 part of test substance 2 in a silica crucible and ignite. To the residue add 2 ml of water, shake and filter. To the filtrate add 0.5 ml of magnesium uranyl acetate TS and allow to stand for a few minutes; a yellow precipitate is produced.
3. Heat test substance 1 with about 0.2 g of sodium carbonate R. To the fused mass add 3 ml of water, shake and filter. Acidify the filtrate with a few drops of hydrochloric acid (~250 g/l) TS and add 2–3 drops of starch TS; a blue-violet colour is produced.

4. Place 1 drop of triketohydrindene/ethanol TS on the test paper and place in an oven at 105 °C for 5 minutes; a violet spot or ring is produced.

LIDOCAINE HYDROCHLORIDE JELLY

Description. The jelly usually contains 20–40 mg of lidocaine hydrochloride in 1.0 g of a suitable water-soluble, viscous base.

Preparation of the sample

1. Add 10–15 ml of water to a separator, add a quantity of the jelly equivalent to 0.15 g of lidocaine hydrochloride and mix. Add 2.0 ml of ammonia (~100 g/l) TS and extract with 2 portions of 30 ml of dichloromethane R. Evaporate the combined dichloromethane extracts to dryness with the aid of a current of warm air and use the residue as test substance 1.

2. Withdraw and weigh an amount equivalent to 0.20 g of lidocaine hydrochloride and use directly as test substance 2, dividing it into 3 equal parts.

Identity tests

Colour and other reactions

1. To test substance 1 add 1 ml of ethanol (~750 g/l) TS and 0.5 ml of cobalt(II) chloride (30 g/l) TS and shake for 2 minutes; a bluish green precipitate is produced.

2. To 1 part of test substance 2 add 2 ml of water and 3 drops of iodine TS and mix; a brown precipitate is produced.

3. Shake 2 parts of test substance 2 with 5 ml of water and add 1 ml of nitric acid (~130 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; a white precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

MAGNESIUM HYDROXIDE ORAL SUSPENSION

Description. The suspension usually contains the equivalent of 55 mg of magnesium oxide in 1.0 ml of a suitable vehicle.

Preparation of the sample. Take a portion of the suspension equivalent to 0.20 g of magnesium hydroxide and use directly as the test solution, dividing it into 2 equal volumes.

Identity tests

Colour and other reactions

1. To 1 volume of the test solution add 5 ml of hydrochloric acid (~70 g/l) TS and filter. To the filtrate add 5 ml of ammonium chloride (100 g/l) TS, 3 ml of disodium hydrogen phosphate (100 g/l) TS and 5 ml of ammonia (~100 g/l) TS; a white precipitate is produced which is soluble in acetic acid (~300 g/l) TS.
2. To 1 volume of the test solution add 5 ml of hydrochloric acid (~70 g/l) TS and 5 ml of sodium hydroxide (~80 g/l) TS; a white, gelatinous precipitate is produced which is insoluble in an excess of sodium hydroxide (~80 g/l) TS. Add a few drops of iodine TS; the colour of the precipitate changes to dark brown.

MAGNESIUM SULFATE POWDER

Description. Brilliant, colourless crystals or a white, crystalline powder; odourless.

Preparation of the sample. Use the powder directly as the test substance.

Identity tests

Colour and other reactions

1. Dissolve 10 mg of the test substance in 2 ml of water and add 1 ml of ammonia (~100 g/l) TS; a white precipitate is produced. Add 1 ml of ammonium chloride (100 g/l) TS; the precipitate dissolves. Add 1 ml of disodium hydrogen phosphate (100 g/l) TS; a white precipitate is produced.
2. Dissolve 10 mg of the test substance in 2 ml of water and add 3 drops of titan yellow TS and 2 ml of sodium hydroxide (~80 g/l) TS; a distinct pink colour is produced.
3. Dissolve 0.05 g of the test substance in 5 ml of water and add 1 ml of hydrochloric acid (~70 g/l) TS and 1 ml of barium chloride (50 g/l) TS; a white precipitate is produced.

METHYLROSANILINIUM CHLORIDE SOLUTION

Description. The solution usually contains 10 mg of methylrosanilinium chloride (gentian violet) in 1.0 ml of a suitable vehicle.

Preparation of the sample. Take a volume of the solution equivalent to 25 mg of methylrosanilinium chloride and use directly as the test solution.

Identity tests

Colour and other reactions

1. Add 2 drops of the test solution to about 1 ml of sulfuric acid (~1760 g/l) TS and shake; an orange or brown-red colour is produced. Cautiously dilute with water; the colour changes to brown, then to green and finally to blue.
2. Dilute the remaining test solution to 10 ml with water and add 5 drops of hydrochloric acid (~420 g/l) TS. To 5 ml of this solution (keep the unused portion for test 3) add, drop by drop, 0.6 ml of tannic acid (100 g/l) TS; a deep blue precipitate is produced.
3. To the remaining solution from test 2 add about 1 ml of hydrochloric acid (~420 g/l) TS and 0.5 g of zinc R powder; a gas is evolved and the solution fades rapidly. Filter and, on a filter-paper, place 1 drop of the filtrate adjacent to 1 drop of ammonia (~100 g/l) TS; a blue colour is produced at the zone of contact.

METOCLOPRAMIDE HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 5 mg of metoclopramide hydrochloride in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.06 g of metoclopramide hydrochloride and use directly as the test solution, dividing it into 6 equal volumes.

Identity tests

Colour and other reactions

1. To 2 volumes of the test solution add 1 ml of hydrochloric acid (~70 g/l) TS and 2 ml of sodium nitrite (10 g/l) TS; a yellow colour is produced.
2. To 2 volumes of the test solution add 1 drop of sulfuric acid (~1760 g/l) TS, 1 drop of potassium bromide (100 g/l) TS and 2 drops of potassium bromate (15 g/l) TS; a green colour is produced.
3. Mix 2 drops of potassium bromide (100 g/l) TS with 4 drops of potassium bromate (15 g/l) TS and dilute to about 1 ml with water. Add 2 drops of sulfuric acid (~1760 g/l) TS and 1 volume of the test solution; a yellowish precipitate is produced.
4. To 1 volume of the test solution add 1 ml of nitric acid (~130 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

METOCLOPRAMIDE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 10 mg of metoclopramide hydrochloride.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.35 g of metoclopramide hydrochloride.
2. Grind the tablets and weigh out the above-calculated equivalent amount as powdered material, shake it with 10 ml of water and filter. Use the filtrate as the test solution, dividing it into 3 equal volumes.

Identity tests

Colour and other reactions

1. To 1 volume of the test solution add 1 ml of hydrochloric acid (~70 g/l) TS and 2 ml of sodium nitrite (10 g/l) TS; a yellow colour is produced.
2. Mix 2 drops of potassium bromide (100 g/l) TS with 4 drops of potassium bromate (15 g/l) TS and dilute to 1 ml with water. Add 2 drops of sulfuric acid (~1760 g/l) TS and 1 volume of the test solution; a yellowish precipitate is produced.
3. To 1 volume of the test solution add 1 ml of nitric acid (~130 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash it with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

METRONIDAZOLE SUPPOSITORIES

Description. Each suppository usually contains 500 mg–1 g of metronidazole.

Preparation of the sample. Dissolve a quantity of the suppository equivalent to 0.5 g of metronidazole in 20 ml of hot water. Allow to stand overnight to crystallize. Filter to separate the crystals, dry them at 105 °C for about 3 hours and use as the test substance.

Identity tests

Description of the test substance. A white or pale yellow, crystalline powder.

Melting point of the test substance. About 161 °C.

Colour and other reactions

1. To 20 mg of the test substance add 0.05 g of 4-dimethylaminobenzaldehyde R dissolved in 2 ml of hydrochloric acid (~70 g/l) TS; the solution is almost colourless or has a yellow tint. Add 0.05 g of zinc R powder; a deep red colour is produced.
2. Boil 20 mg of the test substance with 5 ml of sodium hydroxide (~80 g/l) TS; the solution shows the following colours in turn: pink, pink-violet, red-violet, red, red-brown, yellow-brown and yellow.

MICONAZOLE NITRATE CREAM

Description. The cream usually contains 20 mg of miconazole nitrate in 1.0 g of a suitable cream base.

Preparation of the sample

1. Withdraw and weigh an amount equivalent to 0.10 g of miconazole nitrate and use directly as the test substance, dividing it into 2 equal parts.
2. Dissolve 1 part of the test substance in sufficient methanol R to produce 50 ml, filter, and use as the test solution.

Identity tests

Colour and other reactions

1. To 1 part of the test substance add 2 ml of sodium hydroxide (~80 g/l) TS and 20 g of zinc R powder. Boil gently; ammonia is produced which shows an alkaline range on moistened pH-indicator paper R.
2. Place 5 ml of the test solution in a porcelain crucible, add 0.1 g of anhydrous sodium carbonate R, evaporate to dryness over a water-bath and ignite. Cool, dissolve the residue in 3 ml of nitric acid (~130 g/l) TS and add a few drops of silver nitrate (40 g/l) TS; a white precipitate is produced. Separate the precipitate, wash with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.
3. Cool 3 ml of the test solution in ice and add 0.4 ml of potassium chloride (100 g/l) TS, 0.1 ml of diphenylamine/sulfuric acid TS and 10 ml of sulfuric acid (~1760 g/l) TS; an intense blue colour is produced.

NICLOSAMIDE TABLETS

Description. Each tablet usually contains 500 mg of niclosamide.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.25 g of niclosamide.
2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material, shake it with 20 ml of acetone R and filter. Evaporate the filtrate to dryness over a water-bath and use the residue as the test substance.

Identity tests

Melting point of the test substance. About 228 °C.

Colour and other reactions

1. To 0.05 g of the test substance add 5 ml of hydrochloric acid (~70 g/l) TS and about 0.1 g of zinc R powder, heat over a water-bath for 10 minutes, cool and filter. To the filtrate add 1 ml of sodium nitrite (10 g/l) TS, shake and cool in ice for 2–3 minutes. Add 1 g of urea R, swirl until dissolved and allow to stand for 10 minutes. Add 2 ml of 2-naphthol TS and 2 ml of sodium hydroxide (~400 g/l) TS; a dark red colour is produced.
2. Place about 0.1 g of the test substance in a mortar and mix quickly with about 0.1 g of ground sodium hydroxide R. Transfer to a porcelain crucible and heat until melted. Cool, dissolve the melt in 4 ml of nitric acid (~130 g/l) TS, filter and to 1 ml of the filtrate add 5 drops of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash with water and add about 0.4 ml of ammonia (~260 g/l) TS; the precipitate dissolves but precipitates again on the addition of about 0.75 ml of nitric acid (~1000 g/l) TS.
3. Shake 20 mg of the test substance with 5 ml of ethanol (~750 g/l) TS; a yellow solution is produced. Add 1 drop of sodium hydroxide (~80 g/l) TS; the yellow colour becomes more intense. Then add 2 drops of hydrochloric acid (~70 g/l) TS; the yellow colour fades and a colourless solution is produced.

NYSTATIN OINTMENT

Description. The ointment usually contains 100 000 IU of nystatin in 1.0 g of a suitable ointment base.

Preparation of the sample. Withdraw and weigh an amount equivalent to 22 000 IU of nystatin, add 25 ml of dimethylformamide R and shake well. Filter, evaporate the filtrate to dryness and use the residue as the test substance.

Identity tests

Colour and other reactions

1. To 5 mg of the test substance add about 2 ml of sulfuric acid (~1760 g/l) TS; a dark violet colour is produced.
2. To 5 mg of the test substance add 1 ml of ethanol (~750 g/l) TS and about 1 ml of hydrochloric acid (~250 g/l) TS, shake and filter. To the filtrate add a few crystals of resorcinol R and heat in a water-bath for 2 minutes; a pink colour is produced.
3. To 5 mg of the test substance add 2 ml of ethanol (~750 g/l) TS, shake and filter. To the filtrate add about 1 ml of hydrochloric acid (~250 g/l) TS and 2 drops of a solution composed of 1 ml of ferric chloride (25 g/l) TS and 10 ml of water; a dark green colour is produced.

NYSTATIN PESSARIES

Description. Each pessary usually contains 100 000 IU of nystatin.

Preparation of the sample

1. Weigh 1 pessary and calculate the amount equivalent to 22 000 IU of nystatin.
2. Grind the pessaries, weigh out the above-calculated equivalent amount as powdered material, shake it vigorously with 25 ml of dimethylformamide R, filter, evaporate the filtrate to dryness and use the residue as the test substance.

Identity tests

Colour and other reactions

1. To 5 mg of the test substance add about 2 ml of sulfuric acid (~1760 g/l) TS; a dark violet colour is produced.
2. To 5 mg of the test substance add 1 ml of ethanol (~750 g/l) TS and about 1 ml of hydrochloric acid (~250 g/l) TS, shake and filter. To the filtrate add a few crystals of resorcinol R and heat in a water-bath for 2 minutes; a pink colour is produced.
3. To 5 mg of the test substance add 2 ml of ethanol (~750 g/l) TS, shake and filter. To the filtrate add about 1 ml of hydrochloric acid (~250 g/l) TS and 2 drops of a solution composed of 1 ml of ferric chloride (25 g/l) TS and 10 ml of water; a dark green colour is produced.

ORAL REHYDRATION SALTS (COMPOSITION A)

Description. Each packet contains a white, crystalline powder composed of:

glucose, anhydrous	20.0 g
sodium chloride	3.5 g
sodium hydrogen carbonate	2.5 g
potassium chloride	1.5 g

Preparation of the sample

1. Weigh the contents of 1 packet; it should weigh between 26.0 g and 29.0 g.
2. Use the contents of 1 packet directly as the test substance.
3. Dissolve the contents of 1 packet in 250 ml of water and use as the test solution.

Identity tests

Melting behaviour. Gently heat a small quantity of the test substance; the melt changes first to yellow and then to brown and an odour of burning sugar is perceptible. Then ignite; the melt swells, ignites and chars.

Colour and other reactions

1. The test solution is slightly alkaline when tested with pH-indicator paper R.
2. Add a few drops of the test solution to 5 ml of hot potassio-cupric tartrate TS; a copious red precipitate is produced (glucose).
3. Acidify 5 ml of the test solution with acetic acid (~300 g/l) TS, add 2 ml of magnesium uranyl acetate TS and scratch the sides of the beaker with a glass rod; a light yellow, crystalline precipitate is slowly produced (sodium).

   Alternative test:
   Introduce the test solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour (sodium).
4. To 5 ml of the test solution add 0.5 ml of nitric acid (~130 g/l) TS and 0.5 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves (chlorides).
5. To 5 ml of the test solution add 2 drops of phenolphthalein/ethanol TS; a pink colour is produced. Heat to boiling; a gas is evolved and the colour of the solution changes to red-violet (hydrogen carbonate).
6. To 5 ml of the test solution add 1 ml of acetic acid (~60 g/l) TS and 4 drops of sodium cobaltinitrite (100 g/l) TS; a yellow-orange precipitate is produced (potassium).

ORAL REHYDRATION SALTS (COMPOSITION B)

Description. Each packet contains a white, crystalline powder composed of:

glucose, anhydrous	20.0 g
sodium chloride	3.5 g
sodium citrate dihydrate	2.9 g
potassium chloride	1.5 g

Preparation of the sample

1. Weigh the contents of 1 packet; it should weigh between 26.5 g and 29.5 g.
2. Use the contents of 1 packet directly as the test substance.
3. Dissolve the contents of 1 packet in 250 ml of water and use as the test solution.

Identity tests

Melting behaviour. Gently heat a small quantity of the test substance; the melt changes first to yellow and then to brown and an odour of burning sugar is perceptible. Then ignite; the melt swells, ignites and chars.

Colour and other reactions

1. The test solution is slightly alkaline when tested with pH-indicator paper R.
2. Add a few drops of the test solution to 5 ml of hot potassio-cupric tartrate TS; a copious red precipitate is produced (glucose).
3. Acidify 5 ml of the test solution with acetic acid (~300 g/l) TS, add 2 ml of magnesium uranyl acetate TS and scratch the sides of the beaker with a glass rod; a light yellow, crystalline precipitate is slowly produced (sodium).

   Alternative test:
   Introduce the test solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour (sodium).
4. To 5 ml of the test solution add 0.5 ml of nitric acid (~130 g/l) TS and 0.5 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves (chlorides).
5. To 5 ml of the test solution add 3 ml of calcium chloride (55 g/l) TS; no precipitate is produced. Boil the solution; a white solid is produced which is soluble in acetic acid (~300 g/l) TS (citrates).
6. To 5 ml of the test solution add 1 ml of acetic acid (~60 g/l) TS and 4 drops of sodium cobaltinitrite (100 g/l) TS; a yellow-orange precipitate is produced (potassium).

PARACETAMOL SUPPOSITORIES

Description. Each suppository usually contains 100 mg of paracetamol.

Preparation of the sample

1. Weigh 1 suppository and calculate the amount equivalent to 0.5 g of paracetamol.

2. Dissolve a quantity of the suppositories equivalent to 0.5 g of paracetamol in 25 ml of light petroleum R by warming carefully over a water-bath. Collect the residue by decanting, wash with 3 portions of 25 ml of light petroleum R and use as the test substance.

Identity tests

Description of the test substance. A white, crystalline powder.

Melting point of the test substance. About 170 °C.

Eutectic temperature of the test substance. With benzanilide R, about 137 °C; with phenacetin R, about 114 °C.

Colour and other reactions

1. Dissolve 0.10 g of the test substance in 10 ml of water and add 1 drop of ferric chloride (~25 g/l) TS; an intense violet-blue colour is produced.

2. To 0.10 g of the test substance add 1 ml of hydrochloric acid (~70 g/l) TS and boil for 1 minute. Add 10 ml of water and cool; no precipitate is produced. Add 1 drop of potassium dichromate (100 g/l) TS and shake; a violet colour slowly develops and does not change to red (distinction from phenacetin).

PHENYTOIN TABLETS

Description. Each tablet usually contains 50 mg of phenytoin.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.08 g of phenytoin.

2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material and use directly as the test substance, dividing it into 2 equal parts.

Identity tests

Colour and other reactions

1. To 1 part of the test substance add 4 ml of ethanol (~750 g/l) TS, shake and filter. To the filtrate add 4 drops of cobalt(II) chloride (30 g/l) TS and 1 drop of ammonia (~260 g/l) TS; a blue-violet colour is produced.
2. To half of 1 part of the test substance placed on a white test plate add 2 drops of ammonia (~100 g/l) TS and 1 small drop of copper(II) sulfate (160 g/l) TS and mix thoroughly with a glass rod; a pink precipitate is produced.

PHENYTOIN SODIUM CAPSULES

Description. Each capsule usually contains 25–100 mg of phenytoin sodium.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 0.08 g of phenytoin sodium.
2. Empty the capsules, weigh out the above-calculated equivalent amount and use directly as the test substance, dividing it into 4 equal parts.

Identity tests

Colour and other reactions

1. To 2 parts of the test substance add 4 ml of dichloromethane R and 4 drops of cobalt(II) chloride (30 g/l) TS and shake; a voluminous precipitate is produced in a blue-violet coloured solution (distinction from phenytoin).
2. Place half of 1 part of the test substance on a white test plate, add 2 drops of ammonia (~100 g/l) TS and 1 small drop of copper(II) sulfate (160 g/l) TS and mix thoroughly with a glass rod; a pink precipitate is produced.

PHYTOMENADIONE INJECTION

Description. The injection is a sterile solution usually containing 10 mg of phytomenadione in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 20 mg of phytomenadione; if necessary, reduce the volume by evaporation to about 2 ml or dilute to about 2 ml with water. Use directly as the test solution.

Identity tests

Colour and other reactions

1. Dilute about 1 ml of the test solution with 10 ml of ethanol (~750 g/l) TS and add 3 ml of potassium hydroxide/ethanol TS; a green colour is produced. Allow to stand for 1–2 minutes; the colour changes to blue. Again allow to stand for about 15 minutes; the colour changes to red-brown.
2. To about 1 ml of sulfuric acid (~1760 g/l) TS add 3 drops of the test solution; a brown-orange colour is produced. Heat the solution; the colour changes to dark brown.
3. To 3 drops of the test solution add 5 ml of ethanol (~750 g/l) TS and 2–5 drops of potassium permanganate (10 g/l) TS and shake; the colour of the permanganate is immediately discharged.

PIPERAZINE CITRATE SYRUP

Description. The syrup contains piperazine citrate, usually equivalent to 500 mg of piperazine hydrate in 5 ml of a suitable vehicle.

Preparation of the sample. Use the well homogenized contents of one container or the equivalent of 0.5 g of piperazine hydrate and use directly as the test solution, dividing it into 3 equal volumes.

Identity tests

Colour and other reactions

1. To 2 volumes of the test solution add 5 ml of hydrochloric acid (~70 g/l) TS and then add while stirring 1 ml of sodium nitrite (500 g/l) TS. Cool in ice for 15 minutes, stirring if necessary to induce crystallization. Filter, wash the crystals with 10 ml of ice-water and dry at 105 °C; melting point, about 158 °C.
2. To 1 volume of the test solution add 3 ml of calcium chloride (55 g/l) TS; no precipitate is produced. Boil the solution; a white solid is produced which is soluble in acetic acid (~300 g/l) TS.

POTASSIUM CHLORIDE SOLUTION

Description. The solution usually contains 112 mg of potassium chloride in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the contents of the containers equivalent to 0.30 g of potassium chloride and use directly as test solution 1.

2. Dilute 1.0 ml of test solution 1 with sufficient water to produce 10 ml and use as test solution 2.

Identity tests

Colour and other reactions

1. Introduce test solution 1, together with hydrochloric acid (~250 g/l) TS, into a non-luminous flame using a magnesia stick or a nichrome or a platinum wire sealed to a glass rod; the flame acquires a violet colour which, when viewed through a suitable blue glass, appears reddish violet.
2. To 1.0 ml of test solution 2 add 3 ml of water and 5 drops of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash with water and add a few drops of ammonia (~100 g/l) TS; the precipitate dissolves.
3. Heat 2–3 drops of test solution 1 with a few drops of sodium hydroxide (~80 g/l) TS; no odour of ammonia is perceptible.

PROCAINE BENZYLPENICILLIN POWDER FOR INJECTION

Description. Each vial contains a sterile powder usually equivalent to 1–3 g of procaine benzylpenicillin.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amount equivalent to 30 mg of procaine benzylpenicillin.
2. Empty the vial, weigh out the above-calculated equivalent amount and use directly as the test substance, dividing it into 6 equal parts.

Identity tests

Colour and other reactions

1. Dissolve 1 part of the test substance in 3 ml of water, add 0.1 g of hydroxylamine hydrochloride R and 1 ml of sodium hydroxide (~80 g/l) TS and allow to stand for 5 minutes. Add 1.3 ml of hydrochloric acid (~70 g/l) TS and 10 drops of ferric chloride (25 g/l) TS; a violet-red colour is produced.
2. Dissolve 1 part of the test substance in 2 ml of water and add 2–3 drops of ferric chloride (25 g/l) TS; a cream-coloured, flocculent precipitate is produced.
3. To half of 1 part of the test substance add a solution of 10 mg of paraformaldehyde R in about 1 ml of sulfuric acid (~1760 g/l) TS; an almost colourless solution is produced. Heat the solution in a water-bath for 2 minutes; a brownish violet colour is produced.

4. Dissolve 1 part of the test substance in 2 ml of water and add 5 drops of iodine TS; a brown precipitate is produced.
5. Dissolve 1 part of the test substance in 2 ml of water and add 5 drops of potassio-mercuric iodide TS; a white precipitate is produced.
6. Dissolve 1 part of the test substance in 1 ml of water and add 5 drops of hydrochloric acid (~70 g/l) TS, 10 drops of sodium nitrite (10 g/l) TS and 1 ml of sodium hydroxide (~80 g/l) TS. Then add 5 mg of 2-naphthol R; a red colour is produced.

PYRIDOXINE HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 25 mg of pyridoxine hydrochloride in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.05 g of pyridoxine hydrochloride and use directly as the test solution, dividing it into 4 equal volumes.

Identity tests

Colour and other reactions

1. Dilute 1 volume of the test solution to 2 ml with water and add 2 ml of ferric chloride (25 g/l) TS; a red-brown colour is produced. Add 4 ml of hydrochloric acid (~70 g/l) TS; the colour of the solution changes to yellow.
2. To 0.5 ml of sulfanilic acid TS add 3 drops of sodium nitrite (10 g/l) TS, 1 ml of sodium hydroxide (~80 g/l) TS and 1 volume of the test solution and allow to stand for 2 minutes; a golden yellow colour is produced. Add 2 ml of acetic acid (~300 g/l) TS; the colour remains almost the same or changes to orange.
3. Dilute 1 volume of the test solution to 2 ml with water and add 0.5 ml of nitric acid (~130 g/l) TS and 1 ml of silver nitrate (40 g/l) TS; a white precipitate is produced. Separate the precipitate, wash with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves almost completely (some excipients may interfere with the reaction).
4. To 1 volume of the test solution add 1 ml of water, 1 drop of copper(II) sulfate (160 g/l) TS and 1 ml of sodium hydroxide (~80 g/l) TS; the colour of the solution changes to deep blue.

Degradation tests

Discoloration and a change in the physical state of the test solution usually indicate gross degradation.

PYRIMETHAMINE TABLETS

Description. Each tablet usually contains 25 mg of pyrimethamine.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.25 g of pyrimethamine.
2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material, shake it with 50 ml of ethanol (~750 g/l) TS heated to 60 °C, filter and evaporate to dryness. Dry the residue to constant weight at 105 °C and use as the test substance.

Identity tests

Melting behaviour. The test substance melts at about 239 °C.

Colour and other reactions

1. Dissolve 0.05 g of the test substance in 5 ml of sulfuric acid (~100 g/l) TS and add 0.2 ml of freshly prepared potassio-mercuric iodide TS; a creamy white precipitate is produced.
2. To 1 ml of methyl orange/ethanol TS add 5 ml of water and 2 ml of ethyl acetate R and shake; the ethyl acetate layer remains colourless. Add a solution of 5 mg of the test substance dissolved in 5 ml of sulfuric acid (~5 g/l) TS, shake and allow to separate (about 30 minutes); a yellow colour is produced in the ethyl acetate layer.
3. Ignite about 0.1 g of the test substance with 0.5 g of anhydrous sodium carbonate R, cool, add 5 ml of hot water and heat in a water-bath for 5 minutes. Filter, neutralize the filtrate with nitric acid (~130 g/l) TS and add 5 drops of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

RESERPINE INJECTION

Description. The injection is a sterile solution usually containing 1 mg of reserpine in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 2.0 mg of reserpine and use directly as the test solution, dividing it into 4 equal volumes.

Identity tests

Colour and other reactions

1. To 1 volume of the test solution add 1 ml of dichloromethane R, shake and allow to stand. Separate the dichloromethane layer and evaporate

it to dryness over a water-bath. Mix the residue with 5 mg of 4-dimethylaminobenzaldehyde R and 4 drops of glacial acetic acid R. Then add 4 drops of sulfuric acid (~1760 g/l) TS; a green colour is produced. Add about 1 ml of glacial acetic acid R; the colour changes to red.

2. To 2 volumes of the test solution add 1 drop of sulfuric acid (~100 g/l) TS and a few drops of sodium nitrite (10 g/l) TS; a yellowish green solution is produced which exhibits a greenish fluorescence.
3. To 1 volume of the test solution add a few drops of ammonium vanadate (5 g/l) TS; a green colour is produced.

SODIUM CHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 9 mg of sodium chloride in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the containers equivalent to about 30 mg of sodium chloride; if necessary, reduce the volume by evaporation to about 3 ml or dilute to about 3 ml with water. Use directly as the test solution.

Identity tests

Colour and other reactions

1. Introduce a few drops of the test solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.
2. To 2 ml of the test solution add 3 drops of nitric acid (~130 g/l) TS and 3 drops of silver nitrate (40 g/l) TS and shake; a white, curdy precipitate is produced. Separate the precipitate, wash with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

SODIUM CROMOGLICATE CAPSULES FOR INHALATION

Description. Each capsule usually contains 20 mg of sodium cromoglicate mixed with lactose in a 1 : 1 ratio.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 0.06 g of sodium cromoglicate.
2. Empty the capsules, weigh out the above-calculated equivalent amount and use directly as the test substance, dividing it into 3 equal parts.

Identity tests

Colour and other reactions

1. To 1 part of the test substance add 2 ml of water and 2 ml of sodium hydroxide (~200 g/l) TS. Boil for 1 minute; a yellow solution is produced. Allow to stand for 30 minutes and add 0.5 ml of diazobenzenesulfonic acid TS2; a deep red colour is produced.
2. To 1 part of the test substance add 2 ml of potassio-cupric tartrate TS and heat; a red precipitate is produced.
3. Moisten 1 part of the test substance with 2 drops of hydrochloric acid (~70 g/l) TS and introduce the mixture into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.

SODIUM HYDROGEN CARBONATE INJECTION

Description. The injection is a sterile solution usually containing 14 mg of sodium hydrogen carbonate in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 0.30 g of sodium hydrogen carbonate and use directly as the test solution, dividing it into 3 equal volumes.

Identity tests

Colour and other reactions

1. Introduce a few drops of the test solution into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.
2. Dilute 1 volume of the test solution with 3 ml of water and add 2 drops of phenolphthalein/ethanol TS; a pink colour is produced. Heat to boiling; a colourless and odourless gas evolves and the colour of the solution changes to red-violet.
3. Dilute 1 volume of the test solution with 3 ml of water and add a few drops of magnesium sulfate (50 g/l) TS; no precipitate is produced. Boil the mixture; a white precipitate is produced.

SODIUM LACTATE INJECTION

Description. The injection is a sterile solution usually containing 500 mg of monosodium lactate in 1.0 ml of a suitable vehicle.

Preparation of the sample. Dilute a volume of the solution equivalent to 0.5 g of sodium lactate to 5 ml with water and use directly as the test solution.

Identity tests

Colour and other reactions

1. To 0.5 ml of the test solution add 2 ml of sulfuric acid (~100 g/l) TS and 3 ml of potassium permanganate (10 g/l) TS; the colour of the permanganate is discharged and an odour of acetaldehyde is perceptible.
2. To 1 ml of the test solution add 4 ml of water and mix. Add 1 ml of acetic acid (~300 g/l) TS and 1 ml of ammonium oxalate (25 g/l) TS; no precipitate is produced (distinction from calcium lactate).
3. To a few drops of the test solution add a small volume of hydrochloric acid (~250 g/l) TS and introduce the mixture into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.

   Alternative test:
   To 5 drops of the test solution add 1 ml of water, acidify with acetic acid (~300 g/l) TS and add 2 ml of magnesium uranyl acetate TS; a light yellow, crystalline precipitate is produced.
4. To 2 ml of the test solution add, along the wall of the test-tube, 5 ml of a reagent composed of 0.10 g of catechol R dissolved in 10 ml of sulfuric acid (~1760 g/l) TS; a deep red colour is produced at the junction of the two liquids.

SULFASALAZINE TABLETS

Description. Each tablet usually contains 500 mg of sulfasalazine.[1]

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 10 mg and 0.05 g of sulfasalazine.
2. Grind the tablets, weigh out the above-calculated equivalent amounts as powdered material and use directly, 10 mg as test substance 1 and 0.05 g as test substance 2.

Identity tests

Colour and other reactions

1. To test substance 1 add 5 ml of water, shake and filter. To the filtrate add 1 ml of sodium hydroxide (~80 g/l) TS; an orange-red to deep red colour is produced.

[1] Referred to as salazosulfapyridine in *Basic tests for pharmaceutical substances*, 1986.

2. To test substance 2 add 2 drops of ethanol (~750 g/l) TS and 4 ml of hydrochloric acid (~70 g/l) TS and mix. Then add 0.2 g of zinc R powder, heat in a water-bath for 5 minutes and filter.
   (*a*) To 1 ml of the filtrate add 4 ml of water and 2 drops of ferric chloride (25 g/l) TS; a red colour is produced.
   (*b*) To 1 ml of the filtrate add 1 ml of sodium nitrite (10 g/l) TS and allow to stand in ice for 1 minute. Add 2 ml of sodium hydroxide (~80 g/l) TS and 2 drops of 2-naphthol TS; an intense red colour is produced.

VERAPAMIL HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 2.5 mg of verapamil hydrochloride in 1.0 ml of a suitable vehicle.

Preparation of the sample. Pool the contents of the ampoules equivalent to 15 mg of verapamil hydrochloride and use directly as the test solution, dividing it into 3 equal volumes.

Identity tests

Colour and other reactions

1. To 1 volume of the test solution add about 0.2 ml of mercuric chloride (65 g/l) TS; a white precipitate is produced.
2. To 1 volume of the test solution add 0.5 ml of sulfuric acid (~100 g/l) TS and 4 drops of potassium permanganate (10 g/l) TS; a violet precipitate is produced which immediately dissolves to produce a very pale yellow solution.
3. Acidify 1 volume of the test solution with nitric acid (~130 g/l) TS and add 2 ml of silver nitrate (40 g/l) TS; a white, curdy precipitate is produced. Separate the precipitate, wash with water and add an excess of ammonia (~100 g/l) TS; the precipitate dissolves.

Degradation tests

Discoloration and a change in the physical state of the test solution usually indicate gross degradation.

WARFARIN SODIUM TABLETS

Description. Each tablet usually contains 1–5 mg of warfarin sodium.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.10 g of warfarin sodium.

2. Grind the tablets, weigh out the above-calculated equivalent amount as powdered material.
3. Extract the powdered material with two quantities, each of 15 ml, of water. Filter and to the filtrate add 2 drops of hydrochloric acid (~70 g/l) TS. Filter, wash the precipitate with water and dry at 110 °C for 3 hours; melting point, about 162 °C. Use the crystals as the test substance.

Identity tests

Colour and other reactions

1. Dissolve 20 mg in 1 ml of water and add 5 drops of sodium hydroxide (~80 g/l) TS and 5 drops of iodine TS; a yellow precipitate is produced and a characteristic odour of iodoform is perceptible.
2. Moisten 10 mg with 1 drop of hydrochloric acid (~70 g/l) TS and introduce the mixture into a non-luminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires an intense yellow colour.

EVALUATION OF CRUDE DRUGS

❖ ***Drug evaluation may be defined as the determination of a drug's identity, purity and quality.***

✓ ***Identity*** – Identification of biological source of the drug.

✓ ***Quality*** – quality of the active constituents present.

✓ ***Purity*** – the extent of foreign organic material present in a crude drug.

Importance of evaluation of crude drugs:

✓ Determination of Biochemical variation in the drugs

✓ Identification of deterioration due to treatment and storage

✓ Reporting Substitution and adulteration, as a result of carelessness, ignorance, and fraud

METHODS OF DRUG EVALUATION

- ***Morphological evaluation***
- ***Microscopical evaluation***
- ***Physical evaluation***
- ***Chemical evaluation***
- ***Biological evaluation***

MORPHOLOGICAL EVALUATION

- Organoleptic evaluation, study of morphology and other sensory characters.
- Based on color, odour, taste, size, shape, texture etc.

Part of drug	Examples
Bark	Cinnamon
Underground part	Ginger, turmeric
Leaves	Digitalis
Flowers	Saffron
Fruits	Fennel
Seeds	Nuxvomica
Entire plant	Vinca

Character	Example
Aromatic odour	Umbelliferous fruits
Sweet taste	Liquorice
Fractured surface	Cinchona
Pungent taste	Ginger
Brown colour	Cinnamon
Wavy shape	Rauwolfia
7-8 mm width, 25-60 mm length	Senna leaf

Study of sensory characters

Flowers: Floral parts: stigmas, corollas, anther, ovary, receptacle.

Leaves and leaflets: Length, width, apex, margin, base, venation, the texture of the leaf and the hairs in upper and lower surface. The feel of the surface described as soft, hairy smooth.

Bark: Flat or curved pieces, Single quill, Double quills, Barks have two surfaces, an outer and inner, The inner surface is usually lighter in color than the outer surface

- **Odor and taste:** distinct, indistinct, aromatic, balsamic, spicy
- **Taste:** Acidic (sour), Saccharine (sweet), Saline (salty), Alkaline, Bitter, Tasteless, Distinctive sensations to the tongue like Mucilaginous and oily (soft feeling), Astringent, Pungent (warm biting sensation), Acrid (irritant sensation), Nauseous (those tending to excite vomiting).

COLOUR AND EXTERNAL MARKINGS	
White	Starch
Pale yellow	Ginger, Squill, White pepper
Deep yellow	Peeled liquorice
Pale green	Lobelia
Greenish brown	Most of the leaf herbs
Light brown	Nuxvomica, fennel
Dark brown	Clove buds
Dark reddish brown	Cinchona
Brick red	Cinnamon bark inner

MICROSCOPIC OR ANATOMICAL EVALUATION

- This method allows a more detailed examination of a drug and it can be used to identify organized drugs by their known histological characters.
- Before examination through a microscope the material must be suitably prepared.
- This can be done by powdering, cutting thin sections of the drug or preparing a macerate.
- This method is used to identify organized drug by their known histological characters through Transverse section (T.S.) or Longitudinal Section (L.S.) or Radial Longitudinal Section (R.L.S.) or Tangential Longitudinal Section (T.L.S.).

LEAF CONSTANTS

- Palisade Ratio
- Stomatal Number
- Stomatal Index
- Vein- islet number
- Vein termination number
- Trichomes
- Calcium oxalate crystals
- Lycopodium spore method

Examples:

✓ Powder of clove stalk contain sclereids and calcium oxalate crystals, but cloves does not.

✓ Sclerenchyma absent in *Rauwolfia Serpentina* root but present in *Rauwofia micratha*, *R. densiflora*.

✓ Stone cell present in frangula bark and absent in cascara bark.

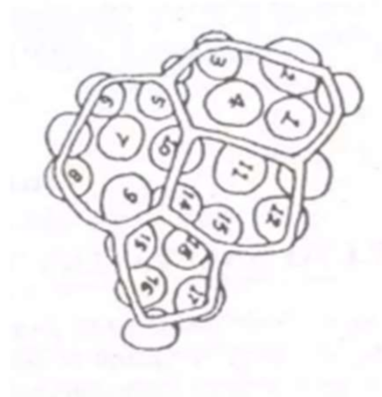
✓ Lignified Trichome and plasmodesmeta in nux-vomica.

1. Palisade ratio

- The average number of palisade cells beneath each epidermal cell is known as the palisade ratio.
- It is an important parameter for determination and characterization of leafy drugs.
- Determined using Camera lucida.

Eg;

- *Atropa belladonna*: 5-10
- *Adhatoda vasica*: 5.5-6.5
- *Cassia angustifolia*: 5.5-10
- *Digitalis lanata*: 2.5-6.5



2. Stomatal number

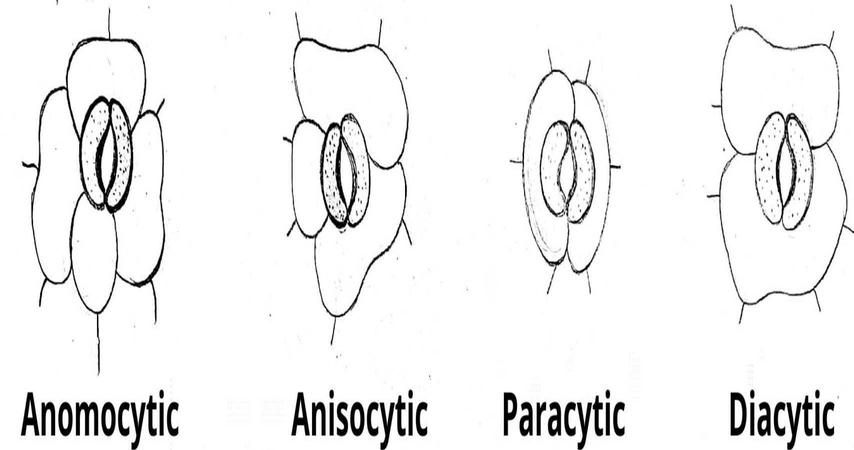
- It is defined as average number of stomata per square millimeter area of epidermis.

Eg;

- *Atropa belladonna*: UE: 7-10, LE: 77-115
- *Datura metel*: UE:147-160, LE: 200-209
- *Ocimum sanctum*: UE: 64-72, LE: 175-250

Stomata

- Stomata are minute pores or openings in the epidermis layer of the leaves, young stems, and some other parts of green plants which form an important constituent of their functioning.
- There are several types of stomata, distinguished by the forms and arrangement of the Surrounding cells.



e.g.:

✓ **Anomocytic** (Ranunculaceous) irregular celled: Digitalis

✓ **Anisoytic** (Cruciferous) unequal – celled: Datura

✓ **Diacytic** (Caryophyllaceous) cross – celled: Mentha

✓ **Paracytic** (Rubiaceous) parallel celled: Senna

3. Stomatal index

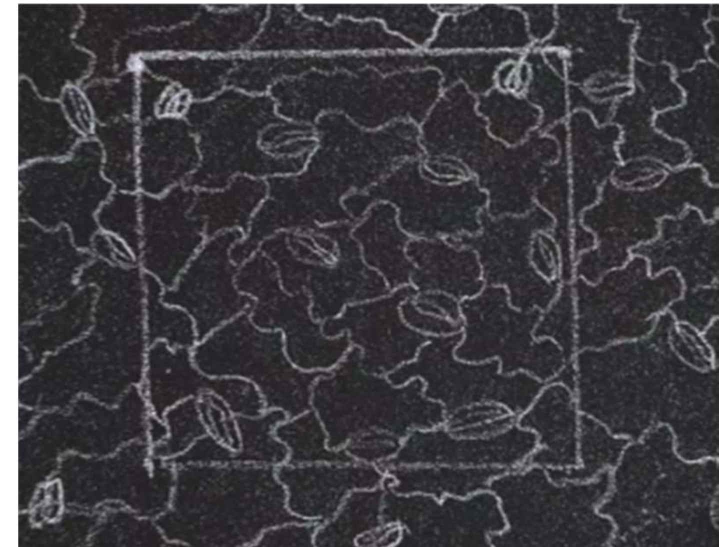
- Stomatal index (I) is the average number of stomata cells present per square millimeter of the epidermis of the leaf.
- It is the percentage proportion of the number of stomata to the total number of epidermal cells.
- Stomatal index can be calculated by using the following equation:

$$S.I = \frac{S}{E + S} * 100$$

- S= Number of stomata per unit area
- E= Number of epidermal cells in the same unit area.

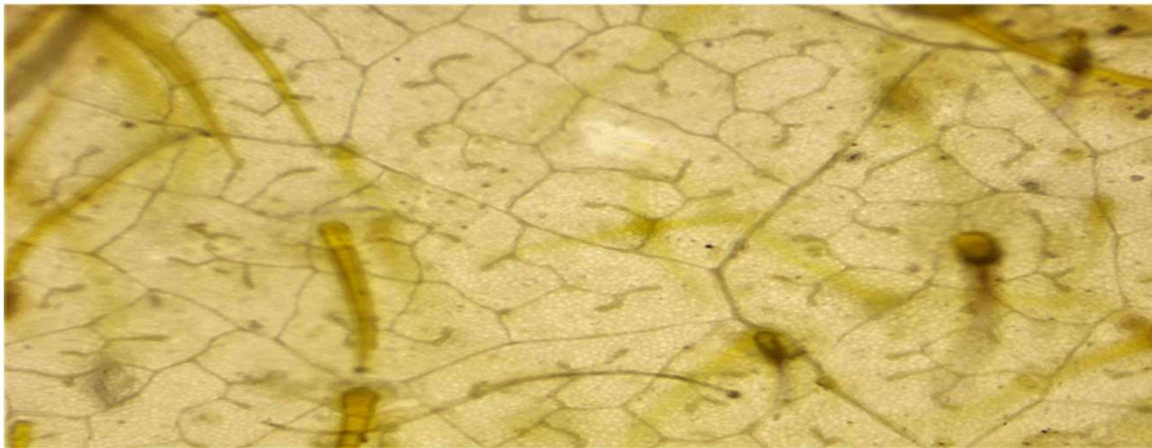
Examples:

- *Atropa belladonna*:-2.3-3.9 to 10.5 (Upper Surface), 20.2 to 23.0 (Lower Surface)
- *Digitalis Purpurea*- 2.7 to 4.0 (Upper Surface), 19.2- 25.2 (lower Surface).



4. Vein- islet number

- It is defined as average number of Vein Islet per square millimeter of the leaf surface midway between midrib and the margin.
- It is constant for a given species of the plant and is used as a character for the identification of the allied species.

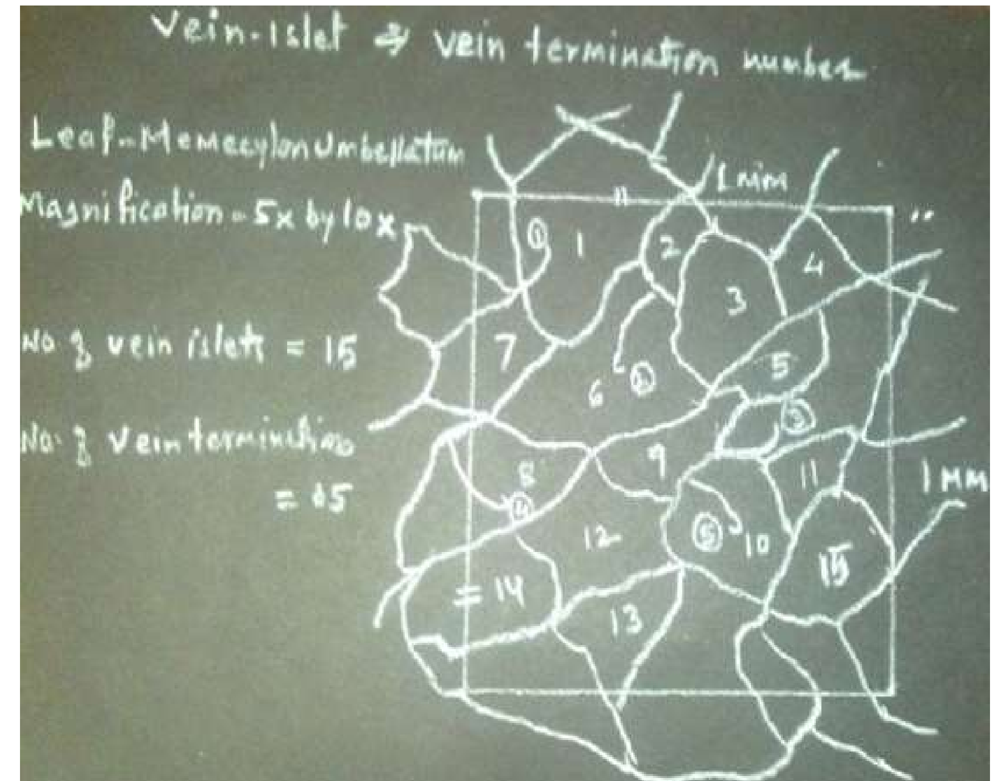


Examples:

NAME OF DRUG	Vein-islet Range
Andrograohis paniculata	9-12
Bacopa monniera	6-13
Cannabis sativa	18-24
Digitalis purpurea	2.5-3
Eucalpytus globules	8-13.5

5. Vein- termination number

- It is defined as average number of Vein terminations per square millimeter of the leaf surface midway between midrib and the margin.

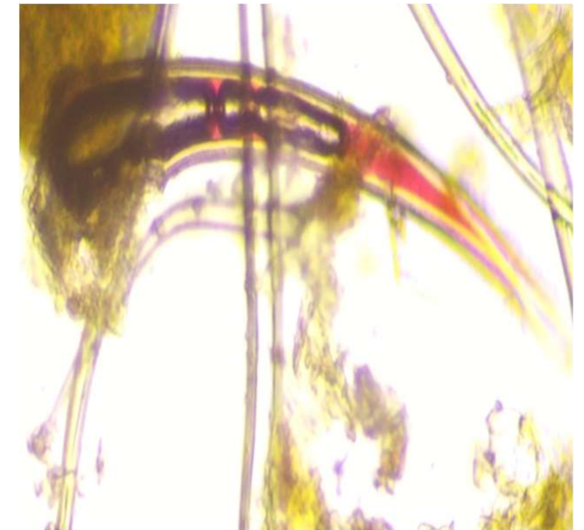


Examples:

- *Atropa belladonna* — 6.3-10.3
- *Atropa acuminate* — 1.4-3.5

6. Trichomes

- Trichomes are the tubular elongated or glandular outgrowth of the epidermal cells. Trichomes are also called as plant hairs. Trichomes consists of two parts root and body. Trichomes present in most of plant parts and are function less but some times perform secretory function.

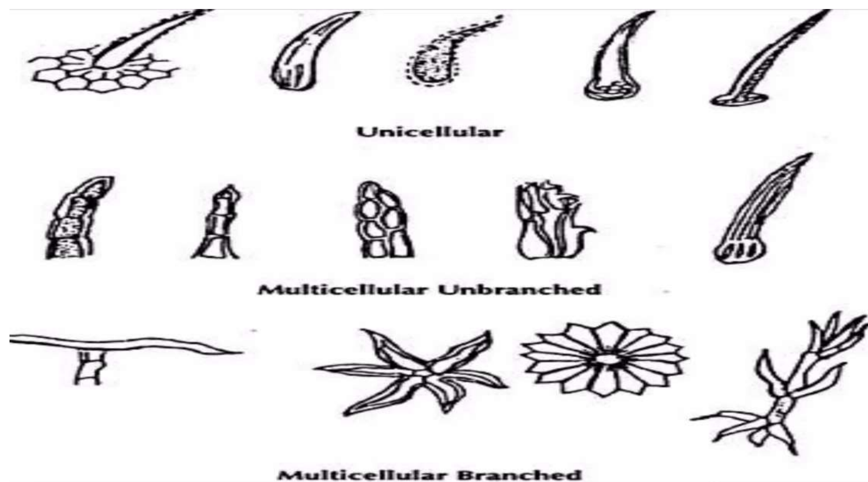


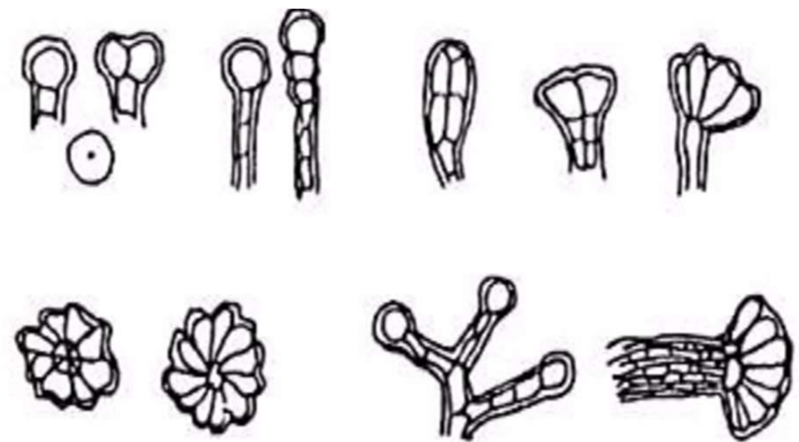
- Trichomes are divided and subdivided as follows;
- **Covering Trichomes:**
  - ✓ Unicellular Trichomes: Nux vomica, Cannabis
  - ✓ Uniseriate Multicellular Unbranched Trichomes: Datura
  - ✓ Biseriate Multicellular unbranched Trichomes: *Calendula officinalis*
  - ✓ Multiseriate Multicellular unbranched Trichomes: Male fern
  - ✓ Multicellular branched Trichomes: *Verbascum Thapsus*

- **Glandular Trichomes:**
  - ✓ Unicellular Glandular Trichomes: Vasaka
  - ✓ Multicellular Glandular Trichomes: *Digitalis purpurea*

Other types are;

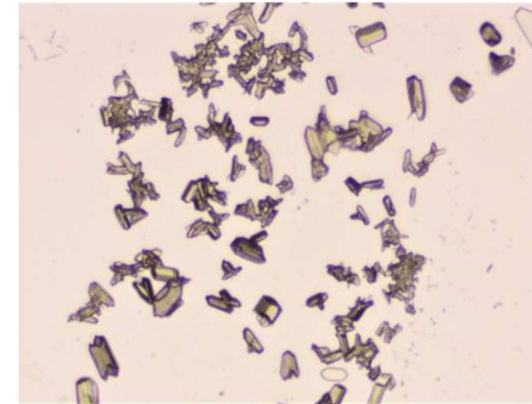
Glabrous, glabrate, hirsute, hispid, articulate, downy, pilose, puberulent, pubescent, strigillose, strigose, tomentellous, tomentose, villosulous, villous.





7. Calcium oxalate crystals

- The inorganic crystalline compounds by virtue of their specific shapes can be utilized for the identification of herbal drugs.



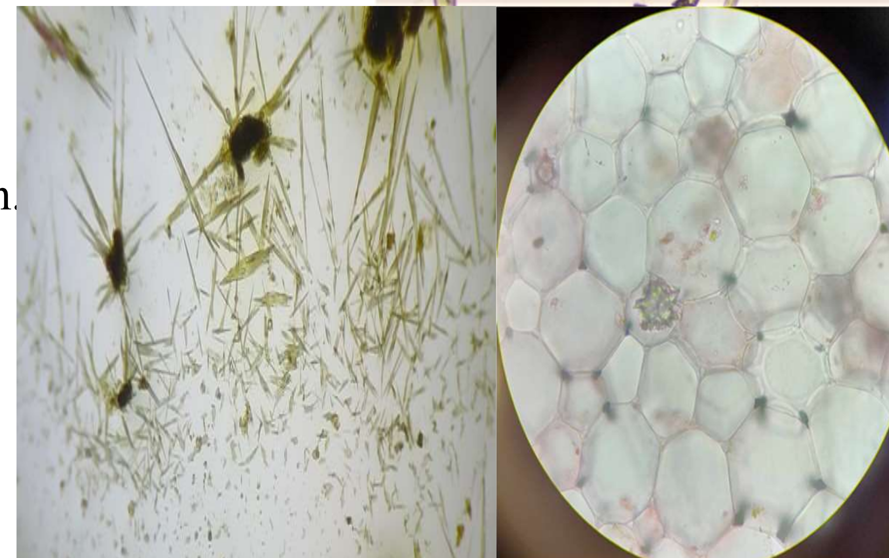
- Due to this reason they are called as **diagnostic characters of the plant**.

✓ **Cubical** (cube shape): Senna, Glycyrrhiza.

✓ **Rhombic** (diamond shape)

✓ **Tetragonal**: onion.

✓ **Acicular** (long slender, pointed, bundles): Squill, Cinnamon.



✓ **Rosettes** –clusters (aggregation of crystals): Clove, Arjuna.

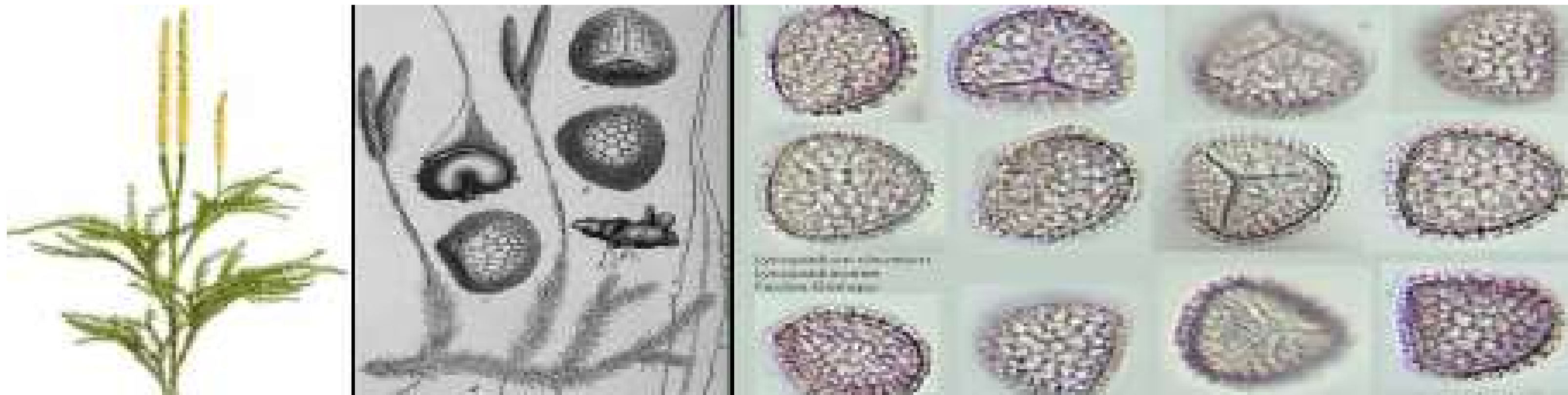
✓ **Microsphenoidal** (minute in structures): Henbane.

8. LYCOPODIUM SPORE METHOD

- Quantitative method
- It is used when especially chemical and other methods of evaluation of drugs fail to determine quality.
- Lycopodium spores are obtained from club moss, *Lycopodium clavatum* Linn., belonging to family Lycopodiaceae.
- The spores are yellow in colour, spheroidal, tetrahedral in shape with reticulate surface.
- They have uniform average diameter of 25 microns. One milligram contains average 94000 spores.
- They have uniform moisture content, hence the weight remains the same.
- This is the reason, why these spores are used to evaluate powdered drugs by comparison. The spores are also resistant to pressure.

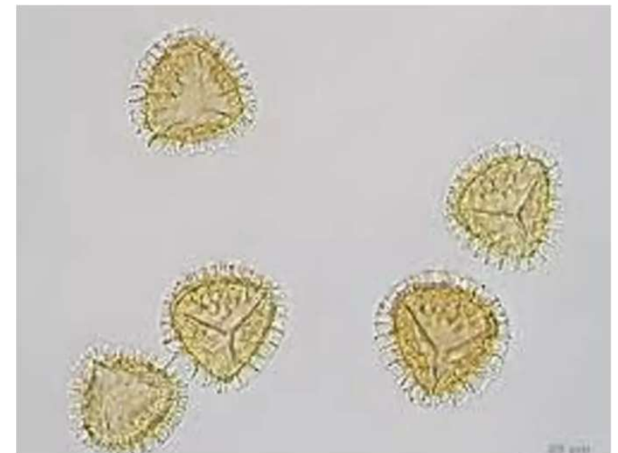
A powdered drug can be evaluated by comparison with lycopodium spores if it contains;

1. Well defined characteristic particles which can be counted (starch grains, pollen grains etc.)
2. Single layered cells or tissues, the area of which may be traced under suitable magnification and actual area calculated (eg: epidermal area of senna leaf).
3. The objects of uniform thickness, the length of which can be measured under suitable magnification and actual area calculated (eg: trichomes).



PROCEDURE

- Dry the powdered drug at 105^0 C and determine its steady weight at room temperature.
- Weigh accurately 200 mg of test material and 100 mg of lycopodium spore powder and mix with a glass rod or spatula uniformly.
- Make a thin smooth paste by adding 5 ml suspending medium (oil).
- Place a drop of above suspension on a glass slide, spread with a needle and put coverslip and observe under microscope in 10X.
- Count the characteristic particles of test and spores in the field.
- Take a count of 25 fields.
- Repeat the step in four times to get an average set of counts.



- Calculate the percentage purity using the formula;

$$\% \, purity = \frac{\mathrm{n} * \mathrm{w} * 94000 * 100}{\mathrm{s} * \mathrm{m} * \mathrm{p}}$$

n= number of characteristic particles in 25 fields

s= number of lycopodium spores in the same 25 fields

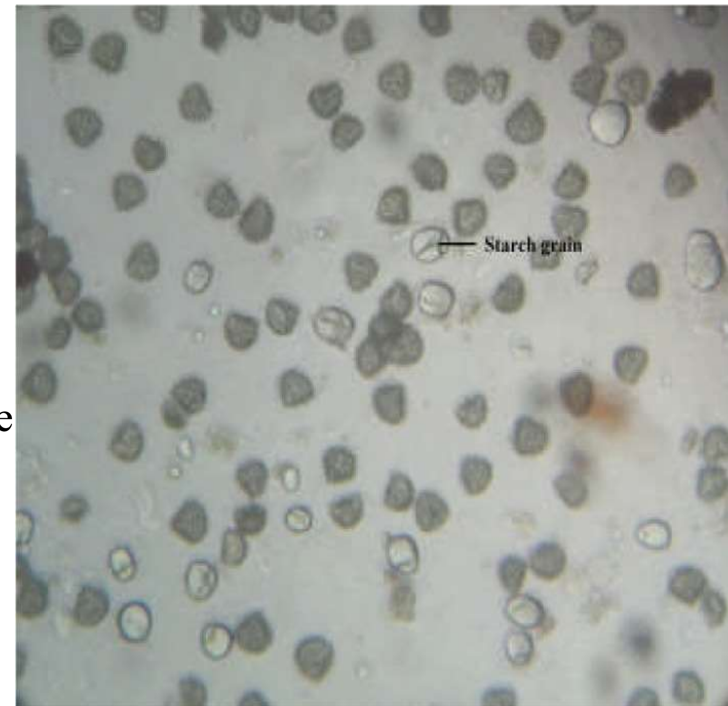
w= weight in mg of lycopodium spore powder taken

m= weight of the test sample taken in mg

p= standard value for number of characteristic particles per mg of pure sample

Eg: in case of starch grain it is 286000

94000= number of spores per mg of lycopodium spore powder

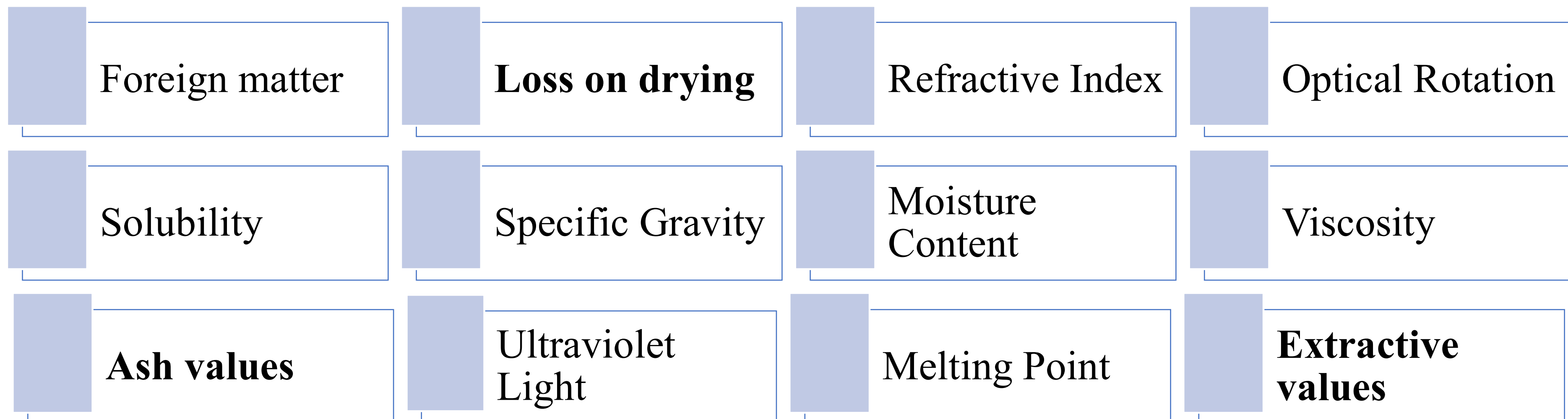


Applications

- Determine the size of the particles in powder such as fragments of leaves, isolated fibers.
- Lycopodium spore method can be used to evaluate powdered clove, ginger, cardamom, nutmeg, umbelliferous fruits etc.
- Determine the percentage of each powder particles in a mixture
- Determine the length of lignified ribs of trichomes in Nuxvomica
- Determine the Nuxvomica content of two veterinary medicines.

PHYSICAL EVALUATION

- For determining physical standards of a crude drug.
- In crude plant evaluation, physical methods are often used to determine the ***solubility, specific gravity, optical rotation, viscosity, refractive index, melting point, water content, degree of fibre elasticity***, and other physical characteristics of the herb material.



1. Solubility

- Drugs-specific behavior towards solvents is taken into consideration. This is useful for the examination of many oils, oleoresins, etc.

DRUG	SOLUBILITY
Castor oil	Soluble in 3 volumes of alcohol
Balsam of Peru	Soluble in chloral hydrate solution
Asafoetida	Soluble in carbon disulphide
Alkaloid bases	Soluble in chloroform
colophony	Soluble in light petroleum

2. Optical Rotation

- Many substances of biological origin, having a chiral centre, can rotate the plane of polarised light either to right (dextro rotatory)or to the left (laevo).
- The extent of rotation is expressed in degrees, plus(+) indicating rotation to the right and minus(-) indication rotation in the left. Such compound are optically active and hence called optical rotation.

Drugs	Angles of Optical Rotation
Caraway oil	+ 75° to +80°
Clove oil	0° to +6.0°
Honey	+3° to -15°

3. Refractive Index

- When a ray of light passes from one medium to another medium of different density, it is bent from its original path.
- Thus, the ratio of velocity of light in vaccum to its velocity in the substance is said to the Refractive index of the second medium.
- It is measured by means of refractometer. RI of a compound varies with the wavelength of the incident light, temperature and pressure.

Eg: **Arachis oil: 1.4678- 1.470**

Clove oil: 1.527-1.535

4. Specific Gravity

- It is also known as relative density.
- The ratio of the mass of a solid or liquid to the mass of an equal volume of distilled water at 4°C (39°F) or of a gas to an equal volume of air or hydrogen under prescribed conditions of temperature and pressure.
  - Eg: **cottonseed oil: 0.88–0.93**

Coconut oil: 0.925

Castor oil: 0.95

5. Viscosity

- Viscosity is the resistance of a fluid to flow.
- The viscosity of a liquid is constant at a given temperature and is an index of its composition.
- Hence, it is used as a means of standardizing liquid drugs.
  - Eg: **Liquid paraffin: not less than 64 centistokes**

Pyroxylin: 1100-2450 centistokes

6. Melting Point

- The melting point of a solid is the temperature at which it changes state from solid to liquid.
- Plant constituents have very sharp and constant melting points.
- It is one of the parameters to judge the purity of crude drugs containing lipids as constituents.
- The purity of the crude drugs can be ascertained by determining their melting points in the range shown against each of them.

Eg: **Beeswax: 62–65°C**

Wool fat: 34–44°C

Agar: 85°C

7. Moisture content

- The moisture content of a drug will be responsible for the decomposition of crude drugs either producing chemical change or microbial growth.
- So the moisture content of a drug should be determined and controlled.
- The moisture content is determined by heating a drug at 105°C in an oven to a constant weight.

Eg: **Digitalis: 5% w/w**

Ergot: 8% w/w

8. Foreign Organic Matter

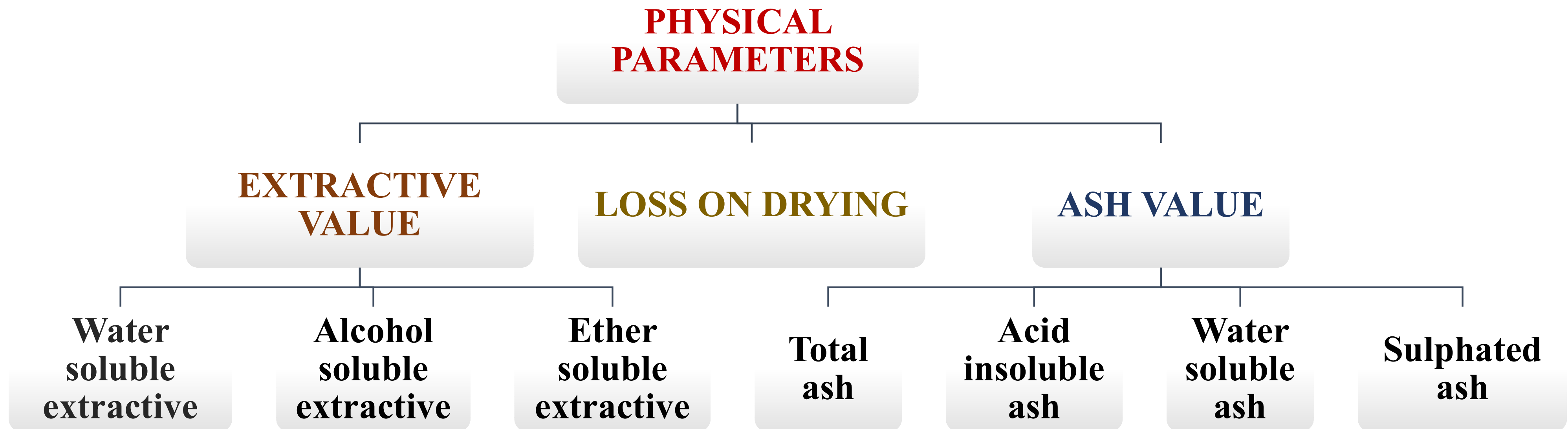
- The parts of the organ or organs other than those parts of drugs mentioned in the definition and description of the drug are known as foreign organic matters.
- They may be insect, molds, earthy material, animal excreta, etc.
- Each and every vegetable drug has its own limits.

Eg: **Garlic should not contain more than 2%**

Saffron should not contain more than 2%

Satavari should not contain more than 1%, etc.

IMPORTANT PHYSICAL PARAMETERS



ASH VALUE

- The residue left after incineration of the drug contains inorganic elements present in the plant is called as physiological ash.
- The determination of ash is useful for detecting low-grade products, exhausted drugs, and excess sandy or earthy matter.
- Ash values are helpful in determining the quality as well as purity of a crude drug, especially when the drug is present in powdered form.
- The object of ashing crude drugs is to remove the traces of organic matter which may interfere in an analytical determination.
- On incineration, the crude drugs normally produce ash which is usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.
- The total ash of a crude drug reveals the care taken during its preparation. A higher limit of acid-insoluble ash is incorporated especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.
- Ash value is a criterion to judge the identity or purity of the crude drug.
- The ash value varies within wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs.

1. Total Ash value

- Organic and carbon matter present in the drug is converted to ash at temperature of 450°C or above.
- It mostly contains carbonates, Phosphate, Silicate and Silica.
- Total ash value can be used further study water soluble and acid insoluble ash.

Procedure

Take about 2 or 3 g, accurately weighed, of the ground drug in a tarred platinum or silica dish previously ignited and weighed.

↓

Incinerate by gradually increasing the heat until free from carbon, cool, and weigh.

↓

If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water

↓

Calculate the percentage of ash with reference to the air dried drug.

2. Water-soluble ash value

- It is introduced by separating the **water soluble material** which is direct to yield water soluble ash.
- In this case most of water insoluble salt may contribute in total ash, or remove to find out the water soluble contents.

Procedure

The total ash obtained was boiled for 5 minutes with 25ml of water.

↓

Collected the insoluble matter on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450 °c.

↓

Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash.

↓

Calculated the percentage of water-soluble ash with reference to the air-dried drug.

3. Acid insoluble ash value

- Total ash may be treated with **dilute hydrochloric acid** which removes any inorganic salts to yield many silica in the residue at acid-insoluble ash.

Procedure

The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid.

↓

Collected the insoluble matter on an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°c.

↓

Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the acid insoluble ash.

↓

Calculated the percentage of acid insoluble ash with reference to the air-dried drug.

4. Sulphated ash value

- The crude drug is incinerated at a temperature about 600°C with dilute sulphuric acid before ignition. This process converts all amides and carbonates to sulphate salt.

Procedure

Heated a platinum crucible to redness for 10 minutes, allowed to cool in a desiccator and weighed.

↓

Put 3g of the powdered drug, accurately weighed, into the crucible, ignited gently at first, until the substance is thoroughly charred..

↓

Cooled and moistened the residue with 1 ml sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800±25 °C until all black particles have disappeared.

↓

The crucible was cooled, few drops of sulphuric acid was added and again heated.

↓

The ignition was carried out as before, cooled and weighed to get a constant weight. Calculated the percentage of sulphated ash with reference to the air-dried drug

EXTRACTIVE VALUE

- Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method.
- Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs.
- Crude drugs contain a number of constituents and these have a selective solubility in different solvents.
- ***Alcohol, water and ether are used as solvents to prepare ethanol-soluble extractive, water soluble extractive (chloroform water), ether soluble extractive*** respectively.
- Extractive values indicate the presence of different constituents and TLC fingerprints can be developed for identification and semi-quantitative analysis from these extracts.

Procedure

5 g of accurately weight powdered air-dried drug was macerated with 100 ml of alcohol/water/ether in a closed flask for twenty-four hours, shaking frequently during six hours and allow standing for eighteen hours

↓

It was then filtered rapidly, taking precautions against loss of solvent.

↓

25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish and dried at 105°C to a constant weight and weighed.

↓

The percentage of alcohol/water/ether soluble extractive was calculated with reference to the air-dried drug.

Significance

- ✓ Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents.
- ✓ Extractive values are primarily useful for the determination of exhausted or adulterated drugs and determines the quality as well as purity of the drug.
- ✓ Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating.

LOSS ON DRYING

- It is the loss of weight expressed as percentage w/w. It determines the amount of volatile matter of any kind (including water) that can be driven off under the conditions specified.

$$\text{Loss on drying (\%)} = \frac{\text{initial weight of sample - weight of sample after drying} \times 100}{\text{Initial weight of sample}}$$

PROCEDURE

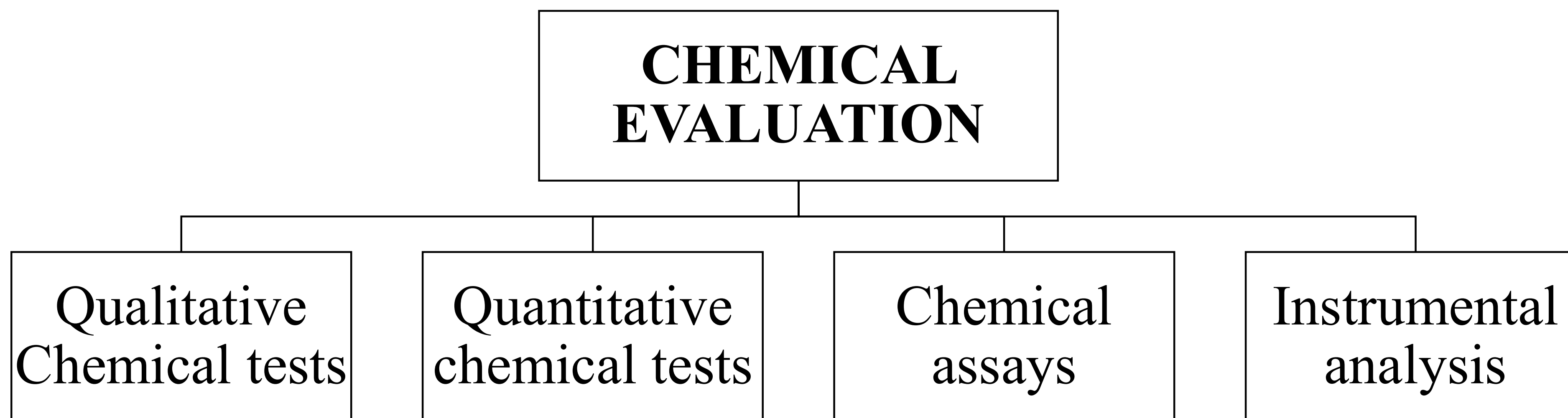
Accurately weighed about 1.5 g of powdered drug in a tarred porcelain dish and dried in an oven at 105 °C to constant weight, cooled in desiccator and weighed. From the difference in weights, percentage loss on drying was calculated.

SIGNIFICANCE

Loss on drying is a widely used test method to determine the moisture content of a sample, although occasionally it may refer to the loss of any volatile matter from the sample.

CHEMICAL EVALUATION

- Evaluation by chemical means;



2. Quantitative chemical tests

a) Acid value
b) Saponification value
c) Ester value
d) Acetyl value
e) Peroxide value
f) Hydroxyl value

These tests are helpful in the evaluation of drugs like volatile oil (acetyl and ester value), balsams (acid, esters, saponification value), resins (acid value) etc.

3. Chemical assays

- Assayed for a particular group of constituents.
- Commonly used techniques are **titrimetric and gravimetric methods**.

By titrimetric methods;

- ✓ Alkaloids can be estimated from alkaloidal drugs; eg: quinine from cinchona, reserpine from rauwolfia, atropine from belladonna
- ✓ Anthraquinone and cardiac glycosides.
- ✓ Estimation of carvone in dill oil, cineole in eucalyptus oil, aldehydes in lemon oil.

4. Instrumental analysis

INSTRUMENTAL ANALYSIS

CHROMATOGRAPHY

- Thin layer chromatography
- Paper chromatography
- High performance thin layer chromatography (HPTLC)
- Column chromatography
- High performance liquid chromatography (HPLC)
- Gas chromatography
- Gel permeation chromatography
- Affinity chromatography

SPECTROSCOPY

- Ultraviolet and Visible spectroscopy
- Infrared spectroscopy
- Flourimetry
- Nuclear Magnetic Resonance spectroscopy
- Mass spectroscopy
- Xray diffraction

BIOLOGICAL EVALUATION

- ✓ For the drugs cannot be evaluated using chemical & physical methods.
- ✓ Response produced on a test drug is compared with standard drug solution.
- ✓ Performed on living animals, isolated organ and tissue, animal preparations & micro-organisms.

Indication of Biological Evaluation:

- ✓ When the chemical nature of the drug is not known but is has an biological action.
- ✓ When chemical methods are not available.
- ✓ When the quantity of the drug is small, it cannot be evaluated chemically.
- ✓ Drugs which have different chemical composition but same biological activity. Example: Cardiac glycosides are evaluated by this method on cats, frogs or pigeons.

Examples;

- Antipyretic activity
- Anti-inflammatory activity
- Hypoglycaemic activity
- Antiulcer activity
- Analgesic activity
- Microbiological assays
- Anthelminthic activity

PHARMACOLOGICAL EVALUATION

- **Bitterness value**: Units equal to bitterness of std solution of quinine hydrochloride.
- **Heamolytic activity**: Ox blood comparison with std saponin.
- **Swelling index**: In water.
- **Foaming index**: Foam height produced by 14 gm material under std. conditions.

TOXICOLOGICAL EVALUATION

- **Pesticide residue**: estimation of chloride, phosphorus, lead, inorganic pesticides.
- **Arsenic & heavy metals**: limit tests
  - **Arsenic**: Stain produced in the $HgBr_2$ paper compared to std. stain.
  - **Heavy metals**: Atomic emission spectroscopy.
- **Microbial contamination**: maximum possible limits are determined. (E.coli, Salmonella, Enterobacteria)
- **Aflatoxins**: naturally occurring mycotoxins produced by Aspergillus species, determined by chromatographic methods using std aflatoxins B_1, B_2, G_1, G_2 mixtures.

11. Determination of bitterness value

Medicinal plant materials that have a strong bitter taste ("bitters") are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice.

Bitter substances can be determined chemically. However, since they are mostly composed of two or more constituents with various degrees of bitterness, it is first necessary to measure total bitterness by taste.

The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride R. The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R in 2000 ml.

Safe drinking-water should be used as a vehicle for the extraction of plant materials and for the mouth-wash after each tasting. Taste buds dull quickly if distilled water is used. The hardness of water rarely has any significant influence on bitterness.

Sensitivity to bitterness varies from person to person, and even for the same person it may be different at different times (because of fatigue, smoking, or after eating strongly flavoured food). Therefore, the same person should taste both the material to be tested and the quinine hydrochloride solution within a short space of time. The bitter sensation is not felt by the whole surface of the tongue, but is limited to the middle section of the upper surface of the tongue. A certain amount of training is required to perform this test. A person who does not appreciate a bitter sensation when tasting a solution of 0.058 mg of quinine hydrochloride R in 10 ml of water is not suitable to undertake this determination.

The preparation of the stock solution of each individual plant material (S_T) should be specified in the test procedure. In each test series, unless otherwise indicated, the determination should start with the lowest concentration in order to retain sufficient sensitivity of the taste buds.

Recommended procedure

Caution: This test should not be carried out until the identity of the plant material has been confirmed.

Preparation of solutions

Stock and diluted quinine hydrochloride solutions
Dissolve 0.100g of quinine hydrochloride R in sufficient safe drinking-water to produce 100 ml. Further dilute 5 ml of this solution to 500 ml with safe drinking-

Table 1
Determination of bitterness value: serial dilution for the initial test

	Tube no.								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
S_q (ml)	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
Safe drinking-water (ml)	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Quinine hydro-chloride in 10 ml of solution (= *c*) (mg)	0.042	0.044	0.046	0.048	0.050	0.052	0.054	0.056	0.058

S_q, stock solution of quinine hydrochloride.

Table 2
Determination of bitterness value: serial dilution for the second test

	Tube no.									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
S_T (ml)	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.0
Safe drinking-water (ml)	9.00	8.00	7.00	6.00	5.00	4.00	3.00	2.00	1.00	-

S_T, stock solution of the plant material being examined.

water. This stock solution of quinine hydrochloride (S_q) contains 0.01 mg/ml. Use nine test-tubes for the serial dilution for the initial test as indicated in Table 1.

Stock and diluted solutions of the plant material
Prepare the solution as specified in the test procedure for the given plant material (S_T). Use 10 test-tubes for the serial dilution for the test as indicated in Table 2.

Method
After rinsing the mouth with safe drinking-water, taste l0ml of the most dilute solution swirling it in the mouth mainly near the base of the tongue for 30 seconds. If the bitter sensation is no longer felt in the mouth after 30 seconds,

spit out the solution and wait for 1 minute to ascertain whether this is due to delayed sensitivity. Then rinse with safe drinking-water. The next highest concentration should not be tasted until at least 10 minutes have passed. The threshold bitter concentration is the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds. After the first series of tests, rinse the mouth thoroughly with safe drinking-water until no bitter sensation remains. Wait for at least 10 minutes before carrying out the second test.

In order to save time in the second test, it is advisable to ascertain first whether the solution in tube no. 5 (containing 5 ml of S_T in 10 ml) gives a bitter sensation. If so, find the threshold bitter concentration of the material by tasting the dilutions in tubes 1-4. If the solution in tube no. 5 does not give a bitter sensation, find the threshold bitter concentration by tasting the dilutions in tubes 6-10.

All solutions and the safe drinking-water for mouthwashing should be at 20-25 °C.

Calculate the bitterness value in units per g using the following formula:
2000 x c

$$\frac{2000 \times c}{a \times b}$$

where a = the concentration of the stock solution (S_T) (mg/ml),
b = the volume of S_T (in ml) in the tube with the threshold bitter concentration,
c = the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

12. Determination of haemolytic activity

Many medicinal plant materials, especially those derived from the families Caryophyllaceae, Araliaceae, Sapindaceae, Primulaceae, and Dioscoreaceae contain saponins. The most characteristic property of saponins is their ability to cause haemolysis: when added to a suspension of blood, saponins produce changes in erythrocyte membranes, causing haemoglobin to diffuse into the surrounding medium.

The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, saponin R, which has a haemolytic activity of 1000 units per g. A suspension of erythrocytes is mixed with equal volumes of a serial dilution of the plant material extract. The lowest concentration to effect complete haemolysis is determined after allowing the mixtures to stand for a given period of time. A similar test is carried out simultaneously with saponin R.

Procedures proposed for the determination of the haemolytic activity of saponaceous medicinal plant material are all based on the same principle although the details may vary, e.g. the source of erythrocytes, methods for the preparation of the erythrocyte suspension and the plant material extract, the defined haemolytic activity of the reference material of saponin, and the experimental method. In order to obtain reliable results, it is essential to standardize the experimental conditions, and especially to determine the haemolytic activity by comparison with that of saponin R.

Recommended procedure

To prepare the erythrocyte suspension fill a glass-stoppered flask to one-tenth of its volume with sodium citrate (36.5 g/l) TS, swirling to ensure that the inside of the flask is thoroughly moistened. Introduce a sufficient volume of blood freshly collected from a healthy ox and shake immediately. Citrated blood prepared in this way can be stored for about 8 days at 2-4°C. Place 1 ml of citrated blood in a 50-ml volumetric flask with phosphate buffer pH 7.4 TS and carefully dilute to volume. This diluted blood suspension (2% solution) can be used as long as the supernatant fluid remains clear and colourless. It must be stored at a cool temperature.

To prepare the reference solution, transfer about 10 mg of saponin R, accurately weighed, to a volumetric flask and add sufficient phosphate buffer pH 7.4 TS to make 100ml. This solution should be freshly prepared.

The extract of plant material and dilutions should be prepared as specified in the test procedure for the plant material concerned, using phosphate buffer pH 7.4 TS.

Table 3
Determination of haemolytic activity: serial dilution for the preliminary test

	Tube no.			
	1	2	3	4
Plant material extract (ml)	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4 TS (ml)	0.90	0.80	0.50	-
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00

Preliminary test
Prepare a serial dilution of the plant material extract with phosphate buffer pH 7.4 TS and blood suspension (2%) using four test-tubes as shown in Table 3.

As soon as the tubes have been prepared, gently invert them to mix, avoiding the formation of foam. Shake again after a 30-minute interval and allow to stand for 6 hours at room temperature. Examine the tubes and record the dilution at which total haemolysis has occurred, indicated by a clear, red solution without any deposit of erythrocytes. Proceed as follows.

- If total haemolysis is observed only in tube no. 4, use the original plant material extract directly for the main test.
- If total haemolysis is observed in tubes 3 and 4, prepare a two-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS.
- If total haemolysis is observed in tubes 2, 3 and 4, prepare a five-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS.
- If, after 6 hours, all four tubes contain a clear, red solution, prepare a ten-fold dilution of the original plant material extract with phosphate buffer pH 7.4 TS and carry out the preliminary test again as described above.
- If total haemolysis is not observed in any of the tubes, repeat the preliminary test using a more concentrated plant material extract.

Main test
Prepare a serial dilution of the plant material extract, undiluted or diluted as deter-mined by the preliminary test, with phosphate buffer pH 7.4 TS and blood suspension (2%) using 13 test-tubes as shown in Table 4.

Carry out the dilutions and evaluations as in the preliminary test but observe the results after 24 hours. Calculate the amount of medicinal plant material in g, or of the preparation in g or ml, that produces total haemolysis.

To eliminate the effect of individual variations in resistance of the erythrocyte suspension to saponin solutions, prepare a series of dilutions of saponin R in the same manner as described above for the plant material extract. Calculate the quantity of saponin R in g that produces total haemolysis.

Table 4
Determination of haemolytic activity: serial dilution for the main test

	Tube no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Plant material extract (diluted if necessary) (ml)	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
Phosphate buffer pH 7.4 TS (ml)	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.05	
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Calculate the haemolytic activity of the medicinal plant material using the following formula:

$$1000 \text{x} \frac{a}{b}$$

where 1000 = the defined haemolytic activity of saponin R in relation to ox blood,
a = quantity of saponin R that produces total haemolysis (g),
b = quantity of plant material that produces total haemolysis (g).

13. Determination of tannins

Tannins (or tanning substances) are substances capable of turning animal hides into leather by binding proteins to form water-insoluble substances that are resistant to proteolytic enzymes. This process, when applied to living tissue, is known as an "astringent" action and is the reason for the therapeutic application of tannins.

Chemically, tannins are complex substances; they usually occur as mixtures of polyphenols that are difficult to separate and crystallize. They are easily oxidized and polymerized in solution; if this happens they lose much of their astringent effect and are therefore of little therapeutic value.

Recommended procedure

To prepare the plant material extract, introduce the quantity specified in the test procedure for the plant material concerned, previously powdered to a known fineness and weighed accurately, into a conical flask. Add 150ml of water and heat over a boiling water-bath for 30 minutes. Cool, transfer the mixture to a 250-ml volumetric flask and dilute to volume with water. Allow the solid material to settle and filter the liquid through a filter-paper, diameter 12cm, discarding the first 50ml of the filtrate.

To determine the total amount of material that is extractable into water, evaporate 50.0ml of the plant material extract to dryness, dry the residue in an oven at 105°C for 4 hours and weigh (T_1).

To determine the amount of plant material not bound to hide powder that is extractable into water, take 80.0ml of the plant material extract, add 6.0g of hide powder R and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_2).

To determine the solubility of hide powder, take 6.0g of hide powder R, add 80.0 ml of water and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T_0). Calculate the quantity of tannins as a percentage using the following formula:

$$\frac{[T_1 - (T_2 - T_0)]x500}{w}$$

where w = the weight of the plant material in grams.

14. Determination of swelling index

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose.

The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized). Using a glass-stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture (in ml) is then read.

The mixing of whole plant material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent.

Recommended procedure

Carry out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125 mm, marked in 0.2-ml divisions from 0 to 25 ml in an upwards direction. Unless otherwise indicated in the test procedure, add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature, or as specified. Measure the volume in ml occupied by the plant material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of plant material.

15. Determination of foaming index

Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

Recommended procedure

Reduce about 1 g of the plant material to a coarse powder (sieve size no. 1250), weigh accurately and transfer to a 500-ml conical flask containing 100ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume.

Pour the decoction into 10 stoppered test-tubes (height 16cm, diameter 16mm) in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml, and adjust the volume of the liquid in each tube with water to 10ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows.

- If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.
- If a height of foam of 1 cm is measured in any tube, the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1 cm in every tube, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

Calculate the foaming index using the following formula:

$$\frac{1000}{a}$$

where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

16. Determination of pesticide residues

Medicinal plant materials are liable to contain pesticide residues which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administration of fumigants during storage. It is therefore recommended that every country producing medicinal plant materials (naturally grown or cultivated) should have at least one control laboratory capable of performing the determination of pesticides in accordance with the procedure outlined below.

Since many medicinal preparations of plant origin are taken over long periods of time, limits for pesticide residues should be established following the recommendations of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) which have already been established for food and animal feed (9). These recommendations include the analytical methodology for the assessment of specific pesticide residues.

Classification of pesticides

Different classifications of pesticides exist (*10, 11*). A classification based on the chemical composition or structure of the pesticide is most useful for analytical chemists, for example:

- chlorinated hydrocarbons and related pesticides: aldrin, HCH (hexachlorocy-clohexane or benzene hexachloride, BHC), hexachlorobenzene (HCB), chlordane, DDT (clofenotane), dieldrin, endrin, heptachlor, lindane, methoxychlor, camphechlor (toxaphene);
- chlorinated phenoxyalkanoic acid herbicides: 2,4-D; 2,4,5-T;
- organophosphorus pesticides: carbophenothion (carbofenotion), coumaphos (coumafos), demeton, dichlorvos, dimethoate, ethion, fenchlorphos (fenclofos), malathion, methyl parathion, parathion;
- carbamate insecticides: carbaryl (carbaril);
- dithiocarbamate fungicides: ferbam, maneb, nabam, thiram, zineb, ziram;
- inorganic pesticides: aluminium phosphide, calcium arsenate, lead arsenate;
- miscellaneous: bromopropylate, chloropicrin, ethylene dibromide, ethylene oxide, methyl bromide;
- pesticides of plant origin: tobacco leaf and nicotine; pyrethrum flower, pyrethrum extract and pyrethroids; derris root and rotenoids.

Only the chlorinated hydrocarbons and related pesticides (e.g. aldrin, chlordane, DDT, dieldrin, HCH) and a few organophosphorus pesticides (e.g. carbophenothion) have a long residual action. Most other pesticides have very short residual actions. Therefore it is suggested that, where the length of exposure to pesticides is unknown, the medicinal plant material should be tested for the presence of organically bound chlorine and phosphorus, or the content of these two substances should be determined. Alternative classifications of pesticides may be based on their intended use, for example:

- insecticides;
- fungicides and nematocides;

- herbicides;
- other pesticides (e.g. acaricides, molluscicides, rodenticides);
- fumigants (e.g. ethylene oxide, ethylene chlorohydrin, methyl bromide).

Methods for the determination of pesticide residues

Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues. Samples are extracted by a standard procedure, impurities are removed by partition and/or adsorption, and the presence of a moderately broad spectrum of pesticides is measured in a single determination. However, these techniques are not universally applicable. Some pesticides are satisfactorily carried through the extraction and clean-up procedures, others are recovered with a poor yield, and some are lost entirely. In chromatography, the separations may not always be complete, pesticides may decompose or metabolize, and many of the metabolic products are still unknown. Consequently, as a result of limitations in the analytical technique and incomplete knowledge of pesticide interaction with the environment, it is not yet possible to apply an integrated set of methods that will be satisfactory in all situations.

It is therefore desirable to test plant materials of unknown history for broad groups of compounds rather than for individual pesticides. A variety of methods meet these requirements. Chlorinated hydrocarbons and other pesticides containing chlorine in the molecule, for example, can be detected by the measurement of total organic chlorine; insecticides containing phosphate can be measured by analysis for total organic phosphorus, while pesticides containing arsenic and lead can be detected by measurement of total arsenic or total lead, respectively. Similarly, the measurement of total bound carbon disulfide in a sample will provide information on whether residues of the dithiocarbamate family of fungicides are present.

If the pesticide to which the plant material has been exposed is known or can be identified by suitable means, an established method for the determination of that particular pesticide residue should be employed.

General aspects of analytical methodology

The samples should be tested as quickly as possible after collection, before any physical or chemical changes occur. If prolonged storage is envisaged, the samples should preferably be stored in air-tight containers under refrigeration.

Light can cause degradation of many pesticides, and it is therefore advisable to protect the samples and any extracts or solutions from undue exposure.

The type of container or wrapping material used should not interfere with the sample or affect the analytical results.

Solvents and reagents used in the analytical method should be free from substances that may interfere with the reaction, alter the results or provoke degradation of the pesticide residue in the sample. It is usually necessary to employ specially purified solvents or to distil them freshly in an all-glass

apparatus. Blank determinations with the solvents should be carried out, concentrating and testing them as specified in the test procedure for the plant material concerned.

The simplest and quickest procedure should be used to separate unwanted material from the sample (clean-up procedure) in order to save time when many samples have to be tested.

The process of concentrating solutions should be undertaken with great care, especially during the evaporation of the last traces of solvent, in order to avoid losses of pesticide residues. For this reason, it is often not advisable to remove the last traces of solvent. Agents, such as mineral oil or other oils of low volatility, that may help to preserve the solution could be added to retard the loss of the relatively volatile pesticides, especially when the last traces of solvent are being evaporated. However, these agents, while satisfactory in colorimetric procedures, are usually not desirable in gas chromatographic methods. It may be necessary to evaporate heat-labile compounds using a rotary vacuum apparatus.

Maximum limit of pesticide residues for medicinal plant materials

The toxicological evaluation of pesticide residues in medicinal plant materials should be based on the likely intake of the material by patients. In general, the intake of residues from medicinal plant materials should account for no more than 1% of total intake from all sources, including food and drinking-water. Certain plant materials may contain extremely high levels of pesticide residues, but the levels remaining after extraction are usually much lower, because of the low solubility in water or ethanol. It is therefore important to determine the actual quantity of residues consumed in the final dosage form.

Where the nature of the pesticide to which the plant material has been exposed is unknown, it is sufficient to determine the content of total chlorine and to base the calculation on the acceptable residue level (ARL) of the most toxic chlorine-containing pesticide (e.g. aldrin or dieldrin).

An ARL (in mg of pesticide per kg of plant material) can be calculated on the basis of the maximum acceptable daily intake of the pesticide for humans (ADI), as, recommended by FAO and WHO, and the mean daily intake (MDI) of the medicinal plant material.

Some countries have established national requirements for residue limits in plant materials. Where such requirements do not exist, the following formula may be used:

$$ARL = \frac{ADI \times E \times 60}{MDI \times 100}$$

where ADI = maximum acceptable daily intake of pesticide (mg/kg of body weight);

E = extraction factor, which determines the transition rate of the pesticide from the plant material into the dosage form;

MDI = mean daily intake of medicinal plant product.

The 60 in the numerator represents mean adult body weight, while the denominator incorporates a consumption factor of 100 reflecting the fact that no more than 1% of the total pesticide residue consumed should be derived from medicinal plant material.

This formula is based on the acceptable daily intake (ADI) determined by FAO and WHO.

Determination of total chlorine and phosphorus
Most pesticides contain organically bound chlorine or phosphorus.

Recommended procedure

Preparation of samples
Reduce the plant material to a fine powder, and extract with a mixture of water and acetonitrile R. Most pesticides are soluble in this mixture, while most cellular constituents (e.g. cellulose, proteins, amino acids, starch, fats and related compounds) are sparingly soluble and are thus removed. A number of polar and moderately polar compounds may also be dissolved; it is therefore necessary to transfer the pesticides to light petroleum R. For pesticides containing chlorine, further purification is seldom required, but for those containing phosphorus, further purification by column chromatography may be necessary, eluting with mixtures of light petroleum R and ether R.

Preparation of the column
Use Florisil R grade 60/100 PR (or equivalent), activated at 650°C, as the support. If this material is obtained in bulk, transfer it immediately after opening to a 500-ml glass jar or bottle with a glass stopper or foil-lined, screw-top lid. Store in the dark. Before use, heat at not less than 130°C, cool in a desiccator to room temperature and heat once again to 130°C after 2 days.

Prepare a Florisil column (external diameter, 22 mm) which contains, after settling, 10 cm of activated Florisil topped with about 1 cm of anhydrous sodium sulfate R. Pre-wet the column with 40-50 ml of light petroleum R. Place a graduated flask under the column to receive the eluate.

Method
Grind the material to pass through a sieve no. 710 or 840 and mix thoroughly. Place 20-50 g of the ground sample into a blender, add 350 ml of acetonitrile R with a water content of 35% (to 350 ml of water add sufficient acetonitrile R to produce 1000 ml). Blend for 5 minutes at a high speed. Filter under vacuum

through an appropriate funnel, diameter 12 cm, fitted with filter-paper, into a 500-ml suction flask.

Transfer the filtrate to a 250-ml measuring cylinder and record the volume. Transfer the measured filtrate to a 1-litre separating funnel and carefully add 100ml of light petroleum R. Shake vigorously for 1-2 minutes, add 10 ml of sodium chloride (400 g/l) TS and 600 ml of water. Hold the separating funnel in a horizontal position and mix vigorously for 30-45 seconds. Allow to separate, discard the aqueous layer and gently wash the solvent layer with two 100-ml portions of water. Discard the washings, transfer the solvent layer to a 100-ml glass-stoppered cylinder, and record the volume. Add about 15 g of anhydrous sodium sulfate R and shake vigorously. The extract must not remain in contact with this reagent for longer than 1 hour. Transfer the extract directly to a Florisil column; if necessary, reduce the volume first to 5-10 ml. Allow it to pass through the column at a rate of not more than 5 ml per minute. Carefully rinse the cylinder with two portions, each of 5 ml, of light petroleum R, transfer them to the column, rinse with further small portions of light petroleum R if necessary, and then elute at the same rate with 200 ml of ether/light petroleum TS1. Change the receiver and elute with 200 ml of ether/light petroleum TS2. Again change the receiver and elute with 200 ml of ether/light petroleum TS3. Evaporate each eluate to a suitable volume, as required, for further testing.

- The first eluate contains chlorinated pesticides (aldrin, DDE, TDE (DDD), o,p'-and p,p'-DDT, HCH, heptachlor, heptachlor epoxide, lindane, methoxychlor), polychlorinated biphenyls (PCB), and phosphated pesticides (carbophenothion, ethion, and fenchlorphos).
- The second eluate contains chlorinated pesticides (dieldrin and endrin) and phosphated pesticides (methyl parathion and parathion).
- The third eluate contains phosphated pesticide (malathion).

Combustion of the organic matter

Combustion of the organic matter in oxygen is the preparatory step for the determination of chlorine and phosphorus. The pesticide is extracted from the sample and purified, if necessary. The extract is concentrated, evaporated to dryness, transferred to a sample holder, and burned in a suitable conical flask flushed with oxygen. The gases produced during combustion are then absorbed in a suitable solution. The absorbed chlorine is determined as chloride and the absorbed phosphorus as orthophosphate, both colorimetrically.

Apparatus

The combustion is carried out in a 1-litre conical flask made of borosilicate glass, into the stopper of which is fused one end of a piece of platinum wire about 1 mm in diameter. To the free end of the wire is attached a piece of platinum gauze measuring about 1.5 x 2 cm to provide a means of holding the sample clear of the absorbing liquid during combustion.

Sample holder for chlorine-containing residues. For a small quantity of solid material, use a sample holder made from a piece of halide-free filter-paper about 5 cm long and 3 cm wide; for a small volume of liquid, preferably use a sample

holder in the form of a cone made from cellulose acetate film. Prepare the cone as follows: wearing cloth gloves and using a suitable cardboard template cut the film in a circle of 4 cm radius.

Manually pin the two edges together to form a cone. Seal the joined edges using heat to form a seam about 5 mm wide. Immerse the seam in acetone R to about one half of its width for 10 seconds. Remove and dry it immediately in a stream of hot air. Using forceps, wash the cone by dipping in a 1-litre beaker containing warm sodium hydroxide (~240 g/l) TS for 10 seconds at a temperature of about 60°C. Rinse the cone thoroughly with water and allow to drain dry on a piece of aluminium foil. Place each cone in a clean funnel (diameter 65 mm).

Sample holder for phosphorus-containing residues. Use a piece of halide-free filter-paper about 4 cm square as the sample holder.

Combustion of chlorine-containing residues
Transfer an aliquot of the extract as prepared above onto the sample holder which is placed in a funnel using a solvent that will not dissolve the sample holder. Allow the solvent to evaporate. Wearing rubber gloves, remove the sample holder and its dry contents from the funnel, and fold it over and up to form a small packet, about 1 cm^2 in area, and secure it in the centre of the platinum gauze. Insert a narrow strip of filter-paper, about 1 x 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 30 ml of water to the combustion flask. Moisten the neck of the flask with water. Fill the flask thoroughly with oxygen by means of a tube with its end just above the liquid. Ignite the free end of the paper strip and immediately insert the stopper. Hold the stopper firmly in place. When vigorous burning has begun, tilt the flask to prevent incompletely burned material from falling into the liquid. Immediately after combustion is completed, shake the flask vigorously for 10 minutes to dissolve the combustion products. Place a small quantity of water around the rim of the flask, and carefully withdraw the stopper. Rinse the stopper, platinum wire, platinum gauze and sides of the flask with water. Transfer the liquid and liquids used for rinsing to a 50-ml volumetric flask and dilute to volume with water.

Combustion of phosphorus-containing residues
Dip the sample holder made from filter-paper into methanolic sodium hydroxide TS, then suspend it in a current of heated air. Immediately transfer about 0.2 ml of an aliquot of the extract as prepared above to the sample holder with the aid of 0.2-ml portions of chloroform R using a micropipette. Allow the solvent to evaporate from the paper, fold it to form a small packet about 1 cm^2 in area and place it in the centre of the platinum gauze. Insert a strip of filter-paper, about 1 x 3 cm, as a fuse into the top of the holder, between the folds of the packet. Add 10 ml of sulfuric acid (~37 g/l) TS to the combustion flask and continue with the combustion as described above. Transfer the solution and the liquid used for rinsing to a 25-ml volumetric flask and dilute to volume with water.

Determination of chlorides

Apparatus
The determination is made with a spectrophotometer capable of measuring absorbance at 460 nm using absorption cells with path-lengths of 2 cm and 10 cm.

Method
Place 15 ml of the solution obtained after combustion in a 50-ml conical flask together with 1 ml of ferric ammonium sulfate (0.25 mol/l) VS and 3 ml of mercuric thiocyanate TS. Swirl the contents of the flask and allow to stand for 10 minutes. Transfer a portion of the solution to a 2-cm cell and measure the absorbance at 460 run using water in the reference cell. The reading should be made promptly to minimize absorption of chloride from the air.

Prepare a standard solution of sodium chloride R containing 5 µg of chloride per ml. Transfer aliquots of this solution (0 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml) into a series of 50-ml conical flasks and dilute to 15 ml with water. Develop the colour and measure the absorbances as described above. Plot the absorbances against the chloride content of the dilutions in µg per ml and interpolate the chloride content of the solutions of the material tested.

Determination of phosphates
The phosphomolybdate method is based on the reaction of phosphate ions with ammonium molybdate to form a molybdophosphate complex, which is subsequently reduced to form a strongly blue-coloured molybdenum complex. The intensity of the blue colour is measured spectrophotometrically. This method is applicable for the determination of any phosphates that have undergone a prior separation procedure.

Naturally occurring phosphates are present in most samples, and are often not removed during the clean-up procedure. In order to obtain background values, therefore, it is necessary to proceed with the determination for all samples, even those with no phosphate-containing pesticides. These background values should be subtracted from the results obtained on testing pesticide residues. Extracts of most uncontaminated materials contain about 0.05-0.1 mg/kg of phosphorus. Therefore, no contamination with organophosphate pesticides can be assumed for results in this range.

Apparatus
The determination is made with a spectrophotometer capable of measuring absorbance at 820 nm using an absorption cell with a path-length of 1 cm.

Method
Place 7 ml of the solution obtained after combustion in a calibrated 10-ml test-tube. Add 2.2 ml of sulfuric acid (300 g/l) TS and mix the solution well. Add 0.4 ml of ammonium molybdate (40 g/l) TS and swirl the mixture. Then add 0.4 ml of aminonaphtholsulfonic acid TS and swirl again. Heat the solution to 100°C for

12 minutes (±2 minutes), cool, and transfer a portion to a 1-cm cell. Measure the absorbance at 820 nm using water in the reference cell.

Prepare standard dilutions with a known content of phosphate and measure the absorbance as described above. Plot the absorbances against the phosphate content of the dilutions in µg per ml and interpolate the phosphate content of the solutions of the material tested.

Qualitative and quantitative determination of organochlorine pesticides

Recommended procedure

Preparation of sample

Place 20 g of powdered plant material (sieve no. 180), accurately weighed, in a 500-ml beaker (tall form), mix with 98 ml of water and allow to macerate for at least 30 minutes. Add 200 ml of acetone R; the resulting volume of extraction solvent will be 295 ml. Extract for 5 minutes, while cooling and using a high-speed mixer. Filter the homogenized mixture through a porcelain filter (Büchner funnel, diameter 70mm) fitted with a filter-paper, using a slight vacuum, into a 250-ml graduated cylinder, allowing the process to last no longer than 1 minute, and then measure the volume *(V)* of the filtrate in ml.

Method

Transfer the filtrate prepared as above to a 500-ml separating funnel. Add a quantity of sodium chloride R equivalent in grams to one-tenth of the volume of the filtrate, then add 100 ml of dichloromethane R. Shake vigorously for 5 minutes, allow the phases to separate and discard the lower (aqueous) layer. Dry the acetone-dichloromethane phase, transfer it to a 500-ml conical flask, add 25 g of anhydrous sodium sulfate R and swirl occasionally. Next, filter the solution into a 500-ml flask with a ground-glass stopper using a glass funnel (diameter 100 mm) containing purified glass-wool and anhydrous sodium sulfate R. Rinse the separating funnel, the conical flask and the glass funnel twice with 10 ml of ethyl acetate R. Add 5 ml of 2,2,4-trimethylpentane R, and concentrate the crude extract to about 2 ml in a rotary vacuum evaporator in a water-bath at 30-40°C. Expel the remaining solvent in a gentle stream of air.

To purify by gel chromatography, macerate 50g of suitable beads (e.g. S-X3 bio-beads) in an elution mixture of cyclohexane R and ethyl acetate R (1:1) and pour them into a chromatographic column (length 600 mm, diameter 25 mm) adapted for use with a vacuum pump. Rinse the gel bed with the elution mixture under air-free conditions. Dissolve the extract in the flask with 5.0 ml of ethyl acetate R. Add 2 g of anhydrous sodium sulfate R, swirl gently and add 5.0 ml of cyclohexane R. Filter the completely dissolved crude extract through a rapid filter into a 10-ml test-tube with a ground-glass stopper and close the tube immediately. Then transfer 5.0 ml of the filtrate onto the gel column. Elute with the elution mixture at an average rate of 5.0 ml/minute. Plant material components leave the gel column first, followed by the active ingredients of pesticides. Fractionation must be determined for each column, using appropriate reference substances.

Discard the first fraction (about 100 ml) containing the impurities. Collect the organochlorine pesticides appearing in the next eluate (about 70ml) in a flask with a ground-glass stopper. Add l0ml of 2,2,4-trimethylpentane R and concentrate the solution to about 5 ml in a rotary vacuum evaporator and a water-bath at 30-40°C. Pipette another 5 ml of 2,2,4-trimethylpentane R into the flask and carefully evaporate the solution to about 1 ml (do not allow to become completely dry).

Calculate the amount of plant material in g in the purified extract using the following formula:

$$\frac{V}{590} \text{ x sample weight in g}$$

where V = volume of filtrate.

To purify further, transfer 1 g of previously deactivated silica gel for column chromatography (70-230 mesh) containing 1.5% of water, to a chromatographic column (length 25 cm, internal diameter 7 mm). Put 10 mm of anhydrous sodium sulfate R on top of the content of the column and cover with purified glass-wool. Before use rinse the column with 5 ml of hexane R. Allow the solvent to reach the surface of the column filling, then transfer quantitatively, by means of a pipette, the purified extract obtained by gel chromatography from the flask to the prepared silica gel column and rinse with 1 ml of hexane R. Set the flask aside for subsequent elutions.

Using a 10-ml volumetric flask as the receiver, elute any residues of polychlorinated biphenyls from the column with 10 ml of hexane R (eluate 0). Add 2 ml of an elution mixture composed of toluene R/hexane R (35:65) to the flask and swirl. Quantitatively transfer the solution to the column. Using another 10-ml volumetric flask as the receiver, elute the majority of the organochlorine pesticides from the silica gel column using 6 ml of the same elution mixture. Dilute the contents of the flasks to volume with the elution mixture (eluate 1).

Rinse the flask with 2 ml of toluene R and transfer it quantitatively to the column. Collect the eluate in a third 10-ml volumetric flask. Add 8 ml of toluene R to the flask, swirl and transfer the solution to the silica gel column; elute the remaining organochlorine pesticides using the same receiver. Dilute the contents of the flask to volume with toluene R (eluate 2).

Evaluate the test solutions by capillary gas chromatography using an electron capture detector (ECD). Confirm the findings obtained for the main column (first separation system) with a second capillary column of different polarity (second separation system).

Determination by gas chromatography
A capillary gas chromatograph with an ECD is used for the measurement. Helium R is used as the carrier gas and a mixture of argon R and methane R (95:5) as an auxiliary gas for the detection.

First separation system
Use a vitreous silica column, 30 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 5% phenyl, 95% methyl-polysiloxane. Use the following temperature programme:

- heat at 60°C for 0.5 minutes;
- increase the temperature at a rate of 30°C per minute to 160°C and maintain this temperature for 2 minutes;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use a "split/split-free" injector to inject the sample solution and maintain the injection port at a temperature of 240°C. Inject a volume of 1 µl at a rate of 30 seconds ("split-free"). The detector temperature should be 300°C.

Second separation system
Use a vitreous silica column, 15 m long with an internal diameter of 0.25 mm, packed with a chemically bound phase of 7% cyanopropyl, 7% phenyl, 86% methyl-polysiloxane. Use the following temperature programme:

- heat at 60°C for 0.2 minutes;
- increase the temperature at a rate of 30°C per minute to 180°C and maintain this temperature for 1 minute;
- increase the temperature at a rate of 2°C per minute to 250°C and maintain this temperature for 5 minutes.

Use an on-column injector to inject a volume of 1 µl of the sample solution. The detector temperature should be 300°C.

Use the "external standard" method for the qualitative and quantitative evaluation of the organochlorine pesticides in the test solutions with reference solutions of the following pesticides: α-, β-, γ- and δ-hexachlorocyclohexane (HCH); hexachlorobenzene; quintozene; aldrin; dieldrin; endrin; α- and β-endosulfan; endosulfan sulfate; heptachlor, heptachlorepoxide; camphechlor; TDE, DDE and DDT (both *o,p'*- and *p,p'*-isomers); methoxychlor.

Measure the peak height of the pesticides obtained in the chromatograms and calculate the concentration of the residues in mg/kg using the following formula:

$$\frac{h_t \text{ x}10}{w} x \frac{w_r}{h_r}$$

where h_t = peak height obtained for the test solution in mm,
w = quantity of sample in the purified extract (g),
w_r = quantity of pesticide in ng in the reference solution injected,
h_r = peak height obtained for the reference solution in mm.

Analysis of esters of organophosphorus compounds

The extraction and the clean-up procedures can be performed as described above, but the detection requires a phosphorus flame ionization detector (P-FID).

Determination of specific pesticide residues in plant material

General recommendations
For the total determination, mix thoroughly 1 kg of plant material.

In order to obtain reliable chromatographic results, do one or more of the following:

- repeat the separation using another column;
- use a different separation system;
- use a different detector system;
- apply a coupling technique;
- prepare a derivative;
- perform chromatography with a mixture of the sample and a reference substance;
- change the sample preparation;
- use a fractionated elution during the column-chromatography clean-up procedure of the plant extract and test every fraction by chromatography; and
- compare the distribution coefficient of the material with that of a reference substance.

Prior to the quantitative determination of the material to be tested, check whether there is a linear relationship between the values obtained for the reference substance and its concentration over the range 0.1-2 times the standard concentration. Otherwise, prepare another concentration range or evaluate the results using a reference curve. Use any suitable mechanical or manual technique for the chromatographic determination.

Store the reference solutions protected from light to prevent decomposition. Use glass vessels closed with glass stoppers and keep them in a container saturated with the solvent employed to avoid any increase in concentration due to evaporation. Check the loss by evaporation by interim weighing of the vessels.

Use concentrated reference solutions within 6 months and diluted reference solutions within 4 weeks.

Rate of recovery
The rate of recovery (R) is the percentage of the reference material, originally added to the plant material, that is determined using the method described below.

Determination of desmetryn, prometryn, and simazine residues

Preparation of the plant material extract
Place 10.0 g of powdered plant material in a 500-ml conical flask and add 125.0 ml of chloroform R. Shake the mixture for 60 minutes and filter under reduced pressure through a filter-paper (medium grade) into a round-bottomed flask. Wash the residue with 3 successive volumes each of 25.0 ml of chloroform R.

Method
Concentrate the combined filtrates to a volume of 3-5 ml using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the extract to a chromatographic column as prepared below, rinsing the round-bottomed flask twice with 5.0 ml of chloroform R.

Preparation of chromatographic column
Use a glass tube (internal diameter 20-22 mm) with a restricted orifice and protected with a sintered-glass plate (e.g. P10 or P16, glass filter G4; or P40, glass filter G3). Fill the column with chloroform R, then pour purified aluminium oxide R into it to form a 100-mm thick layer. The support material should remain covered with chloroform R. After transferring the extract and the rinsing liquids to the column, elute with 150.0 ml of chloroform R, at a rate of 1-2 drops per second, collecting the eluate in a round-bottomed flask. The first purifying process is completed when no further eluate drips from the column.

Evaporate the eluate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. To the residue add 10.0 ml of light petroleum R and transfer the mixture to a chromatographic column containing a layer of purified aluminium oxide R, 50 mm thick, in light petroleum R. Elute the mixture with 90.0 ml of light petroleum R, using this to rinse the round-bottomed flask, at a rate of 1-2 drops per second. Discard the eluate. Dissolve any remaining residue which has not dissolved in light petroleum R in 10.0 ml of a mixture composed of 60 volumes of chloroform R and 40 volumes of light petroleum R and transfer the solution to the column. Rinse the round-bottomed flask twice more with 10.0 ml of the solvent mixture. Transfer the liquid used for rinsing to the column. Elute with 120.0 ml of the same solvent mixture, at a rate of 1-2 drops per second and collect the eluate in a round-bottomed flask. The second purifying process is completed when no further eluate drips from the column.

Evaporate the eluate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. To prepare a purified extract for the determination by gas chromatography, dissolve the residue in sufficient acetone R to produce a volume of 10.0 ml. If an especially purified extract is required, proceed as described below.

To the residue add 10.0 ml of light petroleum R and 10.0 ml of dimethyl sulfoxide R. Shake the mixture and transfer it to a separating funnel. Extract the dimethyl sulfoxide layer twice with 10.0 ml of light petroleum R. Discard the petroleum ether extract. Then add 100 ml of water to the dimethyl sulfoxide layer and extract 3 times, each with 20.0 ml of chloroform R. Extract the combined chloroform extracts twice with 20.0 ml of water and evaporate them to dryness using a rotary vacuum evaporator and a water-bath at 40°C. Transfer the residue along with a mixture of 10.0 ml of light petroleum R and 10.0 ml of hydrochloric acid (1 mol/l) VS to a separating funnel and extract the mixture first with 10.0 ml and then with 5.0 ml of hydrochloric acid (1 mol/l) VS. Discard the petroleum ether layer and adjust the pH of the combined aqueous solutions to a value between 7 and 8 using sodium hydroxide (1 mol/l) VS. Extract the solution 3 times, each with 20.0 ml of chloroform R. Dry the combined chloroform extracts with anhydrous sodium sulfate R and filter into a round-bottomed flask, rinsing the funnel 3 times with 10.0-ml portions of chloroform R. Evaporate the filtrate to dryness using a rotary vacuum evaporator and a water-bath at 40°C. Dissolve the residue in sufficient acetone R to produce 10.0 ml of especially purified extract to be used for the determination by gas chromatography.

Use the extracts as indicated below for the following plant materials:

No.	Material	No.	Material
1	Flores Calendulae	10	Fructus Foeniculi
2	Flores Chamomillae	11	Herba Millefolii
3	Folia Melissae	12	Herba Plantaginis anceolatae
4	Folia Menthae piperitae	13	Radix Althaeae
5	Folia Salviae	14	Radix Angelicae
6	Folia Thymi	15	Radix Levistici
7	Fructus Carvi	16	Radix Petroselini
8	Fructus Coriandri	17	Radix Valerianae
9	Fructus Cynobasti		

For materials no. 1 and 2, use an especially purified extract (see page 58); for materials no. 3-17, use a purified extract (see page 58).

Determination of the rate of recovery

Prepare five individual samples using each of the following procedures:

1. To prepare solution S_2, first dissolve separately 0.040 g of each of the reference substances, desmetryn R, prometryn R and simazine R, in sufficient acetone R to produce 100.0 ml. Then place 5.0 ml of each solution into a 100-ml volumetric flask and dilute the mixture to volume with acetone R (S_2). Place 10.0 g of powdered plant material into a 500-ml conical flask and add 1.0 ml of solution S_2. Shake this mixture

mechanically for 60 minutes; if necessary, repeat the operation manually and then proceed as described under "Preparation of the plant material extract". Use either the purified or especially purified extract for the determination by gas chromatography, as specified in the test procedure for the plant material concerned.

2. Treat 10.0 g of powdered plant material as described under "Preparation of the plant material extract". Use either the purified extract or the especially purified extract for the determination by gas chromatography, as specified in the test procedure for each individual plant material.

Calculate the rate of recovery (R) in % using the following formula:

$$\frac{2\,(a - b)}{c}$$

where a = average quantity in mg/kg of the 5 residues obtained using procedure 1,
b = average quantity in mg/kg of the 5 residues obtained using procedure 2,
c = quantity of reference substances in mg contained in solution S_2 during procedure 1.

The rate should be within the range 70-120%. It is specific for each drug.

Determination by gas chromatography
Perform the determination as described in Volume 1 of *The international pharmacopoeia* (5).

Apparatus
The equipment consists of:

- a glass column 1.2 m long, internal diameter 2 mm;
- a suitable stationary liquid phase;
- a suitable diatomaceous support.

Use nitrogen R as the carrier gas with a flow rate of 30.0 ml/min. The sample injection block should be maintained at 230°C, the column at 190°C and the detector, which should be nitrogen-selective, at 300°C. In addition:

- volume of sample solution to be injected: 2.0 µl;
- separation characteristics: $h \leq 1.2 \times 10^{-3}$ for desmetryn R; $R_S \geq 1.2$ for prometryn R and simazine R;
- relative standard deviation (precision of chromatographic system): $s_r \leq 0.05$ for desmetryn R, prometryn R and simazine R.

Method
Chromatogram T. To determine the separation characteristics, inject solution S_2 (for the preparation of solution S_2 see "Determination of the rate of recovery" above). Chromatograms A_1-A_5. To determine the relative standard deviation, inject solution S_2 and repeat the determination 5 times.

Chromatogram S_2. Inject 1.0 ml of solution S_2 for the determination of the rate of recovery. Dilute 1.0 ml of solution S_2 to 10.0 ml with acetone R and inject it for the chromatographic determination. On the chromatogram the peaks occur in the following sequence: prometryn, simazine, desmetryn.

Chromatogram P_2. Inject the purified extract or the especially purified extract. Determine using an external standard: $a = 0.0005$To convert the values obtained to percentage by weight, multiply the concentration in mg/kg by 10^4.

The total maximum permissible amount of residues due to desmetryn, prometryn and simazine is 2 mg per kg of plant material.

17. Determination of arsenic and heavy metals

Contamination of medicinal plant materials with arsenic and heavy metals can be attributed to many causes including environmental pollution and traces of pesticides.

Limit test for arsenic

The amount of arsenic in the medicinal plant material is estimated by matching the depth of colour with that of a standard stain.

Recommended procedure

Preparation of the sample by acid digestion
Place 35-70g of coarsely ground material, accurately weighed, in a Kjeldahl flask, capacity 800-1000 ml. Add 10-25 ml of water and 25-50 ml of nitric acid (~1000 g/l) TS and then carefully add 20 ml of sulfuric acid (~1760g/l) TS. Heat cautiously so that no excessive foaming takes place. Gradually add nitric acid (~10008/l) TS, drop by drop, until all the organic matter is destroyed. This is achieved when no further darkening of the solution is observed with continued heating, and a clear solution with copious vapours of sulfur trioxide is obtained. Cool, and add 75 ml of water and 25 ml of ammonium oxalate (25 g/l) TS. Heat again until sulfur trioxide vapours develop. Cool, transfer with the help of water to a 250-ml volumetric flask, and dilute to volume with water.

Apparatus
A suitable type of apparatus is constructed as follows. A wide-mouthed bottle of about 120-ml capacity is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of about 200mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). The lower end of the tube is drawn out to an internal diameter of about 1 mm, and there is a hole not less than 2 mm in diameter blown in the side of the tube, near the constricted part. The tube is positioned so that when the bottle contains 70ml of liquid the constricted end is above the surface of the liquid and the hole in the side is below the bottom of the bung. The upper end of the tube has a flat, ground surface at right-angles to the axis of the tube, with slightly rounded-off edges.

One of two rubber bungs (about 25 mm x 25 mm), each with a central hole of exactly 6.5 mm diameter, is fitted at the upper end of the tube. The other bung is fitted with a piece of glass tube about 3 mm long and with an internal diameter of exactly 6.5 mm and with a similar ground surface. One end of each of the tubes is flush with the larger end of the bungs, so that when these ends are held tightly together with a rubber band or a spring clip, the openings of the two tubes meet to form a true tube. Alternatively, the two bungs may be replaced by any suitable construction satisfying the conditions described in the test.

Method

Moisten some cotton-wool with lead acetate (80g/l) TS, allow to dry, and lightly pack into the tube which fits into the wide-mouthed bottle to not less than 25 mm from the top. Between the flat surfaces of the tubes, place a piece of mercuric bromide paper AsR that is large enough to cover their openings (15 mm x 15 mm). The mercuric bromide paper AsR can be fitted by any other means provided that:

- the whole of the evolved gas passes through the paper;
- the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and
- the paper is protected from sunlight during the test.

Place an aliquot (25-50ml) of the solution being tested, prepared as described above, in the wide-mouthed bottle, add 1 g of potassium iodide AsR and l0g of granulated zinc AsR, and place the prepared glass tube assembly quickly in position. Allow the reaction to proceed for 40 minutes. Compare any yellow stain that is produced on the mercuric bromide paper AsR with a standard stain produced in a similar manner with a known quantity of dilute arsenic AsTS. Examine the test and standard stains without delay in daylight; the stains fade with time.

The most suitable temperature for carrying out the test is generally about 40°C but, as the rate of evolution of the gas varies somewhat with different batches of granulated zinc AsR, the temperature may have to be adjusted to obtain an even evolution of gas. The reaction may be accelerated by placing the apparatus on a warm surface, care being taken to ensure that the mercuric bromide paper AsR remains dry throughout.

Between successive tests, the tube must be washed with hydrochloric acid (~250g/l) AsTS, rinsed with water and dried.

Preparation of standard stain

Add 10 ml of stannated hydrochloric acid (~250g/l) AsTS and 1 ml of dilute arsenic AsTS to 50 ml of water. The resulting solution, when treated as described in the general test, yields a stain on mercuric bromide paper AsR referred to as the standard stain (10 µg of As).

Limit test for cadmium and lead

The method of determination is left to the analyst. Nevertheless, the determination must be consistent and sensitive enough to allow comparison with a reference material.

Recommended procedure

Apparatus
The equipment consists of a digestion vessel, consisting of a vitreous silica crucible (DIN 12904), "tall form", height 62mm, diameter 50mm, capacity 75ml, with a vitreous silica cover.

Materials used are:

- *digestion mixture*: 2 parts by weight of nitric acid (~1000g/l) TS and 1 part by weight of perchloric acid (~1170g/l) TS.
- *reference materials*: olive leaves (*Olea europaea*)[1] and hay powder[2].

Clean scrupulously with nitric acid (~1000g/l) TS the digestion vessel and all other equipment to be used for the determination, rinse thoroughly several times with water and dry at 120°C.

Preparation of the sample
For the wet digestion method in an open system, place 200-250mg of air-dried plant material, accurately weighed, finely cut and homogeneously mixed, into a cleaned silica crucible. Add 1.0ml of the digestion mixture, cover the crucible without exerting pressure and place it in an oven with a controlled temperature and time regulator (computer-controlled, if available).

Heat slowly to 100°C and maintain at this temperature for up to 3 hours; then heat to 120°C and maintain at this temperature for 2 hours. Raise the temperature very slowly to 240°C, avoiding losses due to possible violent reactions especially in the temperature range of 160-200°C, and maintain at this temperature for 4 hours. Dissolve the remaining dry inorganic residue in 2.5 ml of nitric acid (~1000g/l) TS and use for the determination of heavy metals.

Every sample should be tested in parallel with a blank.

Method
The contents of lead and cadmium may be determined by inverse voltametry or by atomic absorption spectrophotometry.

The following maximum amounts in dried plant materials, which are based on the ADI values, are proposed:

- lead, 10 mg/kg;
- cadmium, 0.3 mg/kg.

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[1] BCR reference material CRM No. 62 Community Bureau of Reference, obtainable from BCR, Directorate-General X11, Commission of the European Communities, 200 rue de la Loi, B-1049 Brussels, Belgium.
[2] Obtainable from IAEA/V-10, International Atomic Energy Agency, Analytical Quality Control Services, Laboratory Geibersdorf, P.O. Box 1'00, A-Vienna, Austria.

18. Determination of microorganisms

Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. While a large range of bacteria and fungi form the naturally occurring microflora of herbs, aerobic spore-forming bacteria frequently predominate. Current practices of harvesting, handling and production may cause additional contamination and microbial growth. The determination of Escherichia coli and moulds may indicate the quality of production and harvesting practices.

Methods for decontamination are restricted. For example, the use of ethylene oxide has been forbidden within countries of the European Union. Treatment with ionizing irradiation is also forbidden or requires a special registration procedure in some countries.

In addition, the presence of aflatoxins in plant material can be hazardous to health if absorbed even in very small amounts. They should therefore be determined after using a suitable clean-up procedure.

Test for specific microorganisms

The conditions of the test for microbial contamination are designed to minimize accidental contamination of the material being examined; the precautions taken must not adversely affect any microorganisms that could be revealed.

Recommended procedure

Pretreatment of the material being examined
Depending on the nature of the crude medicinal plant material, grind, dissolve, dilute, suspend or emulsify the material being examined using a suitable method and eliminate any antimicrobial properties by dilution, neutralization or filtration.

Water-soluble materials
Dissolve or dilute 10 g or 10 ml of plant material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test, adjust the volume to 100ml with the same medium. (Some materials may require the use of a larger volume.) If necessary, adjust the pH of the suspension to about 7.

Non-fatty materials insoluble in water
Suspend 10g or 10ml of material, unless otherwise specified in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test; dilute to 100ml with the same medium. (Some materials may require the use of a larger volume.) If necessary, divide the material being examined and homogenize the suspension mechanically. A suitable surfactant, such as a solution of polysorbate

80 R containing 1 mg per ml may be added. If necessary, adjust the pH of the suspension to about 7.

Fatty materials

Homogenize 10g or 10ml of material, unless otherwise specified in the test procedure for the material concerned, with 5g of polysorbate 20R or polysorbate 80R. If necessary, heat to not more than 40°C. (Occasionally, it may be necessary to heat to a temperature of up to 45°C, for the shortest possible time.) Mix carefully while maintaining the temperature in a water-bath or oven. Add 85 ml of lactose broth or another suitable medium proven to have no antimicrobial activity in the conditions of the test, heated to not more than 40°C if necessary. Maintain this temperature for the shortest time necessary until an emulsion is formed and, in any case, for not more than 30 minutes. If necessary, adjust the pH of the emulsion to about 7.

Enterobacteriaceae and certain other Gram-negative bacteria

Detection of bacteria

Homogenize the pretreated material appropriately and incubate at 30-37°C for a length of time sufficient for revivification of the bacteria but not sufficient for multiplication of the organisms (usually 2-5 hours). Shake the container, transfer 1g or 1ml of the homogenized material to 100ml of Enterobacteriaceae enrichment broth-Mossel and incubate at 35-37°C for 18-48 hours. Prepare a subculture on a plate with violet-red bile agar with glucose and lactose. Incubate at 35-37°C for 18-48 hours. The material passes the test if no growth of colonies of Gram-negative bacteria is detected on the plate.

Quantitative evaluation

Inoculate a suitable amount of Enterobacteriaceae enrichment broth-Mossel with quantities of homogenized material prepared as described under "Detection of bacteria" above, appropriately diluted as necessary, containing 1.0g, 0.1g and 10µg, or 1.0ml, 0.1 ml and 10µl, of the material being examined. Incubate at 35-37°C for 24-48 hours. Prepare a subculture of each of the cultures on a plate with violet-red bile agar with glucose and lactose in order to obtain selective isolation. Incubate at 35-37°C for 18-24 hours. The growth of well-developed colonies, generally red or reddish in colour, of Gram-negative bacteria constitutes a positive result. Note the smallest quantity of material that gives a positive result. Determine the probable number of bacteria using Table 5.

Escherichia coli

Transfer a quantity of the homogenized material in lactose broth, prepared and incubated as described above, and containing 1 g or 1 ml of the material being examined, to 100 ml of MacConkey broth and incubate at 43-45°C for 18-24 hours.

Table 5
Determination of Enterobacteriaceae and certain other Gram-negative bacteria

Result for each quantity or volume			Probable number of bacteria per g of material
1.0 g or 1.0 ml	0.1g or 0.1 ml	0.01 g or 0.01 ml	
+	+	+	More than 10^2
+	+	–	Less than 10^2 but more than 10
+	–	–	Less than 10 but more than 1
–	–	–	Less than 1

Prepare a subculture on a plate with MacConkey agar and incubate at 43-45°C for 18-24 hours. Growth of red, generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of E. coli. This may be confirmed by the formation of indole at 43.5-44.5°C or by other biochemical reactions. The material passes the test if no such colonies are detected or if the confirmatory biochemical reactions are negative.

Salmonella spp.
Incubate the solution, suspension or emulsion of the pretreated material prepared as described above at 35-37°C for 5-24 hours, as appropriate for enrichment.

Primary test
Transfer 10 ml of the enrichment culture to 100 ml of tetrathionate bile brilliant green broth and incubate at 42-43°C for 18-24 hours. Prepare subcultures on at least two of the following three agar media: deoxycholate citrate agar; xylose, lysine, deoxycholate agar; and brilliant green agar. Incubate at 35-37°C for 24-48 hours. Carry out the secondary test if any colonies are produced that conform to the description given in Table 6.

Table 6
Description of Salmonella colonies appearing on different culture media

Medium	Description of colony
Deoxycholate citrate agar	Well developed, colourless
Xylose, lysine, deoxycholate agar	Well developed, red, with or without black centres deoxycholate agar
Brilliant green agar	Small, transparent and colourless, or opaque, pink or white (frequently surrounded by a pink to red zone)

Secondary test
Prepare a subculture of any colonies showing the characteristics described in Table 6 on the surface of triple sugar iron agar using the deep inoculation technique. This can be achieved by first inoculating the inclined surface of the culture medium followed by a stab culture with the same inoculating needle and incubating at 35-37°C for 18-24 hours. The test is positive for the presence of Salmonella spp. if a change of colour from red to yellow is observed in the deep culture (but not in the surface culture), usually with the formation of gas with or without production of hydrogen sulfide in the agar. Confirmation is obtained by appropriate biochemical and serological tests.

The material being examined passes the test if cultures of the type described do not appear in the primary test, or if the confirmatory biochemical and serological tests in the secondary test are negative.

Pseudomonas aeruginosa
Pretreat the material being examined as described on pages 64-65 but using buffered sodium chloride-peptone solution, pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test, in place of lactose broth. Inoculate 100 ml of soybean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1g or 1 ml of the material being examined. Mix and incubate at 35-37°C for 24-48 hours. Prepare a subculture on a plate of cetrimide agar and incubate at 35-37 °C for 24-48 hours. If no growth of microorganisms is detected, the material passes the test. If growth of colonies of Gram-negative rods occurs, usually with a greenish fluorescence, apply an oxidase test and test the growth in soybean-casein digest medium at 42°C. The following method may be used. Place 2 or 3 drops of a freshly prepared 0.01 g/ml solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride R on filter-paper and apply a smear of the suspected colony; the test is positive if a purple colour is produced within 5-10 seconds. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Staphylococcus aureus
Prepare an enrichment culture as described for *Pseudomonas aeruginosa*. Prepare a subculture on a suitable medium such as Baird-Parker agar. Incubate at 35-37°C for 24-48 hours. The material passes the test if no growth of microorganisms is detected. Black colonies of Gram-positive cocci often surrounded by clear zones may indicate the presence of *Staphylococcus aureus*. For catalase-positive cocci, confirmation may be obtained, for example, by coagulase and deoxyribonuclease tests. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative.

Validation of the tests for specific microorganisms
If necessary, grow separately the test strains listed in Table 7 on the culture media indicated at 30-35°C for 18-24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 so that the test suspensions contain about 10^3; microorganisms per ml. Mix equal volumes of

each suspension and use 0.4 ml (approximately 10^2 microorganisms of each strain) as an inoculum in tests for *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus*, in the presence and absence of the material being examined, if necessary. The test method should give a positive result for the respective strain of microorganism.

Table 7
Test strains and culture media for use in validating the tests for specific microorganisms

Microorganism	**Strain number**[a]	**Medium**
Escherichia coli	e.g. NCIMB 8545 (ATCC 8739, CIP 53.126)	lactose broth
Pseudomonas aeruginosa	e.g. NCIMB 8626 (ATCC 9027, CIP 82.118)	soybean-casein digest medium
Salmonella typhimurium	No strain number is recommended. Species not pathogenic for humans, such as Salmonella abony (NCTC 6017, CIP 80.39), may be used	lactose broth
Staphylococcus aureus	e.g. NCIMB 8625 (ATCC 6538 P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)	soybean-casein digest medium

[a]See section 20, page 78.

Total viable aerobic count

The total viable aerobic count of the material being examined is determined, as specified in the test procedure, for the plant material concerned using one of the following methods: membrane-filtration, plate count or serial dilution.

Pretreatment of the material being examined

Pretreat the material as described in the "Test for specific microorganisms", page 64, but in place of lactose broth use buffered sodium chloride-peptone solution pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test.

Membrane filtration

Use membrane filters with a nominal pore size of not greater than 0.45 μm, the effectiveness of which in retaining bacteria has been established. For example, cellulose nitrate filters are used for aqueous, oily and weakly alcoholic solutions, and cellulose acetate filters for strongly alcoholic solutions. The technique described uses filter discs of about 50mm in diameter. For filters of a different diameter, adjust the volumes of the dilutions and washings accordingly. Sterilize the filtration apparatus and the membrane by appropriate means. They are

designed to permit the solution being examined to be introduced and filtered under aseptic conditions, and the membrane to be transferred to the culture medium.

Transfer 10ml or a solution containing 1g of the material to each of two membrane filters and filter immediately. If necessary, dilute the pretreated material to obtain an expected colony count of 10-100. Wash each membrane, filtering three or more successive quantities of approximately 100ml of a suitable liquid such as buffered sodium chloride-peptone solution, pH 7.0. For fatty materials, a suitable surfactant may be added, such as polysorbate 20R or polysorbate 80R. Transfer one of the membrane filters, intended primarily for the enumeration of bacteria, to the surface of a plate with casein-soybean digest agar and the other, intended primarily for the enumeration of fungi, to the surface of a plate with Sabouraud glucose agar with antibiotics. Incubate the plates for 5 days, unless a more reliable count can be obtained otherwise, at 30-35°C for the detection of bacteria and at 20-25°C for the detection of fungi. Count the number of colonies formed. Calculate the number of microorganisms per g or per ml of the material tested, if necessary counting bacteria and fungi separately.

Plate count
For bacteria. Use Petri dishes 9-10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied casein-soybean digest agar at a temperature not exceeding 45°C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the pretreated material as described above to obtain an expected colony count of not more than 300. Prepare at least two dishes using the same dilution and incubate them at 30-35°C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the plate with the largest number of colonies, up to a maximum of 300.

For fungi. Use Petri dishes 9-10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied Sabouraud glucose agar with antibiotics at a temperature not exceeding 45°C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the pretreated material as described above to obtain an expected colony count of not more than 100. Prepare at least two dishes using the same dilution and incubate them at 20-25°C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculate the results using the dish with not more than 100 colonies.

Serial dilution
Prepare a series of 12 tubes each containing 9-10ml of soybean-casein digest medium. To each of the first three tubes add 1 ml of the 1:10 dilution of dissolved, homogenized material prepared as described on pages 64-65. To the next three tubes add 1 ml of a 1:100 dilution of the material and to the next three tubes add 1 ml of a 1:1000 dilution of the material. To the last three tubes add 1 ml of the diluent. Incubate the tubes at 30-35°C for at least 5 days. No microbial growth

should appear in the last three tubes. If the reading of the results is difficult or uncertain owing to the nature of the material being examined, prepare a subculture in a liquid or a solid medium, and evaluate the results after a further period of incubation. Determine the most probable number of microorganisms per g or ml of the material using Table 8.

If, for the first column, the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per ml is less than 100.

Effectiveness of the culture medium and validity of the counting method
The following strains are normally used (see also section 20):

Staphylococcus aureus	NCIMB 8625 (ATCC 6538-P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)
Bacillus subtilis	NCIMB 8054 (ATCC 6633, CIP 52.62)
Escherichia coli	NCIMB 8545 (ATCC 8739, CIP 53.126)
Candida albicans	ATCC 2091 (CIP 1180.79) or ATCC 10 231 (NCPF 3179, CIP 48.72)

Allow the test strains to grow separately in tubes containing soybean-casein digest medium at 30-35°C for 18-24 hours, except for Candida albicans which needs a temperature of 20-25°C for 48 hours.

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to obtain test suspensions containing about 100 viable microorganisms per ml. Use the suspension of each microorganism separately as a control of the counting methods, in the presence and absence of the material being examined, if necessary.

Table 8
Determination of total viable aerobic count

Number of tubes 100mg or 0.1 ml per tube	with microbial 10mg or 0.01 ml per tube	growth[a] 1mg or 0.001 ml per tube	Most probable number of microorganisms per g or ml
3	3	3	>1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

[a] Amounts in mg or ml are quantities of original plant material.

To validate the method, a count for the test organism should be obtained differing by not more than a factor of 10 from the calculated value for the inoculum. To test the sterility of the medium and the diluent, as well as aseptic performance, carry out the total viable aerobic count using sterile buffered sodium chloride-peptone solution pH 7.0 as the test preparation. There should be no growth of microorganisms.

Microbial contamination limits in medicinal plant materials

Different limits are set according to the use of the material and the material itself.

- For contamination of "crude" plant material intended for further processing (including additional decontamination by a physical or chemical process) the limits, adapted from the provisional guidelines established by an international consultative group (12), are given for untreated plant material harvested under acceptable hygienic conditions:
  - *Escherichia coli*, maximum 10^4 per gram;
  - mould propagules, maximum 10^5 per gram.

- For plant materials that have been pretreated (e.g. with boiling water as used for herbal teas and infusions) or that are used as topical dosage forms:
  - aerobic bacteria, maximum 10^7 per gram;

- yeasts and moulds, maximum 10^4 per gram;
- Escherichia coli, maximum 10^2 per gram;
- other enterobacteria, maximum 10^4 per gram;
- salmonellae, none.

- For other plant materials for internal use:
  - aerobic bacteria, maximum 10^5 per gram;
  - yeasts and moulds, maximum 10^3 per gram;
  - Escherichia coli, maximum 10 per gram;
  - other enterobacteria, maximum 10^3, per gram;
  - salmonellae, none.

Test for aflatoxins

This test is designed to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2, which are highly dangerous contaminants in any material of plant origin.

Recommended procedure

Preparation of samples
Grind or reduce not less than 100g of crude medicinal plant material to a moderately fine powder (sieve no. 355/180). The larger the sample size, i.e. 500g-1kg or more, the greater the possibility of detecting pockets of contamination.

Weigh 50g of the powdered material, transfer to a conical glass-stoppered flask, and add 170 ml of methanol R and 30 ml of water. Using a mechanical device, shake vigorously for not less than 30 minutes. Filter through a medium-porosity filter-paper. If a special clean-up procedure is required (see below), collect 100ml of filtrate (A) from the start of flow; otherwise discard the first 50ml and collect 40ml of filtrate (B).

In order to eliminate interfering plant pigments use a special clean-up procedure: transfer 100 ml of filtrate A to a 250-ml beaker and add 20 ml of zinc acetate/aluminium chloride TS and 80 ml of water. Stir, allow to stand for 5 minutes, add 5 g of a filter aid, such as diatomaceous earth, mix and filter through a medium-porosity filter-paper. Discard the first 50ml and collect 80ml of filtrate (C).

Transfer either filtrate B or C to a separating funnel. Add 40ml of sodium chloride (100 g/l) TS and 25 ml of light petroleum R, and shake for 1 minute. Allow the layers to separate and transfer the lower layer to a second separating funnel. Extract twice with 25 ml of dichloromethane R and shake for 1 minute. Allow the layers to separate and combine each of the lower layers in a 125-ml conical flask. Add several boiling chips and evaporate almost to dryness on a water-bath. Cool the residue, cover the flask and keep it for the determination by thin-layer chromatography or for a further clean-up procedure by column chromatography.

If necessary, remove further interfering compounds using a column 300 mm long with an internal diameter of 10 mm, a stopper and either a medium-pore sintered disc or a glass-wool plug. Prepare a slurry by mixing 2 g of silica gel R with 10 ml of a mixture of 3 volumes of ether R and 1 volume of light petroleum R, pour into the column and wash with 5 ml of the same solvent mixture. Allow the adsorbent to settle and add to the top of the column a layer of 1.5 g of anhydrous sodium sulfate R. Dissolve the residue from above in 3 ml of dichloromethane R and transfer it to the column. Rinse the flask twice with 1-ml portions of dichloromethane R and add them to the column, eluting at a rate not faster than 1 ml/min. Then add successively to the column 3 ml of light petroleum R, 3 ml of ether R and 3 ml of dichloromethane R, and elute at a rate not faster than 3 ml/min. Discard the eluates. Add to the column 6 ml of a mixture of 9 volumes of dichloromethane R and 1 volume of acetone R and elute at a rate not faster than 1 ml/min, preferably without using vacuum. Collect this eluate in a small vial, add a few boiling chips and evaporate just to dryness on a water-bath.

Method

To either of the residues obtained above, add 0.2 ml of a mixture of 98 volumes of chloroform R and 2 volumes of acetonitrile R, close the vial and shake vigorously until the residues are dissolved, preferably using a vortex mixer.

Carry out the test as described in section 6, "Thin-layer chromatography", using silica gel G as the coating substance and a mixture of 85 volumes of chloroform R, 10 volumes of acetone R and 5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 2.5 µl, 5 µl, 7.5 µl and 10 µl of aflatoxin mixture TS, then apply three volumes, each of 10 µl, of the sample residues. Further superimpose on one of these spots 5 µl of aflatoxin mixture TS. Place the plate in an unsaturated chamber and develop. After removing the plate from the chromatographic chamber, allow it to dry in air and examine the chromatogram in a dark room under ultraviolet light (365 nm).

Four clearly separated blue fluorescent spots are obtained from the aflatoxin mixture. Observe any spot obtained from the solutions of the residues that coincides in hue and position with those of the aflatoxin mixture. Any spot obtained from the solutions of the residues with the superimposed aflatoxin mixture should be more intense than the corresponding spot for the test solution, and should show no sign of separation or tailing, which would be a sign of dissimilar compounds.

Interpretation of results

No spots corresponding to aflatoxin should be obtained from any of the sample residues. If any such spot is obtained, compare its position with the spots obtained from the aflatoxin mixture to identify the type of aflatoxin present. An approximate estimation of the concentration of aflatoxin in the sample may be obtained by comparing the intensity of the spots with those of the aflatoxin mixtures.

19. Radioactive contamination

A certain amount of exposure to ionizing radiation cannot be avoided since there are many sources, including radionuclides occurring naturally in the ground and the atmosphere. These sources are described extensively in the booklet Facts about low-level radiation (13).

Dangerous contamination may be the consequence of a nuclear accident. The World Health Organization, in close collaboration with several other international organizations, has developed guidelines for use in the event of widespread contamination by radionuclides resulting from a major nuclear accident (14). This publication emphasizes that the health risks from food accidentally contaminated by radionuclides depend not only on the specific radionuclide and the level of contamination but also on the quantity of food consumed.

The range of radionuclides that may be released into the environment as the result of a nuclear accident might include long-lived and short-lived fission products, actinides, and activation products. The nature and the intensity of radionuclides released may differ markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc.).

The amount of exposure to radiation depends on the intake of radionuclides and other variables such as age, metabolic kinetics, and weight of the individual (also known as the dose conversion factor).

Even at maximum observed levels of radioactive contamination with the more dangerous radionuclides, significant risk is associated only with consumption of quantities of over 20 kg of plant material per year so that a risk to health is most unlikely to be encountered given the amount of medicinal plant materials that would need to be ingested. Additionally, the level of contamination might be reduced during the manufacturing process. Therefore, no limits for radioactive contamination are proposed.

Method of measurement
Since radionuclides from accidental discharges vary with the type of facility involved, a generalized method of measurement is so far not available. However, should such contamination be of concern, suspect samples can be analysed by a competent laboratory. Details of laboratory techniques are available from the International Atomic Energy Agency (IAEA).[3]

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[3] International Atomic Energy Agency (IAEA), Analytical Quality Control Services, Laboratory Seibersdorf, PO Box 100, Vienna, Austria.

20. Culture media and strains of microorganisms

Culture media

The following media are satisfactory, but other media may be used if they have similar nutritive and selective properties for the microorganisms to be tested.

Baird-Parker agar
Procedure. Dissolve 10.0 g of pancreatic digest of casein R, 5.0 g of beef extract R, 1.0g of water-soluble yeast extract R, 5.0 g of lithium chloride R, 20.0 g of agar R, 12.0 g of glycine R and 10.0 g of sodium pyruvate R in sufficient water to produce 950 ml. Heat to boiling for 1 minute, shaking frequently and adjust the pH to 6.6-7.0 using sodium hydroxide (0.5 mol/l) VS. Sterilize in an autoclave at 121°C for 15 minutes, cool to 45-50°C and add 10 ml of a sterile 0.01 g/ml solution of potassium tellurite R and 50 ml of egg-yolk emulsion.

Brilliant green agar
Procedure. Dissolve 10.0 g of dried peptone R (meat and casein), 3.0 g of water-soluble yeast extract R, 5.0 g of sodium chloride R, 10.0 g of lactose R, 10.0 g of sucrose R, 20.0 g of agar R, 0.08 g of phenol red R and 12.5 mg of brilliant green R in sufficient water to produce 1000 ml. Heat to boiling for 1 minute. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 6.7-7.1. Immediately before use, sterilize in an autoclave at 121°C for 15 minutes, cool to 50°C and pour into Petri dishes.

Buffered sodium chloride-peptone solution pH 7.0
Procedure. Dissolve 3.56 g of potassium dihydrogen phosphate R, 7.23 g of disodium hydrogen phosphate R, 4.30 g of sodium chloride R and 1.0 g of dried peptone R (meat and casein) in sufficient water to produce 1000ml. Polysorbate 20 R or polysorbate 80 R may be added, 0.001-0.01 g per ml. Sterilize in an autoclave at 121°C for 15 minutes.

Casein-soybean digest agar
Procedure. Dissolve 15.0g of pancreatic digest of casein R, 3.0g of papaic digest of soybean meal R, 5.0 g of sodium chloride R and 15.0 g of agar R in sufficient water to produce 1000 ml. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 7.1-7.5. Sterilize in an autoclave at 121°C for 15 minutes.

Cetrimide agar
Procedure. Dissolve 20.0 g of pancreatic digest of gelatin R, 1.4g of magnesium chloride R, 10.0 g of potassium sulfate R, 0.3 g of cetrimide R, 13.6 g of agar R and 10.0 ml of glycerol R insufficient water to produce 1000 ml. Heat to boiling for 1 minute with shaking. Using sodium hydroxide (0.05 mol/l) VS adjust the pH to 7.0-7.4. Sterilize in an autoclave at 121°C for 15 minutes.

Deoxycholate citrate agar
Procedure. Dissolve 10.0 g of beef extract R, 10.0 g of dried peptone R (meat), 10.0 g of lactose R, 20.0 g of sodium citrate R, 1.0 g of iron(III) citrate R, 5.0 g of sodium deoxycholate, 13.5 g of agar R and 20 mg of neutral red R in sufficient

ICH GUIDELINES

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is unique in bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration. Since its inception in 1990, ICH has gradually evolved, to respond to the increasingly global face of drug development. ICH's mission is to achieve greater harmonisation worldwide to ensure that safe, effective, and high-quality medicines are developed and registered in the most resource-efficient manner. Harmonisation is achieved through the development of ICH Guidelines via a process of scientific consensus with regulatory and industry experts working side-by-side. Key to the success of this process is the commitment of the ICH regulators to implement the final Guidelines.

With ICH's establishment as an international non-profit Association under Swiss law on October 23, 2015, ICH's mission has been embodied in its Articles of Association as follows:

- To make recommendations towards achieving greater harmonisation in the interpretation and application of technical guidelines and requirements for pharmaceutical product registration and the maintenance of such registrations;
- To maintain a forum for a constructive dialogue on scientific issues between regulatory authorities and the pharmaceutical industry on the harmonisation of the technical requirements for pharmaceutical products;
- To contribute to the protection of public health in the interest of patients from an international perspective;
- To monitor and update harmonised technical requirements leading to a greater mutual acceptance of research and development data;
- To avoid divergent future requirements through harmonisation of selected topics needed as a result of therapeutic advances and the development of new technologies for the production of medicinal products;
- To facilitate the adoption of new or improved technical research and development approaches which update or replace current practices;
- To encourage the adequate implementation and integration of common standards through the dissemination of, the communication of information about and coordination of training on, harmonised guidelines and their use;
- And to develop policy for the ICH Medical Dictionary for Regulatory Activities Terminology (MedDRA) whilst ensuring the scientific and technical maintenance,

development and dissemination of MedDRA as a standardised dictionary which facilitates the sharing of regulatory information internationally for medicinal products used by humans.

ICH Guidelines

The ICH topics are divided into the four categories below and ICH topic codes are assigned according to these categories.

Quality Guidelines

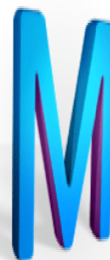
Harmonisation achievements in the Quality area include pivotal milestones such as the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management.

Safety Guidelines

ICH has produced a comprehensive set of safety Guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years.

Efficacy Guidelines

The work carried out by ICH under the Efficacy heading is concerned with the design, conduct, safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/genomics techniques to produce better targeted medicines.



Multidisciplinary Guidelines

Those are the cross-cutting topics which do not fit uniquely into one of the Quality, Safety and Efficacy categories. It includes the ICH medical terminology (MedDRA), the Common Technical Document (CTD) and the development of Electronic Standards for the Transfer of Regulatory Information (ESTRI).

Home \ ICH Guidelines \ Quality Guidelines

Quality Guidelines

Harmonisation achievements in the Quality area include pivotal milestones such as the conduct of stability studies, defining relevant thresholds for impurities testing and a more flexible approach to pharmaceutical quality based on Good Manufacturing Practice (GMP) risk management.

Q1A - Q1F
Stability

Q2 Analytical
Validation

Q3A - Q3E
Impurities

Q4A - Q4B
Pharmacopoeias

Q5A - Q5E Quality of Biotechnological
Products

Q6A- Q6B
Specifications

Q7 Good Manufacturing
Practice

Q8 Pharmaceutical
Development

Q9 Quality Risk
Management

Q10 Pharmaceutical Quality
System

Q11 Development and Manufacture of Drug
Substances

Q12 Lifecycle
Management

Q13 Continuous Manufacturing of Drug Substances and Drug
Products

Q14 Analytical Procedure Development

Safety Guidelines

ICH has produced a comprehensive set of safety Guidelines to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity. A recent breakthrough has been a non-clinical testing strategy for assessing the QT interval prolongation liability: the single most important cause of drug withdrawals in recent years.

S1A - S1C Carcinogenicity Studies

S2 Genotoxicity Studies

S3A - S3B Toxicokinetics and Pharmacokinetics

S4 Toxicity Testing

S5 Reproductive Toxicology

S6 Biotechnological Products

S7A - S7B Pharmacology Studies

S8 Immunotoxicology Studies

S9 Nonclinical Evaluation for Anticancer Pharmaceuticals

S10 Photosafety Evaluation

S11 Nonclinical Paediatric Safety

S12 Non-clinical Biodistribution Considerations for Gene Therapy Products

Home \ ICH Guidelines \ Efficacy Guidelines

Efficacy Guidelines

The work carried out by ICH under the Efficacy heading is concerned with the design, conduct, safety and reporting of clinical trials. It also covers novel types of medicines derived from biotechnological processes and the use of pharmacogenetics/ pharmacogenomics techniques to produce better targeted medicines.

E1 Clinical Safety for Drugs used in Long-Term Treatment

E2A - E2F Pharmacovigilance

E3 Clinical Study Reports

E4 Dose-Response Studies

E5 Ethnic Factors

E6 Good Clinical Practice

E7 Clinical Trials in Geriatric Population

E8 General Considerations for Clinical Trials

E9 Statistical Principles for Clinical Trials

E10 Choice of Control Group in Clinical Trials

E11 - E11A Clinical Trials in Pediatric Population

E12 Clinical Evaluation by Therapeutic Category

E14 Clinical Evaluation of QT

E15 Definitions in Pharmacogenetics / Pharmacogenomics

E16 Qualification of Genomic Biomarkers

E17 Multi-Regional Clinical Trials

E18 Genomic Sampling

E19 Safety Data Collection

E20 Adaptive Clinical Trials

E21 Inclusion of Pregnant and Breastfeeding Individuals in Clinical

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Those are the cross-cutting topics which do not fit uniquely into one of the Quality, Safety and Efficacy categories. It includes the ICH medical terminology (MedDRA), the Common Technical Document (CTD) and the development of Electronic Standards for the Transfer of Regulatory Information (ESTRI).

M1 MedDRA Terminology

M2 Electronic Standards

M3 Nonclinical Safety Studies

M4 Common Technical Document

M5 Data Elements and Standards for Drug Dictionaries

M6 Gene Therapy

M7 Mutagenic Impurities

M8 Electronic Common Technical Document (eCTD)

M9 Biopharmaceutics Classification System-based Biowaivers

M10 Bioanalytical Method Validation and Study Sample Analysis

M11 Clinical electronic Structured Harmonised Protocol (CeSHarP)

M12 Drug Interaction Studies

M13 Bioequivalence for Immediate-Release Solid Oral Dosage Forms

M14 Use of real-world data for safety assessment of medicines

M15 General Principles for Model-Informed Drug Development

Stability Studies

- Stability testing is an obligatory requirement in the registration process for all medicinal products, including Herbal Medicinal Products (HMPs).
- The HMPs are subjected to systematic stability testing is order to establish and ensure consistent therapeutic efficacy and safety throughout their shelf life. The tests are performed to define storage conditions and the product's shelf-life.
- Stability testing is an important component of herbal drugs and products development process.
- Drugs regulatory agencies across the globe have recommended guidelines for the conduct of stability studies of HMPs, which require that stability data should be included during product registration.
- Numerous chemical constituents in an herbal drug are liable to varied chemical reactions under the influence of conditions during shelf life.
- These reactions can change chemical composition of the HMPs and finally altered therapeutic profile.
- The challenges in stability of HMPs are chemical complexity, chemical variability in crude drug, selection of marker, influence of enzymes etc.

Contents

1. The need for stability studies
2. Regulatory Basis of Herbal Drug Stability Testing
3. Scientific Basis of Herbal Drug Stability Testing
4. Herbal Drugs and Preparation
5. Mechanisms affecting Stability
6. ICH zones and long- term stability conditions
7. Types of Stability testing methods
8. Stability Testing Storage Conditions for drugs
9. Challenges in Stability Testing

10. Special conditions to take account for HMPs
11. Protocol

12.Summary
13. Bibliography

The need for Stability Studies

Well- being of the patient and manufacturer by ensuring product quality

For selection of adequate formulation, determination of shelf- life and storage conditions

Preparation and substantiation of the claimed shelf- life for the registration dossier

To provide
on how
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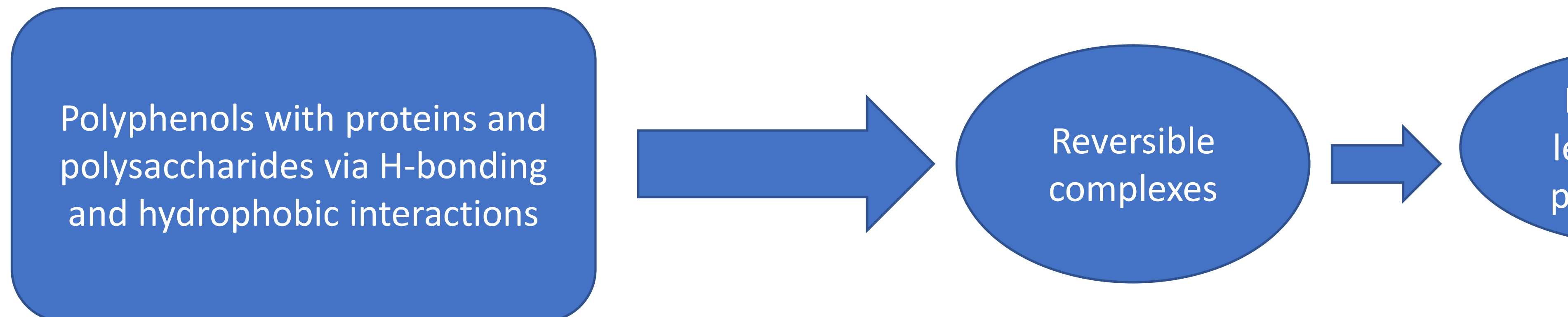
Regulatory Basis of Herbal Drug Stability Testin

- **Guidelines provided by drugs regulatory agencies such as European Me Agency (EMEA, 2010), International Conference on Harmonization (ICH and World Health Organization (WHO,2009) require stability data of an product prior its approval.**
- **Various approaches for assessment of shelf life include assay of marker (active or analytical), biological assays, and/or chromatographic chemoprofiling or fingerprinting of control and stability samples of a pr under different stability conditions.**

Scientific Basis of Herbal Drug Stability Te

- Stability studies on herbal drugs involve quantitative monitoring of active const
  and or analytical marker.
- Constituents belonging to different chemical groups in an HMP may undergo va
  molecular or inter-molecular reactions under the influence of heat, humidity ar
  during its manufacture, transportation and storage.
- These interactions between different groups are liable to produce products tha
  more or less active and/or toxic.

Example:



- Water solubility of tannins is increased in the presence of water solub glycosides as with paeniflorin and glycyrrhizin.
- Alkaloids are precipitated in the presence of tannins.
- Polysaccharides, tannins and lignins form mono-dentate and bi-denta complexes with heavy metal ions.
- Cations form strong complexes with Polysaccharides in alkaline mediu weak complexes in other media.

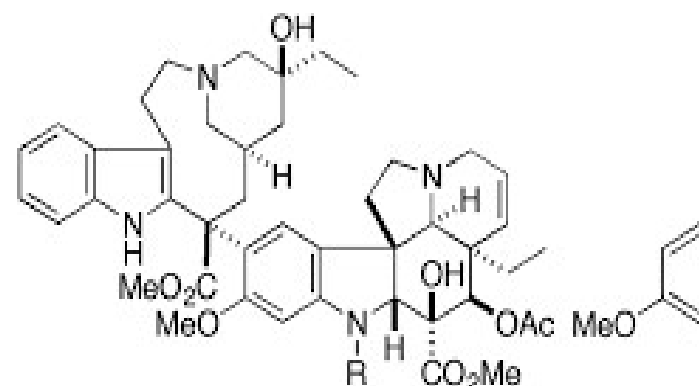
So it is required to ensure that the overall chemical composition of a d remains unchanged during its shelf-life

Vinca rosea (Catharanthus roseus)

Effect of UV light on total contents of *Vinca rosea*

UV- light

- Presence of UV light B increases total flavanoid content
- VINBLASTIN rapidly biotransforms to VINCRISTINE in presence of UV-A light.
- presence of UV-A light increases concentration of VINBLASTIN



Herbal Drugs and Preparation

Herbal products are complex in nature due to high number of constituents of different chemical classes.

Classified entirely on basis of the active pharmaceutical Ingredients.

EMEA, has subdivided herbal preparations into three categories based on the active constituents in the product.

1. Standardized Extract

- Silymarines in *Silybum marianum*

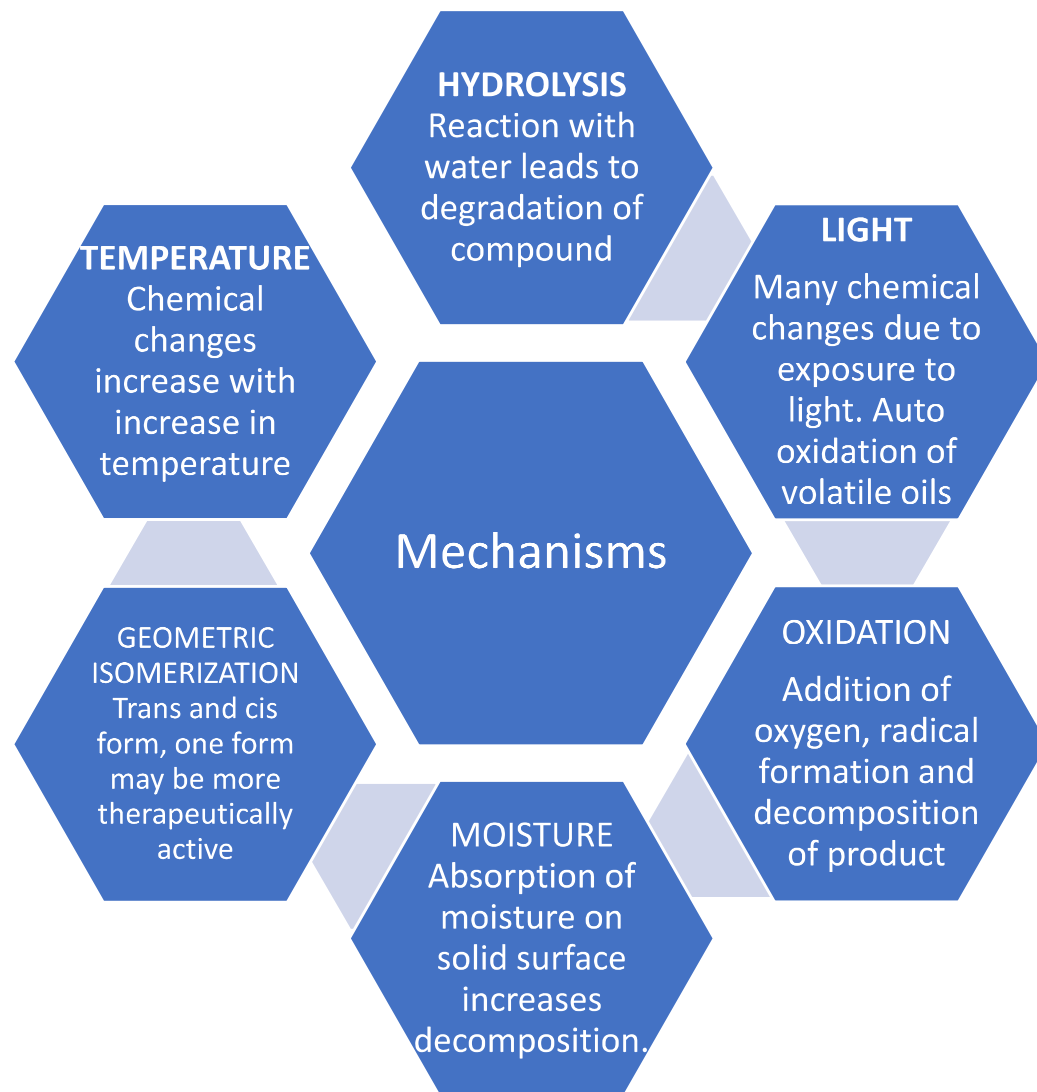
2. Quantified Extracts

- Hypericines in *Hypericum perforatum*

3. Others

- Drug- Extract Ratio

Mechanisms affecting Stability



ICH zones and long- term stability conditions

CLIMATIC ZONE	CLIMATE	COUNTRIES	MAT/ MAPWP	LONG TERM TESTING CONDITION
I	Temperate	UK Northern Europe, US	<15ºC/ , <11hPa	21℃/ 45%RH
II	Subtropical & Mediterranean	Japan, Southern Europe	>15-22ºC/ >11-18hPa	25℃/ 60%RH
III	Hot & Dry	Iraq, India	>22ºC/ <15hPa	30℃/ 35%RH
IVa	Hot & Humid	Iran, Egypt		30℃/ 65%RH
IVb	Hot & Very Humid	Brazil, Singapore	>22ºC/ >27hPa	30℃/ 75%RH

Types of Stability testing methods

- Accelerated Testing: **Product subjected to high temperature, humidity, light, etc. 40ºC/ 75% R H. At 3rd and 6th month.**
- **Real Time(long-term) Testing:** Longer duration. 25-30ºC & 35- 75% RH(depending on climati zone), for 3rd, 6th, 9th, 12th, 18th, 24th, and 36th month.
- **Intermediate Testing:** Conducted when accelerated studies fail. At 25ºC for longer duration of time.
- **Stress Testing:** Includes effects of temperature, i.e., above 40ºC and ≥75% RH.
- **Forced Degradation Testing:** performed to provide intrinsic stability assessment of drug.

Stability Testing Storage Conditions for drugs as per ICH and WHO

Intended Storage Condition	Stability Test Method	Test temp. and humidity (period in months) as per ICH	Test temp. and h (period in month per WHO
Room temperature	Long term	25±2°C/60±5% RH	25±2°C/60±5% R 30±2°C/65±5% R 30±2°C/75±5% R
	Intermediate	30±2°C/65±5% RH	30±2°C/65±5% R
	Accelerated	40±2°C/75±5% RH	40±2°C/75±5% R
Refrigerated	Long term	5°C/ambient	5±3°C
	Accelerated	25±2°C/60±5% RH(6)	25±2°C/60±5% R 30±2°C/65±5% R
Freezer	Long term	-20°C/ambient (12)	-20°C5±5°C

Test Schedule for Stability of new Products

Method and climatic zone	Environment	Time points for sampling
Long term for climatic zones I and IV	25°C/60% RH	3,6,9,12,18,24,36 months
Long term for climatic zones III	30°C/35% RH	3,6,9,12,18,24,36 months
Long term for climatic zone IVa/Intermediate for zones I and II	30°C/65% RH	3,6,9,12,18,24,36 months
Long term for climatic zone IVb/Intermediate for zones I and II	30°C/75% RH	3,6,9,12,18,24,36 months
Accelerated condition for all zones	40°C/75% RH	3, 6 months

Challenges in Stability Testing

Active substances in HMPs consist of complex mixtures of constituents & most of markers & their therapeutic effects are unknown

Many herbal compounds are unstable hence, a set of test criteria including qualitative and quantitative parameters has been recognized as quality indicating

In combined for
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studies are condu
one year for suc

Selection of batches and Testing conc

FORMAL STABILITY STUDIES	LONG TERM STABILITY STUDIES	ONGOING STABILITY STU
▪ Conducted on at least three primary batches. ▪ Stability performed on each individual strength and container size till bracketing.	▪ On at least three batches performed under natural conditions.	▪ All products have to tested at least one b year. ▪ Wherever appropria products are also to tested. ▪ Applies to every dos packaging size and t

Special conditions to take account for H

Specification Limit:

- Limit of assay of ±5% of declared value shall be applied to standardized extracts.
- For drugs with low marker concentration, the range shall be widene to ±10% or even higher.
- Due to influence of climate, natural variation in markers shall be take into account.
- Since marker content cannot be defined to a specific level, the relativ changes from starting value are (95-105% or 90-110%) from initial value.
- An analytical marker is stable in herbal substances(monograph) and in solid dosage forms but unstable in liquid dosage form. This can be solved by various means: a stable marker shall be chosen, etc.

Protocols for Stability Testing

1. **Selection of Batches and Samples**
2. **Test Attributes**
3. **Analytical procedures**
4. **Acceptance Criteria**
5. **Storage Conditions & Storage period**
6. **Testing Frequency**
7. **Sampling Plan**
8. **Container closure system**
9. **Evaluation**
10. **Statements labelling**

RESEARCH GUIDELINES FOR EVALUATING THE SAFETY AND EFFICACY OF HERBAL MEDICINES

World Health Organization
Regional Office for the Western Pacific
Manila

The World Health Organization is a specialized agency of the United Nations with primary responsibility for international health matters and public health. Through this organization, which was created in 1948, the health professions of some 180 countries exchange their knowledge and experience with the aim of making possible the attainment by all citizens of the world by the year 2000 of a level of health that will permit them to lead a socially and economically productive life.

By means of direct technical cooperation with its Member States, and by stimulating such cooperation among them, WHO promotes the development of comprehensive health services, the prevention and control of diseases, the improvement of environmental conditions, the development of human resources for health, the coordination and development of biomedical and health services research, and the planning and implementation of health programmes.

These broad fields of endeavour encompass a wide variety of activities, such as developing systems of primary health care that reach the whole population of Member countries; promoting the health of mothers and children; combating malnutrition; controlling malaria and other communicable diseases including tuberculosis and leprosy; coordinating the global strategy for the prevention and control of AIDS; having achieved the eradication of smallpox, promoting mass immunization against a number of other preventable diseases; improving mental health; providing safe water supplies; and training health personnel of all categories.

Progress towards better health throughout the world also demands international cooperation in such matters as establishing standards for biological substances, pesticides and pharmaceuticals; formulating environmental health criteria; recommending international nonproprietary names for drugs; administering the International Health Regulations; revising the International Statistical Classification of Diseases and Related Health Problems; and collecting and disseminating health statistical information.

Reflecting the concerns and priorities of the Organization and its Member States, WHO publications provide authoritative information and guidance aimed at promoting and protecting health and preventing and controlling disease.

RESEARCH GUIDELINES FOR EVALUATING THE SAFETY AND EFFICACY OF HERBAL MEDICINES

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Manila
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FOREWORD

Herbal medicines have been used for thousands of years. The practice continues today because of its biomedical benefits and place in cultural beliefs in many parts of the world. The economic reality of the inaccessibility of modern medication for many societies has also played a major role in the broad use of herbal medicines.

The World Health Organization has recognized the contribution and value of the herbal medicines used by a large segment of the world's population. A growing interest in usage has created the need for greater precision in preparation and evaluation and has stimulated research into herbal medicines' various uses and applications.

The Western Pacific Region has a rich tradition of preparation and use of herbal medicines. In 1992, the WHO Regional Office for the Western Pacific invited a group of experts to develop criteria and general principles to guide research work on evaluating herbal medicines. These guidelines have been prepared for research on different forms of herbal medicines, including those in traditional use. Basic scientific principles as well as any special requirements related to the use of herbal medicines in traditional practice have been incorporated in these guidelines.

These guidelines are published to support the application of evaluation principles by modern science to a tradition of herbal medicine that is still extremely vibrant and of growing interest throughout the world.

S.T. Han, MD, Ph.D.
Regional Director

1. INTRODUCTION

Background

Herbal medicines, as the major remedy in traditional medical systems, have been used in medical practice for thousands of years and have made a great contribution to maintaining human health. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. The use of these medicines has a particularly rich tradition among the peoples of the Western Pacific Region. In recent years, this has extended far beyond its original ethnic setting. The attention paid by health authorities to the use of herbal medicines has increased considerably, both because they are often the only medicine available in less developed areas and because they are becoming a popular alternative medicine in more developed areas.

The World Health Organization is fully aware of the importance of herbal medicines to the health of many people throughout the world, as stated in a number of resolutions adopted by the World Health Assembly and the Regional Committee for the Western Pacific. Thus herbal medicines have been recognized as a valuable and readily available resource for primary health care, and WHO has endorsed their safe and effective use. A comprehensive programme for the identification, cultivation, preparation, evaluation, utilization and conservation of herbal medicines has been developed. Meanwhile, it has been realized that medicinal plants are a valuable resource for new pharmaceutical products and thus a potential source of new drugs as well as for economic development.

WHO supports the appropriate use of herbal medicines and encourages the use of remedies that have been proven to be safe and effective. A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore or improve health. Most herbal medicines still need to be studied scientifically, although the experience obtained from their traditional use over the years should not be ignored. Member States have been seeking the cooperation of WHO in identifying safe and effective herbal medicines for use in their national health care systems. As there is not enough evidence produced by common scientific approaches to answer questions of safety and efficacy about most of the herbal medicines now in use, the rational use and further development of herbal medicines will be supported by further appropriate scientific studies of these products, and thus the development of criteria for such studies.

Goals

- To strengthen research in the evaluation of the safety and efficacy of herbal medicines.
- To strengthen and promote the rational use of herbal medicines.

Objectives

- To ensure the safety and efficacy of herbal medicines used in the health care systems of countries within the Region and elsewhere in the world.
- To provide research criteria for evaluating the safety and efficacy of herbal medicines and to propose a basis for the Member States to develop their own research guidelines for the study of herbal medicines.

- To facilitate the exchange of research experience and other information so that a body of reliable data for the validation of herbal medicines can be accumulated.

Definition of terms

Herbal medicine	A plant-derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants. In some traditions, materials of inorganic or animal origin may also be present.
Characterizing compound	A natural constituent of a plant part that may be used to assure the identity or quality of a plant preparation, but is not necessarily responsible for the plant's biological or therapeutic activity.
Biological activity	A change in the base-line function of an animal or part of an animal brought about by the administration of a test substance.
Therapeutic activity	An intervention that results in the amelioration of the manifestations of human disease.
Processed plant materials	Plant materials treated according to traditional procedures to improve their safety and/or efficacy, to facilitate their clinical use, or to make medicinal preparations.
Medicinal preparations of plant materials	Medicinal preparations that contain one or more of the following: powdered plant materials, extracts, purified extracts, or partially purified active substances isolated from plant materials. In certain cases, materials of animal or mineral origin may also be included in such preparations.

2. GENERAL CONSIDERATIONS IN HERBAL MEDICINE RESEARCH

Legal considerations

Governments should actively promote the rational use of herbal medicines that have been scientifically validated. To do so, they *need a national policy for approving* those that are safe and effective for specified clinical indications. The adoption of such policy will help to overcome some of the legal barriers against the use of herbal medicines which in some countries may still be inadequately standardized.

Legislation concerning procedures for the registration of herbal medicine can play a very important role in ensuring that medicinal plant preparations are of acceptable quality, safety and efficacy. Research on herbal medicines, which is necessary to ensure their improved utilization by the public, would benefit from strong governmental endorsement.

Ethical considerations

Research on herbal medicines must be carried out in accordance with all relevant ethical guidelines.

Research on human subjects

When human subjects are involved, research must be conducted in accordance with four basic principles: justice, respect for persons, beneficence and non-maleficence.

Research on animals

Research on animals must be carried out with respect for their welfare and consideration must be given to using *in vitro* laboratory methods that may reduce experimentation on intact animals.

Respect for the environment

Proper consideration must be given to protection of the environment which supports the natural products that are the basis for herbal medicines and which may yield valuable medicinal products in the future.

Traditional knowledge on herbal medicine

Herbal medicines have been used by the traditional system of medicine for a long time. Prolonged and apparently uneventful use of an herbal medicine may offer testimony of its safety and efficacy. The research approaches should differentiate between herbal medicines which have had documented experience from a long period of use with those whose traditional use has not been established.

Traditional use of herbal medicine is usually an integral part of culture, which was developed within an ethnic group before the development and spread of modern science. Respect for the principle of the traditional system of medicine under study must be an important priority. Traditional knowledge and experience of the

use of herbal medicines should be considered when the research is proposed, prepared and conducted. Consultation with traditional medical practitioners is encouraged. In conducting research on herbal medicines which are based on traditional principles and techniques, such principles and techniques should be considered.

Regulatory requirements

Regulatory requirements may be different in different countries. As a general rule, traditional experience which means that long-term use as well as the medical, historial and ethnological background are well recorded, should be taken into account. *WHO Guidelines for the Assessment of Herbal Medicine** should be consulted for the registration of herbal medicine products.

In the design and conduct of researches on herbal medicine, the country's regulatory requirements must already be considered, particularly those required for registration of herbal medicine products.

Purposes of research

There are many reasons for carrying out research. An evaluation may be carried out to prove scientifically the traditional experience on the safety and efficacy of herbal medicines. It may also be conducted to validate a new-found plant material or a new combination of herbal medicines, or even a new indication, a new dosage form or a new administrative route for an existing herbal medicine. Purified or semi-purified compounds derived from herbal medicine may also be presented for research.

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* *Guidelines for the assessment of herbal medicine programme on traditional medicine.* Geneva: World Health Organization, document, 1991 (WHO/TRM/91.4).

The requirement of evidence as to the safety and efficacy of herbal medicines and the method of research chosen should be adjusted to the original purpose of the research.

Selection of research projects

Research projects should be selected with due consideration for several factors in addition to scientific interest. Three of these are:

(1) potential value of the research results for improving the health of the community with due regard to the prevalence of disease and the feasibility of using alternative treatments;

(2) the medical value of indigenous plants;

(3) technical and financial considerations.

Research approaches

Research on herbal medicines in the past has generally been carried out by individual researchers working independently. One researcher may find an active principle whose pharmacological and toxicological properties are then further studied elsewhere. Finally, yet another group may decide to go directly to human studies.

A single multidisciplinary group may enable more rapid progress. In such a group the first step might be to collect information on folkloric experience whose scientific validity is then investigated. If appropriate pharmacodynamic studies seem to verify the traditional use, the group can begin to conduct more general pharmacological and toxicological tests to assure the safety of the medicinal product, which can then be tested in an initial clinical trial. Additional confirmatory clinical trials may be conducted if warranted.

In certain instances, the isolation of an active substance may be useful in order to provide an exact dosage. In many cases, however, the plant preparation as a whole is therapeutically effective even though the active principle is not known. The clinical investigation of the therapeutic activity of such crude preparations may be useful, because that activity may depend not only on a single substance but may be influenced by a large number of other components in the herbal medicine.

Assuring access to relevant databases

Databases devoted to herbal medicines and natural products have been established in several countries and areas including China, Hong Kong, Japan and the United States. Easy access to such databases greatly facilitates the efforts of those interested in herbal medicines. Since the maintenance of such databases and access to them are costly, a government financial subsidy may be necessary in order to assure access of researchers and health planners to the information needed to hasten the rational use of herbal medicines in their countries.

Education

Dissemination of knowledge about herbal medicines in the form both of courses for professional health workers and of information for the public can greatly aid the overall effort to promote the rational use of herbal medicines.

3. RESEARCH STUDIES

Literature background

As the various traditions of herbal medicine have their roots in many different cultures and have only recently been investigated scientifically, it must be recognized that knowledge about herbal remedies is apt to be still perpetuated by oral tradition and found in anecdotal observations rather than in systematic laboratory and clinical studies that have been published in the scientific literature. Furthermore, it must also be recognized that while some publications on herbal medicines may not meet the stringent requirements of international peer-reviewed journals, they may still provide potentially useful observations and ideas for further study. Therefore, a thorough literature survey should be the starting point for every serious effort in herbal medicine research.

Protocol preparation

A carefully planned protocol is a prerequisite for preparing any successful research project. A survey of the literature should help to put the objective of the project into sharp focus. A working hypothesis is then formulated and the experimental approach to test this hypothesis is designed. The methods necessary to gather the relevant data must, however, be executed with due consideration for the ethical aspects that govern experiments on both animals and human subjects.

Quality specifications of plant materials and preparation

All research on herbal medicines must specify the quality of the plant material or the preparation being investigated, in order that studies conducted by one investigator may be corroborated by other investigators (see Guidelines A, page 27).

Non-clinical studies

The primary objectives of non-clinical studies are:

- to determine whether such studies support the clinical use of a herbal medicine;
- to characterize the range of pharmacological actions of herbal medicines; and
- to define the chemical characteristics of pharmacologically active natural products and to elucidate their mechanisms or actions.

Pharmacodynamic investigations

Pharmacodynamic investigations are conducted in the light of the expected therapeutic effect of a herbal medicine using appropriate non-human systems.

General pharmacological investigations

General pharmacological investigations are conducted to elucidate various pharmacological activities other than the main pharmacodynamic action. Such investigations usually cover the tests on nervous, cardiovascular and respiratory systems, and if necessary others, and should be performed on conscious or

anaesthetized animals using adequate doses and proper routes of administration.

Toxicological investigations

Toxicological investigations are required to supplement human experience in defining possible toxicity from short-term use, but are particularly important in detecting toxicity that may occur either after prolonged exposure or years after the exposure has been discontinued. Generally, the longer the anticipated human use, the longer the test substance is administered to test animals.

Methods

In the conduct of non-clinical research on herbal medicines, standard methods are usually employed. However, the use of novel technologies and methods resulting from scientific progress should be encouraged.

1. Pharmacodynamic and general pharmacological methods should utilize animal models or bioassays that closely relate to human disease as described by either traditional or modern medicine (see Guidelines B, page 31).

2. Toxicological methods

   Animal and other toxicity studies are conducted according to generally accepted principles, referred to collectively as Good Laboratory Practice (GLP), which should be consulted in order to design appropriate studies (see Guidelines C, page 35).

a. Systemic toxicity tests

Systemic toxicity tests refer to alteration of either physiology, anatomy (gross or microscopic) or clinical chemistry (including haematology) that result from pathological changes in any organ distant from the site at which a herbal medicine is administered.

i. Acute toxicity tests aim to determine toxic manifestations of the test substance that occur when animals are exposed to one or more doses of the test substance within a single 24-hour period.

ii. Long-term toxicity tests aim to determine toxic reactions when animals are exposed to the test drug for periods as long as their lifetime. In such tests, the animals are observed for behavioural changes as well as anatomical, physiological and biochemical manifestations of tissue damage. If pathological changes are detected during the period of drug administration, and the changes are not serious, it may be advisable to determine whether such changes are reversible after the drug is withdrawn. Thus, observations are made at intervals during continuous administration of the drug and then, at intervals after the drug has been withdrawn to determine whether such pathology is reversible.

b. Local toxicity tests are done to determine the local irritation and/or systemic absorption of a herbal medicine used for local

applications (such as respiratory inhalants, drugs applied to skin or mucosa).

c. Special toxicity test - regulatory requirements for special toxicity tests vary among Member States. For herbal medicines containing commonly used herbs which have been used clinically for a long period of time, some countries may not require special tests. Mutagenicity tests, however, are commonly required. If any deviation from traditional use is contemplated (such as new use, new preparation, new route of administration or more prolonged administration), additional toxicity tests such as carcinogenicity, teratogenicity and reproduction studies may be recommended.

Clinical trials using herbal medicines

These guidelines for the clinical evaluation of herbal medicines attempt to recognize the long and diverse history of traditional medicine in the Region and the differences between the diagnostic systems of modern medicine and the various traditional medicines of the Member States. Although special considerations may be required, the general principles of the clinical trials of herbal medicines are similar to those applied to synthetic drugs if clinical trial is regarded to be necessary.

Clinical trials ofherbal medicines may have two types of objectives. One is to validate the safety and efficacy that is claimed for a traditional herbal medicine. The other is to develop new herbal medicines or examine a new indication for an existing herbal medicine or a change of dose formulation, or route of administration. In some cases, trials may be designed to test the clinical activity of a purified or semi-purified compound derived from herbal medicines.

Clinical trial protocol development

The development of a protocol should be the joint effort of representatives from several disciplines such as clinical pharmacologists, pharmacists, biostatisticians, physicians and other relevant health care workers, as well as experts in traditional medicine. Ordinarily, the protocol group is chaired by the chief investigator, who is a physician. The protocol should include the following:

1. The title of the trial.

2. A clear statement on the objectives of the study.

3. The justification of the proposed trial based on the available information on safety and efficacy, including a consideration of the non-clinical data as well as the drug utilization pattern and the disease spectrum for the country concerned.

4. The rationale for the composition of the formula being studied and its relation to the principles of both herbal medicine and pharmacodynamic data.

5. The type of trial (such as controlled, open) and trial design (parallel groups, cross-over techniques), blind technique (double blind, single blind), randomization (methods and procedures).

6. Entry and exclusion criteria for study subjects (which may be based on diagnostic criteria of either modern or traditional medicine).

7. Number of trial subjects needed to achieve the trial objective, based on statistical considerations.

8. The therapeutic or clinical end points that are to be analysed at the conclusion of the trial (the unique nature of traditional medicine, which can relate to subjective wellness or quality of life, should also be

considered when selecting the end points of the trial).

9. Control groups to be used (whether a therapeutic control group or a placebo group is used will depend on the disease being studied and the availability of alternative modern drugs or herbal medicines of proven efficacy).

10. The subjective and objective clinical observations and laboratory tests which will be recorded during the course of the trial.

11. The treatment schedule for the duration of the trial, including dosage form and route of administration and the details of the product being used as a therapeutic control.

12. Criteria for other treatments that may or may not be given to subjects during the trial.

13. Procedures for the maintenance of subject identification code lists, treatment record, randomization list and/or Case Report Form (CRF).

14. Information on establishment of the trial code, where it will be kept and when, how and by whom it can be broken in the event of an emergency.

15. The qualifications and experience of the investigators.

16. The facilities and the sites where studies will be undertaken.

17. Methodology for the evaluation of results (such as statistical methods and reports on patients or participants who withdrew from the trial).

18. Information to be given to trial subjects.

19. Relevant communications with appropriate regulatory authorities.

20. Information given to the staff involved in the trial.

21. Medical care to be made available to patients after the trial.

22. List of literature referred to in the protocol.

When considering the above items, special attention must be given to designing a protocol that eliminates bias and reduces variance.

Phases of a clinical trial

A step-by-step approach is usually followed in the development of new herbal medicines, but may ordinarily be less necessary for a study to validate the safety and efficacy of a traditional herbal medicine.

The point of entry to the trial phases will be determined by the nature and history of the herbal medicines being studied.

Clinical trials are generally designated in terms of a "phase", although study designs appropriate for the clinical evaluation of a herbal medicine may, strictly speaking, fall on the borderline between two of the following classical definitions of the usual phases.

Phase I: First trials for a new compound or a new formulation that are generally carried out with a small number of healthy volunteers or patients suffering from the disease for which the herbal medicine is intended. The main purpose of a phase I trial is to observe tolerance to the herbal medicine and therefore to get an indication of the dose that might be used safely in subsequent studies.

Phase II: Studies on a limited number of patients to determine clinical efficacy and to further confirm safety. Such trials are preferably designed as randomized, double-blind, controlled studies, using for control groups either an existing alternative treatment or a placebo. The dosage schedules established in such studies are then used for a more extensive clinical study.

Phase III: A larger patient group is usually studied at several centres using a randomized double-blind design to validate preliminary evidence of efficacy obtained in earlier studies. Ordinarily, such trials are conducted under conditions which are as close as possible to the anticipated conditions of normal use.

Phase IV: Studies performed after the dosage form is available for general use. The main purpose of such studies is to detect toxic events that may occur so rarely that they are not detected earlier.

Individual countries may design clinical trials that follow the general principles embodied in the four phases mentioned above; namely, first to ensure general safety, then to determine efficacy and finally to use post-marketing surveillance to be certain that rare but serious adverse reactions are not occurring and to confirm the long-term efficacy.

Ethics review board

The trial protocol should be considered by an ethics review board. The board will generally be established at an institutional level but boards existing at a regional or national level can also be used. The board will be an independent body constituted of both medical and non-medical members who are not involved in the experimental activity of the trial under review. The board will verify that the rights of the patients participating in the trial are protected and that the trial is justified in medical and social terms. The board will also consider the suitability of the trial protocol, patient selection and

patient protection, and issues of informed consent of patients. The work of the board should be guided by the World Medical Association's Declaration of Helsinki (Annex 2).

The board will work under standard operating procedures which will be developed by each institution taking into consideration all necessary requirements of local regulatory authorities and related governmental agencies including such rules as those for Good Clinical Practice (GCP).

Responsibilities of investigators

The investigators who participate in the design of the protocol will also be responsible for preparing all necessary material for review by the ethics review board.

The investigators must be aware of such responsibilities as the following:

- the appropriate medical care of patients in the study;
- the ethical requirements for the trial (such as selection of patients, advice to patients);
- a knowledge of the product used in the trial;
- an appreciation of research methodology and the conduct of clinical trials (such as the recording and evaluation of results);
- an appreciation of the importance of careful monitoring of the trial and the need to take necessary action, to alter or terminate the trial if patients appear to be harmed by some aspect of the trial.

Responsibilities of the sponsor

If the product under investigation is supplied by a manufacturer, or if the trial is undertaken at the request of a manufacturer, the manufacturer (sponsor) has obligations to maintain the integrity of the investigators, the protocol group and the ethics review board, and to prevent harm to a patient. The sponsor of a study can be an institution or an individual investigator as well as a manufacturer.

The material supplied for the trial will be prepared according to Good Manufacturing Practices (GMP) to ensure the quality of the material used in the investigation. All data on the product will be made available to the investigator before the trial design is completed.

The sponsor must meet all of the local requirements set by regulatory authorities and government agencies and should be aware of standards of good clinical practice.

Data management

The aim of record keeping and the handling of data is to gather information from the trial without error in a form that can later be analysed and reported. A Case Report Form (CRF) for each patient in the trial must be completed and signed by the investigator and the patient's files, CRFs and other sources of primary data must be kept for future reference. Patient data must be handled in a way that maintains confidentiality and yet ensures accuracy. All efforts should be made to maintain error-free records.

When subjects are randomized to different groups, the randomization procedure used must be documented. In the case of a blinded trial, a code for the medicine actually administered must be kept under appropriate conditions.

Statistical analysis

Biostatistical expertise is required when the trial is designed, and must continue to be available as data are collected, analysed and prepared for the final report on the trial. Statistical considerations will govern the number of patients needed to obtain a significant result from the trial, the number of patients needed depending on the anticipated difference in the result between the treatment groups of the trial. The plan for the statistical analyses to be used at the conclusion of the trial must be determined in advance and specified within the protocol. When results are finally analysed, they should be presented in a form that facilitates clinical interpretation.

Reporting

The Chief Investigator will be responsible for preparing a final report of the trial which should be provided to the sponsor, the ethics review board, and any other authorities determined by local legislation. The results of the trials conducted on a herbal medicine should be published in a timely fashion and must include all significant positive and negative results. Even studies which fail to demonstrate efficacy should be published, as selective publication, showing only results that are favourable, will only lead to a form of misconception known as publication bias.

Evaluation of herbal medicine research

A formal procedure for the systematic evaluation of a research project or programme may greatly contribute to its success. Evaluation should be done at all stages of the study, from the design, through its implementation and completion.

The following elements of the programme or project should be examined: goals, conformity of the protocols with goals, progress of the research towards intended goals, and impact of research.

Technology transfer and education

Herbal medical research

Training in such fields as phytochemistry and pharmacology, which contribute to the rational use of herbal medicines, will help to build a core of competent researchers for the study of herbal medicines. The productivity of such researchers will be enhanced by workshops, seminars, lectures, study tours, and scientific exchange programmes with colleagues from other countries.

The health care professions

Productive use of herbal medicines will be enhanced if the medical, dental, pharmacy and nursing professions provide continuing education on herbal medicines, introduce the subject to their students and include it in their curricula.

The public

The public, too, will benefit if herbalists, manufacturers and distributors of herbal medicines have access to unbiased information about herbal medicines.

4. USING THE GUIDELINES

These research guidelines for evaluating the safety and efficacy of herbal medicines are intended to facilitate the work of research scientists and clinicians in this field and to furnish some reference points for the governmental, industrial and non-profit organizations that provide financial support for their work. It is hoped that these guidelines will be found general enough to enable each Member State to modify them to meet its own specific needs. It must be emphasized that these guidelines are offered as a summary of scientific standards governing various aspects of the study of herbal medicines. As such, they may be useful to the regulatory authorities who control the sale of these products and the governmental agencies and medical authorities who supervise their use in the health care system.

A. Guidelines for quality specifications of plant materials and preparations

To ensure the reliability and repeatability of research on herbal medicines, the identity and quality of the plant material or preparation must be determined and stipulated according to the following headings.

Information for fresh, dried and processed plant materials

Name and characteristics

- name of the plant material in Latin, native languages and English whenever applicable.
- scientific name of the plant with reference to the authors and the family to which it belongs.
- part of the plant used and its condition (such as fresh aerial parts, dried root and rhizome, sliced or decorticated).
- time and method of collection, preliminary preparation and drying. If the material has been processed, the method of processing (such as steamed, stir-baked, carbonized) should be indicated.
- a brief description of the distribution and habitat of the plant; growing wild or cultivated (including possible pesticide used). If more than one plant species is concerned, their differences should be indicated. Drawings or photographs of the plants should be provided.

- characterizing compounds of the plant materials, which may also be the biologically or therapeutically active principle, should be quantified and described with their structural formulae, particularly if they are uncommon. For processed plant material, changes in the quantities of these characterizing compounds should be described.

Quality specifications

Authenticity. A description of the macroscopic, microscopic and sensory characteristics of the plant should be provided, including drawings or photographs if possible. A description should be provided of the physical or chemical tests done to identify the plant substances and chromatogram of the active fraction or characterizing compound should be provided. If this is not possible, it should be sufficient to identify a characteristic mixture of substances ("finger print") of the plant material.

Purity. Limits of foreign organic matter (such as stem and rachis fragments in the leaves or leaflets, leaf fragments in the flowers, etc.) and foreign mineral matter (such as sand and soil adhering to the plant material) should be specified; ash determinations should be provided.

Assay. A physical, chemical or biological assay of any known or active fractions should be described and the biological activity of the plant materials expressed in terms of this assay along with an acceptable range for the assay results.

Packaging, labelling and storage

The conditions for packaging, labelling and storage should all be recorded.

Information for medicinal preparations of plant materials

Among the medicinal preparations now widely used are powders, granules, pills, extracts, tablets and injections. Traditional powders and pills are made of powdered plant materials; tablets, granules, ointments and newer types of pills are mostly made of extracts; injections are made of purified extracts or pure active constituents isolated from the plant material. There are also certain medicinal preparations made of both powdered plant materials and extracts.

Name and formula of the product

- Name in Latin, English and native languages.
- Formula including the name of each ingredient and the quantities used for 1000 g or 1000 ml of the product. A quantity may be given as a range corresponding to a definite quantity of assayed active constituents. Any excipient used should be specified.
- Method of preparation to make 1000 g or 1000 ml of the product. The description of the method should include details of any process, such as solvent used, time and temperature of an extraction and concentration, as well as the process used to reduce the level of microbial contamination.
- The active constituents, as far as they are known, should be stated and their structural formulae given. Any chemical or pharmacological incompatibility should be mentioned.

Quality specifications

Authenticity. A description of macroscopic and sensory characteristics should be given and, if powdered plant materials are used as ingredients, their microscopic characteristics should be described

together with drawings or pictures. Physical or chemical identification tests should be described and thin-layer chromatographic procedures for the characterizing compounds should be described. A drawing or photograph of the chromatogram should be included. For compound preparations, the most important ingredients including the use of a "finger-print" should be obtained by either thin-layer chromatography or high performance liquid chromatography.

Purity. Limit tests for heavy metals in extracts and the test for freedom from methanol in alcoholic preparations should be specified. Limit tests for contaminants such as microorganisms, mycotoxins and pesticides may be needed.

Assay. The content of biologically or therapeutically active constituents, particularly those which influence the efficacy of the product, should be determined and an acceptable range specified. For herbal mixtures, the most characterizing compounds possible should be assayed.

Tests related to the form of the preparation for both non-clinical and clinical tests should follow any available regulatory requirements of Member States or the World Health Organization guidelines.

Packaging, labelling and storage. The conditions for packaging, labelling and storage should all be recorded.

B. Guidelines for pharmacodynamic and general pharmacological studies of herbal medicines

Herbal medicines have various pharmacological effects. The appropriate methods for evaluating the particular herbal medicine tested should be applied. The guidelines present basic concepts and principles which should be of utmost concern.

Animals

Species

Appropriate animals may include mice, rats, guinea pigs, rabbits, cats, dogs, etc. Characteristics of the animals such as strain, sex, age and holding conditions should be specified.

Disease model

Disease models can be made by treating animals with certain chemicals or other modalities. For example, immunologically depressed mice can be made by treating them with an immunosuppressive agent, such as cyclophosphamide. Such animals can be used to evaluate immunostimulating activity of a test medicine.

Animals with genetic defects can also be useful: for example the autoimmune mouse (NZB W/F1, MRL/1) and the hypertensive rat (SHR), etc. For study of those herbal medicines which are used under the principles of traditional medicine, animal models may need to be established according to those principles.

Test assays can use

- whole animals;
- isolated organs and tissues;
- blood and its components;
- *ex vivo* and tissue culture cells; and
- subcellular constituents.

Careful attention must be given to the selection of the test system since *in vitro* assays, although less expensive, may not provide such factors as metabolic activation which may be necessary for the biological activity of a herbal medicine. On the other hand, body fluids from test animals may contain such biologically active metabolites and be used successfully in less complex test systems.

Special attention should be given to the sensitivity, reproducibility and general acceptance of the test animals or test systems selected.

An examination of the literature may help to select the species and test systems considered to be most predictive of clinical results and therefore provide the most useful information.

Administration

Route of administration

Since oral dosage forms of herbal medicines are usually used clinically, the oral route of administration is ordinarily the most suitable for use with test animals. Additional routes may be used to approximate the intended route of administration in man.

Frequency of administration

Ordinarily, doses selected for a study should be established by means of a dose-response relationship but since such relationships often cannot be demonstrated with herbal medicines in whole animals, it may be sufficient to select one or more doses that provide a desired effect.

Selection of doses for animal studies should be in accordance with customary clinical doses.

Control group

It is essential that all studies include a negative (vehicle only) control group of animals and, if possible, a positive control group, that is, a group of animals in which the effect of a drug known to be positive is examined.

C. Guidelines for toxicity investigation of herbal medicines

These guidelines are intended to indicate the standard methods of non-clinical toxicological studies related to assessing the safety of herbal medicines. Not all tests are necessarily required for each herbal medicine intended for human study.

Acute toxicity test

Animal species

Some regulatory agencies require that at least two species be used, one of them to be selected from rodents and the other from non-rodents.

Sex

In at least one of the species, males and females should be used.

Number of animals

In the case of rodents, each group should consist of at least five animals per sex. In the case of non-rodents, each group should consist of at least two animals per sex.

Route of administration

Ordinarily, the oral route is sufficient as this is the normal route of clinical administration. However, some regulatory agencies suggest in addition a parenteral route of administration.

In cases where it is proposed to administer the herbal preparation to a human subject by the parenteral route, it may be sufficient to use this route alone for animal testing.

Dose levels

A sufficient number of dose levels should be used in rodents to determine the approximate lethal dose. In non-rodents, sufficient dose levels should be used for the observation of overt toxic signs.

Frequency of administration

The test substance should be administered in one or more doses during a 24-hour period.

Observation

Toxic signs and the severity, onset, progression and reversibility of the signs should be observed and recorded in relation to dose and time. As a general rule, the animals should be observed for at least seven to fourteen days.

Animals dying during the observation period, as well as rodents surviving to the end of the observation period should be autopsied.

If necessary, a histopathological examination should be conducted on any organ or tissue showing macroscopic changes at autopsy.

Long-term toxicity test

Animal species

Many regulatory agencies require that at least two species be used, one a rodent and the other a non-rodent.

Sex

Normally, the same number of male and female animals should be used.

Number of animals

In the case of rodents, each group should consist of at least ten males and ten females. In the case of non-rodents, each group should consist of at least three males and three females.

When interim examinations are scheduled, the number of animals should be increased accordingly.

Route of administration

Normally, the expected clinical route of administration should be used.

Administration period

The period of administration of the test substance to animals will depend on the expected period of clinical use. The period of administration of the toxicity study may vary from country to country, according to its individual regulations.

The following table reflects commonly used ranges of administration periods:

Expected period of clinical use	*Administration period for the toxicity study*
Single administration or repeated administration for less than one week	2 weeks to 1 month
Repeated administration, between one week to four weeks	4 weeks to 3 months
Repeated administration, between one to six months	3 to 6 months
Long-term repeated administration for more than six months	9 to 12 months

As a rule, the test substance should be administered seven days a week. Administration periods for the toxicity study must be recorded in each result.

Dose levels

Groups receiving at least three different dose levels should be used.

One dose level should not cause toxic changes (no-effect dose) and one dose level that produces overt toxic effects should be included. Within this range the addition of at least one more dose may enhance the possibility of observing a dose-response relationship for toxic manifestations. All studies should include a vehicle control group of test animals.

Observations and examinations

Observations and examinations should be performed on the following items (from 1 to 6):

1. General signs, body weight and food and water intake.

   For all experimental animals, the general signs should be observed daily and body weight and food intake should be measured periodically. If useful, water intake should also be determined. The frequency of measurements should normally be as follows:

   - Body weight: before the start of drug administration, at least once a week for the first three months of administration, and at least once every four weeks thereafter.

   - Food intake: before the start of drug administration, at least once a week for the first three months of administration and at least once every four weeks thereafter. If the test substance is administered mixed in the food, the intake should be measured once a week.

6. In order to maximize the amount of useful information that can be obtained during the administration period, all moribund animals should be sacrificed rather than allowed to die. Prior to sacrifice, clinical observations should be recorded and blood samples collected for haematological and blood chemical analysis. At autopsy, a macroscopic examination of organs and tissues and measurement of organ weights should be recorded. A full histopathological examination should be performed in an attempt to characterize the nature (severity or degree) of all toxic changes.

All survivors should be autopsied at the end of the administration period or of the recovery period after taking blood samples for haematological (including blood chemistry) examinations; organs and tissues should be examined macroscopically and organ weights measured. Histopathological examination of the organs and tissues of animals receiving lower dosage should also be performed, if changes are found on gross or macroscopic examination of their organs and tissues of these animals, or if the highest dose group reveal significant changes. On the other hand, histopathological examination of all rodents will further improve the chances of detecting toxicity.

Recovery from toxicity

In order to investigate the recovery from toxic changes, animals that are allowed to live for varying lengths of time after cessation of the period of administration of the test substance, should be examined.

Local toxicity test

Skin sensitization test

Dermatological preparations to be tested

- solid preparations:

  To be prepared by wetting the preparation with water or a suitable solvent to provide a uniform application.

- semi-solid preparations:

  To be tested as undiluted preparations.

- liquid preparations:

  To be tested as undiluted preparations. However, an aerosol agent can be diluted if necessary.

Experimental animals

Use a species with high susceptibility. Guinea-pigs are considered the most suitable experimental animals.

Test methods (in alphabetical order)

1. Adjuvant and patch test
2. Buehler test
3. Draize test
4. Freund's complete adjuvant test
5. Maximization test
6. Open epicutaneous test

7. Optimization test
8. Split adjuvant test

It is recognized that the above-mentioned methods differ in their probability and degree of response to sensitizing substances. However, it is generally accepted that the use of Freund's complete adjuvant increases sensitivity and therefore the possibility of detecting substances with weak sensitizing potential.

Evaluation of test results

The skin reaction of each animal should be evaluated according to the assessment standard of the particular test method used.

Other local toxicity tests

Other local toxicity tests may be conducted if the herbal medicine is intended for such use i.e. vaginal, rectal, respiratory, etc. irritations tests.

Special toxicity tests

Mutagenicity test

Test methods

I. Reverse mutation test in bacteria

1. Strains:

   Salmonella typhimurium TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* WP2 uvr A, are the most commonly used bacteria.*

2. Dose levels:

   At least five dose levels should be employed.

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* See Notes, page 84.

3. Control groups:

   A solvent group should normally serve as a negative control. Authentic mutagens which require S9 (9000 g supernatant) mixture, as well as those which do not require S9 mixture, should be employed as positive control groups.

4. Metabolic activation:

   Tests in the presence of S9 mixture should also be performed.

5. Test methods:

   Either a preincubation method or a plate incorporation method should be used.

6. Presentation of results:

   The actual number and mean value of revertants should be presented in tables.

II. Chromosomal aberration test with mammalian cells in culture

1. Cells:

   Primary or established cell lines of mammalian cells in culture should be used.

2. Dose levels:

   At least three dose levels should be employed.

3. Control groups:

   Normally a solvent group should serve as a negative control. A substance known to cause chromosomal aberrations should be employed as a positive control.

4. Metabolic activation:

   Tests should also be performed with a suitable method of metabolic activation (such as, S9 mix)

5. Experimental procedure:

   a. Chromosomal preparations should be made at an appropriate time after treatment.

   b. At least two plates should be used for each dose level. Examination should be made for chromosomal structural aberrations and polyploid cells on 100 metaphase cells per plate.

6. Presentation of results:

   The relative frequency of cells with chromosomal aberrations and the frequency of chromosomal aberrations per cell should be presented in tables.

III. Micronucleus test with rodents

1. Animals:

   Male mice should normally be used.

2. Number of animals:

   Each group should consist of at least five animals.

3. Route of administration:

   Administration should be intraperitoneal or via the expected clinical route.

4. Dose levels:

   At least three dose groups should be employed.

5. Control groups:

   As a general rule, a solvent group should serve as a negative control. A positive control group should receive a substance known to induce micronuclei.

6. Frequency of administration:

   Single or repeated administration may be employed.

7. Experimental procedure:

   a. Animals should be sacrificed at an appropriate time after administration of the test substance, and bone marrow smears prepared.

   b. Normally, observation should be made of the incidence of micronuclei in 1000 polychromatic erythrocytes per animal. The relative frequency of polychromatic erythrocytes and total erythrocytes should also be calculated.

8. Presentation of results:

   The incidence of polychromatic erythrocytes with micronuclei and the frequency of polychromatic erythrocytes per total erythrocytes should be presented in tables.

Carcinogenicity test

Experimental animals

1. Species and strains of the animals should be selected in consideration of such factors as resistance against infectious disease, life span, spontaneous tumour incidence, and sensitivity to known carcinogens.

2. Animals of the same species and strain should be used for preliminary and full-scale carcinogenicity studies with the same test substance.

Experimental method

1. Preliminary carcinogenicity study

This study is performed to set the dose levels for the full-scale carcinogenicity study. However, if sufficiently reliable data are available, some or all of the following studies may be omitted.

(1) Single dose toxicity studies

These studies are performed on a small number of animals in order to determine the highest dose to be used in the following repeated dose studies.

(2) Repeated dose toxicity studies

These studies are performed in order to determine the highest dose to be used in the full-scale carcinogenicity study

a. Animals:

At least two species of animals of both sexes should be used. It is desirable to initiate studies with normal animals of the same age, but no more than six weeks in rodents.

b. Number of animals:

Each group should contain about ten males and ten females.

c. Route of administration:

The same route of administration should be used as for the full-scale carcinogenicity study.

d. Dose levels:

At least three dose groups and a control group should be established for each sex.

e. Administration period:

The administration period should be 90 days with the dose usually administered seven days a week. However, if the test substance has delayed toxicity or a cumulative effect, administration for a longer period may be neces-sary.

f. Experimental procedure:

1. For all animals in each group, the general signs should be observed daily and body weight measured at least once a week.

2. Autopsy and gross observations on organs and tissues should be performed on dead animals on each occasion and on surviving animals at the end of the administration period. Organs and tissues with gross changes should be examined histopathologically.

g. Results:

1. The dose in the preliminary carcinogenicity study that inhibits body weight gain by less than 10% in comparison with the control and causes neither death due to toxic effects nor remarkable changes in the general signs and laboratory examination findings of the animals. This is the highest dose to be used in the full-scale carcinogenicity study.

2. It is desirable that the highest dose should be set for each species and sex.

2. Full-scale carcinogenicity study

a. Animals:

At least two species of animals of both sexes should be employed. It is desirable to use animals with normal growth of the same age, up to the age of six weeks.

b. Number of animals:

Each group should comprise at least 50 males and 50 females. Allocation of the animals to each group should be made with the proper random sampling method based on body weight, etc.

c. Route of administration:

The expected route of clinical application should be used, if possible.

d. Dose levels:

At least three dose groups and a control group should be employed for each sex.

e. Control group:

i. A negative control group should be included.

ii. If various vehicles or emulsifiers are required to administer the test substance, the negative control group should receive such vehicles or emulsifiers alone. It is also desirable to establish an untreated control group.

2. Haematological examination

   For rodents, blood samples should be taken before autopsy. For non-rodents, blood samples should be taken before the start of drug administration, at least once during the administration period (for studies of longer than one month), and before autopsy.

   For both haematological and blood chemistry examinations, it is desirable to include as many parameters as possible.

3. Renal and hepatic function tests

   Since the liver and kidneys are the usual organs of metabolism and excretion, they are easily affected by potentially toxic agents; their functions should be monitored in long term toxicity studies.

   For rodents, a fixed number of animals from each group should be selected and urinalysis should be performed before the start of drug administration, and at least once during the administration period.

4. Other function tests

   If appropriate, ECG and visual, auditory tests should be performed. For rodents, ophthalmological examination should be performed on a fixed number of animals from each group at least once during the administration period; for non-rodents, examination should be performed on all animals before the start of drug administration and at least once during the period of administration.

5. Animals found dead during the examination should be autopsied as soon as possible. A macroscopic examination should be made of organs and tissues. In addition, where possible, organ weight measurements and histopathological examinations should be performed in an attempt to identify the cause of death and the nature (severity or degree) of the toxic changes present.

measure red and white blood cells as well as to prepare smear specimens. The smear specimens should be examined in cases suggestive of blood disorders such as anaemia or pathology of lymph nodes, liver or spleen.

iv. At the end of the study, the survivors should be autopsied immediately, the organs and tissues of all animals in each group should be examined macroscopically. Histopathological examination should be performed on all animals in the highest dose group and the control group. If the incidence of neoplastic lesions between organs and tissues of the highest dose group and the control group are found to differ, the relevant organs and tissues of all animals in other dose groups should be examined histopathologically and blood examined as in (iii) above.

Evaluation of results

A test substance is considered to be positive for carcinogenicity when any of the following types of response has been observed in the carcinogenicity study:

1. Development of tumours of a type not seen in the control group.
2. Development of tumours seen with greater frequency in the test group, compared with the control group.
3. Greater varieties of organs and tissues are involved in tumour development in the test group, compared with the control group.
4. Earlier development of tumours in the test group, though in the absence of any significant difference in the incidence of tumours between the test group and the control group.

Reproductive and development toxicity test

Experimental animals

1. Species and strains should be selected in consideration of reproductive and developmental information such as fertility, incidence of spontaneous malformation, and susceptibility to substances known to affect reproduction and development.

2. It is desirable to select species and strains with a low incidence of spontaneous malformations.

3. It is desirable that animals used in studies referred to as Segment I, II and III studies be of the same strain and species.

Experimental methods

1. Segment I. Study on administration of the test substance prior to and in the early stages of pregnancy.

   a. Animals:

      At least one species of animal of both sexes such as rats or mice should be used.

   b. Number of animals:

      In the case of rats or mice, each group should consist of at least 20 males and 20 females.

   c. Route of administration:

      The route of administration ordinarily will be the expected clinical route of administration.

   d. Dose levels:

      Groups with three different doses plus a control group should be employed.

e. Control group:

i. A negative control group should be employed. A positive or a comparative control group is desirable.

ii. When vehicles or emulsifiers are required for the administration of the test substance, a negative control group should normally receive such vehicles or emulsifiers alone. A positive control group should receive a substance known to have potent reproductive and developmental toxicity, and a comparative control group should receive a drug with a similar chemical structure or pharmacological effects as the tested drug.

f. Administration period:

When rats or mice are used, males at least 40 days of age should be dosed daily for 60 days or more before mating, and administration should be continued until successful copulation. Sexually mature females should be dosed daily for at least 14 days before mating, during mating and after successful copulation until the beginning of organogenesis.

g. Experimental procedure:

i. During the experimental period, mortality should be recorded, general signs noted and body weights and food intake should be measured.

ii. A treated male and a treated female should be housed together and observed daily for confirmation of successful copulation.

iii. The mating period between the male and female pairs should be about two weeks. If necessary, a treated male and a non-treated

female, or a treated female and a non-treated male should be housed together and observed daily for confirmation of successful copulation.

iv. After successful copulation, females should be autopsied at term, and examined for the number of corpora lutea, successful pregnancies and mortality of fetuses. Additionally, a gross examination of the organs and tissues for all dams should be made.

v. Males used for mating and females without successful copulation should be autopsied at an appropriate time, and gross observation on organs and tissues should be made.

2. Segment II. Study on administration of the test substance during the period of organogenesis.

a. Animals:

Females of at least one species of rodent and a non-rodent such as rabbits should be used.

b. Number of animals:

Each group should consist of at least 30 animals for rats or mice and at least 12 animals for rabbits.

c. Route of administration:

The route of administration should ordinarily be that expected clinically.

d. Dose levels:

At least three different dosage groups plus a control group should be employed.

e. Control group:

i. A negative control group is necessary and a positive or a comparative control group is generally desirable.

ii. When vehicles or emulsifiers are required for the administration of the test substance, a negative control group should normally receive such emulsifiers alone. A positive control group should receive a substance known to have potent reproductive and developmental toxicity and a comparative control group should receive a drug with a similar chemical structure or pharmacological effects.

f. Experimental procedure

i. During the experimental period, mortality, general signs, body weights and food intake should be measured for all dams.

ii. In the case of rodents such as rats or mice, approximately 2/3 of the dams in each group, and in the case of non-rodents such as rabbits, all the dams in each group should be autopsied at term. They should be examined for successful pregnancy and mortality of fetuses. Body weight measurement and morphological examinations should be made on live fetuses. Gross observations on organs and tissues should be made for dams.

iii. For rats or mice, etc., the remaining approximately 1/3 of the dams should be allowed to deliver their offspring. Dams should be examined for abnormality on delivery.

iv. Litter size, mortality, sex and external changes of neonates should be examined, and body weights should be measured.

v. Offspring should be examined for growth and development, appearance of specific signs, reproductive performance, etc. Growth and development should be recorded and morphological, functional and behavioural examinations should be made. Reproductive performance of the offspring, that is, the ability to establish pregnancy, should be examined. If necessary, observation for a longer period should be made.

vi. At an appropriate time, autopsy and gross observation of the organs and tissues of treated dams should be made on treated dams. If necessary, an examination of the second litters should be done.

3. Segment III. Study on administration of the test substance during the perinatal and lactation periods

a. Animals:

At least one species of female animals such as rats or mice should be used. Species should be selected from among those used in the study of administration of the test substance during organogenesis specified in the segment II study.

b. Number of animals:

Each group should consist of at least 20 animals for rats or mice.

c. Route of administration:

The route of administration should be the expected clinical route as a rule.

d. Dose levels:

At least three dose groups plus a control group should be employed.

e. Control group:

i. A negative group should be employed. A positive or a comparative control group may be employed, if necessary.

ii. When vehicles or emulsifiers are required for administration of the test substance, a negative control group should normally receive such vehicles or emulsifiers alone. A positive control group should receive a substance known to have potent reproductive and developmental toxicity and a comparative control group should receive a drug with a similar chemical structure or pharmacological effects.

f. Administration period:

i. During the experimental period, all the dams in each group should be examined for mortality and general signs and body weights and food intake should be measured.

ii. All the dams in each group should be allowed to deliver and nurse their offspring. Dams should be examined for abnormality on delivery.

iii. Litter size, mortality, sex and external changes of neonates should be examined, and body weights should be measured.

iv. Offspring should be examined for growth and development, appearance of specific signs, reproductive performance, etc. For observation of growth and development,

morphological, functional and behavioural examinations should be made. Reproductive performance of offspring should be examined on the basis of establishment of pregnancy. If necessary, observation for a longer period should be made.

v. At an appropriate time, autopsy and gross observations on organs and tissues should be made on treated dams. If necessary, an examination of the second litters should be done.

Analysis of results

1. The results obtained should be presented in the form of tables and figures with discussion of the results. For presentation, summary tables which give an overview of the results of all groups should be prepared. In addition, appendix tables which provide data for individual animals in each group should be prepared for reference.

2. For statistical analysis of the data obtained before weaning, it is desirable that the litter, instead of the individual fetus or offspring, serve as the unit for analysis.

3. The discussion should address the no-effect dose level of the test substance concerned with the reproduction of the parent animals and development of the next generation. It is desirable to compare the reproductive and developmental toxicity with that of similar drugs.

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI*

RECOMMENDATIONS GUIDING PHYSICIANS IN BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

ADOPTED BY THE 18TH WORLD MEDICAL ASSEMBLY
HELSINKI, FINLAND
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29TH WORLD MEDICAL ASSEMBLY
TOKYO, JAPAN
OCTOBER 1975

35TH WORLD MEDICAL ASSEMBLY
VENICE, ITALY
OCTOBER 1983

AND

THE 41ST WORLD MEDICAL ASSEMBLY
HONG KONG
SEPTEMBER 1989

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Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

1. Basic principles

1.1 Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

1.2 The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

1.3 Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

1.4 Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is

in proportion to the inherent risk to the subject.

1.5 Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

1.6 The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

1.7 Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

1.8 In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

1.9 In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

1.10 When obtaining informed consent for the research project, the physician should be particularly cautious if the subject is in a

dependent relationship to him or her or may consent under duress. In that case, the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

1.11 In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

1.12 The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

2. *Medical research combined with professional care (Clinical research)*

2.1 In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing health or alleviating suffering.

2.2 The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

2.3 In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method.

2.4 The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

2.5 If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

2.6 The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

3. Non-therapeutic biomedical research involving human subjects (Non-clinical biomedical research)

3.1 In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

3.2 The subjects should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3.3 The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

3.4 In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

REPORT OF THE MEETING OF THE WORKING GROUP ON THE SAFETY AND EFFICACY OF HERBAL MEDICINE

Summary

The Working Group on the Safety and Efficacy of Herbal Medicine met in Manila, Philippines, from 5 to 9 October 1992. The main objective of the meeting was to develop research guidelines for evaluating the safety and efficacy of herbal medicines and to make recommendations on further collaboration and activity on the safety and efficacy of herbal medicines.

The meeting was attended by 15 members from ten Member States, two consultants, two secretariat staff from the WHO Regional Office for the Western Pacific and one observer from Japan. Dr Nelia Cortes-Maramba was elected Chairperson, Dr G.N. Vaughan as Vice-Chairman, and Dr Kit Lam Chan and Professor Yun Cheong Kong as Rapporteurs. Dr S.T. Han, WHO's Regional Director for the Western Pacific, delivered addresses at both the opening and the closing ceremonies.

The members presented their papers to review the current status of research works on herbal medicine, and to introduce methodology used for evaluating the safety and efficacy of herbal medicines. The drafts of guidelines for evaluating safety and efficacy of herbal medicines were discussed extensively. The issues covered in the discussion included: the definition of terms used in the guidelines; the development of a coherent scheme for describing various herbal medicines and the plant substances from which they are formulated; the means of incorporating ethical considerations into

the guidelines; the standards for non-clinical and clinical studie: the philosophical difficulties of evaluating herbal medicine rem edies outside the system of medical thought in which they wer developed; and the financial and technical difficulties liable to b encountered in carrying out the terms of the guidelines.

In the course of these discussions, the Working Group develope the research guidelines for evaluating the safety and efficacy c herbal medicines and made recommendations for promoting th dissemination of these guidelines. A summary of these recommen dations follows:

(1) WHO and the Member States should help to promote th use of scientifically validated herbal medicines for th purpose of providing medical care in a cost-effectiv manner with the goal of validating claims for herbal medi cines now in use and seeking collaborative solutions t common national and regional problems. In addition programmes should be established to disseminate researc on herbal medicine, not only to research workers, but t health planners and health care practitioners as well a students in the health care professions.

Member States are also encouraged to make nationa policies that encourage the rational use of herbal medicine and promote their safety and quality. In addition, nationa policies should be made to preserve the environment tha provides valuable medicinal plants.

(2) WHO should disseminate these guidelines as widely a possible among Member States and their institutions con cerned with herbal medicines to solicit their comments an recommendations, so that the guidelines can be revised an made as helpful as possible to each country in forming it own guidelines to meet its own specific needs.

Introduction

Humankind has used plants as therapeutic agents for thousands of years and continues to rely on them for health care, particularly in developing countries where they are usually prescribed by traditional medical practitioners who are respected members of the community. WHO has recognized the potential significance of the practice of traditional medicine, the therapeutic practices of which are based almost entirely on the use of traditional herbal remedies. Thus, resolutions have been adopted both by the the World Health Assembly and the Regional Committee for the Western Pacific to encourage the appropriate development of traditional systems of medicine and to examine the therapeutic properties of their herbal remedies.

Approximately 40% of the world's population lives in the countries served by the WHO Regional Office for the Western Pacific. This Office has actively examined how the resources of herbal medicines, which have a particularly rich tradition in many of its member countries, might be mobilized to help achieve the goal of health for all by the year 2000.

With a view to providing safe and effective herbal medicines and promoting research on herbal medicines for their scientific development, WHO constituted a Working Group on the Safety and Efficacy of Herbal Medicine which met from 5 to 9 October 1992, in Manila.

Objectives

The objectives of the meeting were as follows:

(1) to review the current status of attempts to evaluate the safety and efficacy of herbal medicines;

(2) to discuss the methodology for evaluating the safety and efficacy of herbal medicines;

(3) to outline principles and approaches to be used in developing guidelines for the safety and efficacy of herbal medicines; and

(4) to develop research guidelines for evaluating the safety and efficacy of herbal medicines and to make recommendations on further collaboration and activity on the safety and efficacy of herbal medicines.

Participants

The working group comprised 15 temporary advisers, two consultants and two members of the WHO secretariat. One observer from Japan also attended the meeting. The list of participants is in Annex 3.

Organization

Dr Nelia-Cortes-Maramba and Dr G.N. Vaughan were elected Chairperson and Vice-Chairman respectively of the Working Group and Dr Kit Lam Chan and Professor Yun Cheong Kong were elected as Rapporteurs.

Opening ceremony

Dr S.T. Han, Regional Director of the WHO Regional Office for the Western Pacific Region opened the meeting by pointing out how herbal medicines, which have contributed to human health for thousands of years, continue to do so, particularly in the Western Pacific Region where herbal medical traditions are particularly rich and still vital. He reminded the Working Group that WHO was fully aware of the continuing importance of herbal medicines for the

health of a very large sector of the world's population and that WHO encouraged the use of herbal medicines which have been proven to be safe and effective. He noted that the Working Group had the responsibility to develop the first guidelines in the Region to be applied to research on the evaluation of herbal medicines.

Proceedings

The Working Group recognized its task to develop Research Guidelines for Evaluating Herbal Medicines and was aided by working papers provided by the members of the Working Group and by draft guidelines provided by two consultants and members of the secretariat.

Presentation

The current status of research on herbal medicine in the Region and the methodology used for evaluating the safety and efficacy of herbal medicines were outlined by the members during the presentation of their working papers in the Meeting. The papers are summarized below:

Dr Chen Ken, WHO-WPRO Medical Officer, Traditional Medicine, gave a summary of various global and regional WHO resolutions regarding traditional medicine. He emphasized that the aim of WHO's Traditional Medicine Programme is to promote the appropriate utilization of traditional medicine in relation to a country's health care system, and reviewed WHO's role in developing a comprehensive approach to herbal medicines that includes utilization and administration, inventory survey and conservation, training and research, cultivation and pharmaceutical production, legislation and registration.

In another paper, Dr Chen Ken reviewed the research carried out on herbal medicines within the Western Pacific Region. Such research was classified according to the materials examined, the disciplines involved and the purpose and place of research. The difficulties of carrying out research on the safety and efficacy of herbal medicines were also pointed out. These include inadequate technical facilities, financial shortages, and in some cases different approaches to problems as a result of different cultural and ethical norms. He outlined a set of principles for the development of research guidelines for evaluating the safety and efficacy of herbal medicine.

Mr Noriaki Shigeno, WHO-WPRO Scientist, Pharmaceuticals, outlined the basic requirements for the quality control of both prescription drugs and non-prescription drugs and suggested that herbal medicines may be considered non-prescription drugs.

Dr Kit Lam Chan emphasized the importance of identifying the biologically active constituents of each plant which forms the composition of a herbal medicine preparation in order to explain the therapeutic properties of the preparation. He reviewed evidence of the value of the brine shrimp as a test system for screening for pharmacological activity prior to the use of more specific and sophisticated bioassays, and pointed out how KB cell cultures may provide preliminary insight into the therapeutic selectivity of plant materials and some measure of the difference between possible therapeutic activity and toxicity in the same host system.

Professor Byung Hoon Han pointed out the need for a systemic approach to, and the importance of evaluating the efficacy of herbal medicines, by both *in vitro* and *in vivo* studies. False-positive and false-negative results are inevitable in the *in vitro* assay systems owing to the wide distribution in plants of simple fatty acids, polyvalent organic acids, tannin and metallic ions. Professor Byung Hoon Han also pointed out that the registration for sale of

herbal medicine preparations by the Korean Government relies almost completely on acceptance of a formulation that follows one of the classic traditional medicine texts.

Professor Hoang Bao Chau, reviewed the progress made at the Institute of Traditional Medicine of Viet Nam since 1960. The Institute has worked towards improving the safety and evaluating the efficacy of traditional drugs, and efforts have been made to standardize drugs and overcome toxicity. In the period from 1990 to 1995, the Institute will focus on the safety and efficacy of some herbal medicine preparations with different functional groups as compared with total extracts. Measures taken to integrate studies from various non-clinical disciplines with clinical practice were also mentioned.

Dr Nam-Jae Kim presented the screening methodology for the biochemical and pharmacological evaluation of herbal medicines. He also summarized the present status of the role of herbal medicines in the health care system of the Republic of Korea. He proposed revising the terminology of traditional medicine into an integrated system that could readily be adapted to modern scientific terms, and which would be in accordance with pathological studies.

Professor Yun Cheong Kong focused on the measures that could improve the quality of both the crude plant materials and proprietary herbal medical products. He concluded that education of the public in the basic concepts of traditional medicine and principles guiding its diagnostic methods is the best way to enable people to make their own evaluation of traditional medical practitioners and of the safety and efficacy of the medicines they prescribe.

Dr Liao Fu-long suggested guidelines for the evaluation of the safety and efficacy of both crude and processed forms and preparations of herbal medicines. He also emphasized the importance of

legislative and administrative policies in determining the approval of new herbal medicines.

Professor Lou Zhi-cen, presented a comprehensive scheme for the evaluation of the safety and efficacy of herbal medicines by considering standards for appropriate pharmacodynamic, toxicological and clinical investigations.

Dr Nelia Cortes-Maramba described the research activities conducted by the National Integrated Research Programme on Medicinal Plants in the Philippines and described a survey of 1207 traditional healers regarding their preparation of herbal medicines, the plants and their parts, the methods of processing, and the dose and frequency of administration for various indications. Preclinical studies have been carried out for 30 medicinal plants, and out of 270 plants tested 12 were found to have mutagenic activity.

Dr Motoyoshi Satake described the guidelines for the required types of pharmacological, pharmacokinetic, toxicological and clinical studies that have been developed to assure the quality of herbal medicines (Kampo) registered with the Japanese regulatory authorities. Kampo medicines in Japan are of two types: one, the traditional decoctions prepared from several varieties of chopped herbs, the other specific formulations of extracts compounded on an industrial scale by pharmaceutical manufacturers.

Professor S. Sotheeswaran reviewed the safety and efficacy of some of the plants used in the herbal medicines of the South Pacific and some of their pharmacological activities. He indicated that extracts of many of these medicinal plants have pharmacological properties that confirm their clinical use, such as herbs used to treat wounds that show antimicrobial activity. He also drew attention to the toxic compounds of some of these herbs which have not yet

been fully appreciated and pointed out ways in which the actions of some traditional herbal medicines may interfere with the intended actions of modern drugs.

In the paper of Dr Bounhoong Southavong, the research activities of herbal medicine in Lao People's Democratic Republic were presented. The Research Institute of Medicinal Plants was founded in 1976, and since then has begun to evaluate more than 200 species of newly discovered medicinal plants whose evaluation for safety and efficacy is a continuing activity of the Institute.

Dr Hiroyori Tosa addressed the problem of evaluating the safety and efficacy of Kampo prescriptions, particularly when physicians no longer understand the Kampo philosophy. To increase the accuracy of Kampo prescribing, he proposed that checklists of symptoms, signs, laboratory tests and other examinations should be created and their intensity graded systematically. Composite scores could then be created, which, with statistical analysis, could provide a clinical evaluation of the efficacy of Kampo prescriptions.

Dr G.N. Vaughan reviewed the guidelines and policies at the Therapeutic Goods Administration (TGA), the federal agency in Australia responsible for evaluating the quality, safety and efficacy of all drugs including herbal medicines. "Registration" drugs undergo intensive evaluation to ensure safety, quality and efficacy. "Listed" drugs, on the other hand, which apply to most herbal medicines, undergo an evaluation emphasizing only quality and safety. No therapeutic claims can be made for such drugs. All drugs, however, are subject to Good Manufacturing Practice (GMP).

Professor Wang Bao-qin described how herbal medicines are assessed in the People's Republic of China. In the past five years,

the Government has listed 120 commonly used crude herbal medicines for systematic investigation and strict quality assessment. It is of particular interest that pharmacodynamic studies show that several traditional multi-herbal prescriptions have a greater therapeutic effect than their individual components. Studies are also under way to develop and evaluate new herbal medicines.

Dr Haruki Yamada described his studies on pharmacologically active molecules in Japanese Kampo medicines which call attention to the pharmacological activity of the larger molecules in some of these prescriptions. Several pectic polysaccharides in the "Kampo" prescription, Juzen-Taiho-To, for example, have immunomodulating activity. Such studies support the conviction that attention in the study of classic herbal medicines should not be confined exclusively to compounds of low molecular weight.

Discussion

During the preparation of the guidelines, a number of issues were raised that received particular attention and required considerable discussion.

The definition of the term herbal medicines was accepted to refer to the remedy derived primarily from plant material taken by the patient, although it was recognized that such remedies are not always exclusively of plant origin and that the term herbal medicine can be used to refer to the system of medicine based on plant remedies rather than exclusively to the remedies themselves.

Another difficult problem was the development of a coherent scheme for describing various herbal medicines and the plant substances from which they are formulated. Such remedies come from many cultures and are often thousands of years old. The

Working Group recognized, therefore, that there would probably be important exceptions to any scheme proposed, but hoped that its proposed definitions would be a helpful frame of reference that would not unjustly exclude any useful or potentially useful herbal medicine.

Although the Guidelines do not specifically raise the issue of financing the scientific evaluation of the safety and efficacy of herbal medicines, the issue of such financial support was repeatedly raised by members of the Working Group. All members of the Working Group recognized that the goals of the guidelines required research facilities, personnel and supplies that in turn required the necessary financial support.

In addition to general principles such as feasibility, reliability, repeatability and practicality, the following four points were considered by the members of the Working Group in relation to principles for developing research guidelines for evaluating the safety and efficacy of herbal medicine.

The Working Group wished to make clear the importance of ethical considerations relevant to both human and animal research and to the preservation of the environment that allows medicinal herbs to flourish, but wished to incorporate these considerations into the guidelines in a way that would be acceptable to all groups within the Region and elsewhere in the world.

The Working Group also recognized the difficulty of setting standards for non-clinical studies that might be too rigid on the one hand or too vague on the other, since in a sense the task of writing such guidelines forces the Group to solve the very difficult problem of defining "good science".

Randomized, double-blind, placebo-controlled clinical trials have provided much useful information about drugs used in modern

medicine, but their power lies in their ability to eliminate the biases that seem to beset the advocates of any therapy and that make it so difficult to distinguish a true therapeutic response from the variability inherent in clinical medicine. Similarly, statistical reasoning has helped the modern clinician to recognize that an apparent therapeutic success may merely be a chance occurrence. Thus the guidelines on clinical trials were formulated to show the importance of these principles of modern drug evaluation and not to thwart imaginative research protocols that find other ways of convincing the sophisticated observer that bias and chance results have not been confused with therapeutic effect.

Although the Working Group carried out its charge to develop guidelines on the evaluation of herbal medicines, its members were keenly aware of the philosophical difficulties of evaluating remedies used in traditional systems of medicine. This unfortunate philosophical paradox was also one of the reasons why several members of the Working Group felt so strongly that the philosophical rationale of traditional systems of medicine should be made available in the courses taken by students in the health professions and made available to the public as well.

Conclusions and recommendations

Conclusion

The consensus reached by the Working Group on principles, methodologies and technical considerations is set out in the Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines which were developed during this meeting. The recommendations of the working group are outlined below.

Recommendations

The Working Group recommends that WHO and its Member States help to promote the use of scientifically validated herbal medicines for the purpose of providing medical care in a cost-effective manner.

To carry out this overall goal the following more specific recommendations are made:

1. Research on herbal medicines should be encouraged both by WHO and the individual Member States, with the goals of validating claims for herbal medicines now in use; encouraging the convergence of traditional and modern medicine; and providing collaborative solutions to common national and regional medical problems. When planning research projects on the medical value of herbal medicines, the value of this research as a basis for economic development should also be considered.

2. WHO should encourage the establishment of facilities for the dissemination of research and other information about research on herbal medicines with the goal of providing health care workers and planners with the latest information for use in their own countries and to encourage them to establish regional cooperation and collaboration towards the solution of common medical and educational problems. Facilities, which should be equipped with computers, CD-ROM and other modern information technology and stationed within one country should be made accessible to scientists and planners from other countries.

3. WHO and its Member States should foster programmes that enable students and practitioners in the health care professions to learn about traditional medicines and the benefits that herbal remedies can provide. Education of the public about herbal medicine will help in the selection of remedies of the highest standards.

4. All Member States should develop national policies to foster the rational use of herbal medicines including the development of standards for those products that may eventually result in national or regional formularies; and they should foster efforts both to preserve the environment which provides valuable medicinal plants and to increase knowledge about their own medicinal plants.

WHO should disseminate the Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines as widely as possible among Member States and their institutions concerned with herbal medicines to solicit their comments and recommendations, particularly with regard to the value of these guidelines in helping each country to form its own guidelines.

WHO should help the Member States to develop and improve their national health policies on herbal remedies by taking steps to amend these guidelines periodically to reflect both technical and scientific advances and the experience of other Member States.

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These bacterial strains can be obtained from:

American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, 20852 Maryland, USA.

Institute for Fermentation, OSAKA (IFO), 17-85, Juso-Honmachi 2, Yodogawa-ku, Osaka 532, Japan

The accession numbers are as follows:

Strain	Accession Number	
	ATCC	IFO
Salmonella typhimurium		
TA 1535	ATCC 29629	IFO 14209
TA 1537	ATCC 29630	IFO 14211
TA 98	-	IFO 14211
TA 100	-	IFO 14194
Escherichia coli		
WP2 *uvr* A	-	IFO 14196

Reference: D. Durand et al. *Detection of Carcinogens*. MELE in Microbiology (student manual), Burgess Publishing Company, Minneapolis: 1982.

BIBLIOGRAPHY

Guidelines for the assessment of herbal medicine programme on traditional medicine. Geneva: World Health Organization, document, 1991 (WHO/TRM/91.4).

Guidelines for reproduction studies for safety evaluation of drugs for human use. Bethesda, MD: U.S. Food and Drug Administration, 1966.

1990 Guidelines for toxicity studies of drugs manual/ editorial supervision by New Drugs Division, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare. Tokyo: Yakuji Nippo, 1991.

新药(中药)临床研究指导原则(第一、二批合订本)中华人民共和国卫生部药政局一九八九年。 *Guiding principles for clinical research on new drugs (traditional Chinese medicine)*. Beijing, China: Ministry of Public Health, Bureau of Drug Policy and Administration, 1989.

Proposed WHO guidelines for good clinical practice (GCP) for trials on pharmaceutical products. WHO drug information 1992; 6(4): 170-188.

Provisions for new drugs approval. Beijing, China: Ministry of Public Health, 1988.

新药审批办法：有关中药部分的修订和补充规定(一九九二年九月一日起施行)中华人民共和国卫生部。 *Provisions for new drug approval: supplementary stipulations and revision of issues related to traditional Chinese medicine*, Beijing, China: Ministry of Public Health, 1992.

Selection and scientific validation of medicinal plants for primary health care. Taguig, Metro Manila: Department of Science and Technology, Philippine Council for Health Research and Development, 1991. (Technical report series 12).

Technical guidelines for pharmaceuticals in the European Economic Community, editor, Duilio Poggiolini, New York: Raven Press, 1983.

The rules governing medicinal products in the European community: Volume III. Guidelines on the quality, safety and efficacy of medicinal products for human use, Luxembourg: Office for Official Publications of the European Communities, 1989.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

MEDICINAL PLANTS IN CHINA

WHO Regional Publications, Western Pacific Series No. 2, 1989, 327 pp, 151 colour plates, ISBN 92 9061 102 2, Sw.fr. 50.-/US$ 40.00

Catalogues the 150 species of medicinal plants most commonly used in traditional Chinese medicine. The book was produced in an effort to communicate knowledge about herbal medicine that has accumulated over thousands of years, has been confirmed through both empirical experience and scientific evaluation, and yet has rarely been published outside the Chinese literature.

MEDICINAL PLANTS IN VIET NAM

WHO Regional Publications, Western Pacific Series No. 3, 1990, 410 pp, 200 colour plates, ISBN 92 9061 102 2, Sw.fr. 45.-/US$ 40.50

Catalogues the 200 species of medicinal plants most commonly used in traditional Vietnamese medicine. The book responds to the increasing respect for the value of medicinal plants as a source of efficacious and inexpensive new drugs that offer an important alternative to chemically synthesized medicines.

THE PROMOTION AND DEVELOPMENT OF TRADITIONAL MEDICINE

Report of a WHO Meeting Technical Report Series, No. 622, 1978, 41 pp, ISBN 92 4 120622 5 Sw.fr. 5.-/US$ 4.00

Presents conclusions and practical guidelines supporting the need for radical promotion and development of traditional medicine as one of the surest ways to achieve total health care coverage of the world population. Emphasis is placed on the need for traditional medicine to be evaluated, given due recognition and developed so as to improve its efficacy, safety, availability, and wider application at low cost.

"These guidelines are intended to facilitate the work of research scientists and clinicians in the field of herbal medicine. They will also furnish some reference points for the various governmental, industrial and non-profit organizations involved in research and use of herbal medicines."

Price : Sw. fr. 7.50 ($US 5.25)
For developing countries Sw. fr. 5.00 ($US 3.50)
ISBN 92 9061 110 3

WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems

World Health Organization
Geneva
2004

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Foreword

The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings. However, mass media reports of adverse events tend to be sensational and give a negative impression regarding the use of herbal medicines in general rather than identifying the causes of these events, which may relate to a variety of issues. The safety of herbal medicines has become a major concern to both national health authorities and the general public.

The World Health Organization (WHO) received an urgent request from its Member States, through the national pharmacovigilance centres participating in the WHO International Drug Monitoring Programme and drug regulatory authorities, to assist Member States to strengthen national capacity in monitoring the safety of herbal medicines and in analysing the causes of adverse events, and to share safety information at national, regional and global levels. These guidelines have been developed as WHO’s immediate response to this request, and to support Member States’ efforts in this area in the context of the WHO International Drug Monitoring Programme, which has been in operation since the 1970s. Thus, development of the guidelines has been carried out as a joint project between the Traditional Medicine Team (TRM) and the Quality, Safety: Medicines Team (QSM) in the Department of Essential Drugs and Medicines Policy (EDM) at WHO headquarters.

The recommended approach is to include herbal medicines in existing national pharmacovigilance systems or, where such systems have not yet been developed, to establish comprehensive national pharmacovigilance systems which incorporate coverage of herbal medicines. As described in the Introduction to the guidelines, it is not WHO’s intention to suggest that different systems should be instituted for this purpose. However, in view of the unique characteristics of the provision and use of herbal medicines, there are several technical issues that need to be addressed if adequate and effective monitoring is to be introduced. The guidelines therefore identify the particular challenges posed in monitoring the safety of herbal medicines effectively and propose approaches for overcoming them. Special attention is also given to the reporting system for adverse reactions to herbal medicines, and to the analysis of the causes of the reported adverse reactions.

In order to handle herbal medicines, in particular, to analyse the causes of adverse events, national pharmacovigilance centres (or equivalent institutions) will need to acquire specific technical expertise. This will include trained personnel in relevant technical areas and facilities to analyse the products concerned, for which there is often insufficient information and lack of access to reliable information support. Many countries currently lack this expertise, in particular, access to suitable analytical laboratories. Member States have therefore recommended the establishment of regional laboratories specializing in

the analysis of herbal products. WHO encourages Member States to explore the feasibility of this proposal.

To further the implementation of these guidelines, WHO plans to organize a series of training workshops for Member States, in collaboration with the WHO Collaborating Centres for International Drug Monitoring and for Traditional Medicine. National capacity in monitoring the safety of herbal medicines will be further strengthened through national training workshops on topics such as, broadening reporting schemes, acquiring technical expertise at national pharmacovigilance centres, and promoting awareness. Training of practitioners who provide herbal medicines will also be crucial.

The guidelines also articulate technical issues relating to data management and communication. The Uppsala Monitoring Centre, Uppsala, Sweden (UMC) has proposed the herbal anatomical-therapeutic-chemical classification (HATC) as a coding tool to permit the inclusion of individual herbal products in the global WHO database of adverse drug reaction (ADR) reports for pharmacovigilance purposes. The summary explanation of the proposed system by UMC is annexed to the guidelines. Although the system represents a valuable attempt at coding herbal medicines, it may not be perfect for covering all types of herbal products, in particular, traditional medicines that are used under unique concepts and with unique terminologies. Member States are encouraged to offer suggestions, on the basis of their national experience in the day-to-day operation of national pharmacovigilance, as to how classification could be approached in a more comprehensive manner and in a way that meets their national circumstances. WHO, in collaboration with UMC, will work with Member States to continue development of the system.

Currently, the majority of adverse events related to the use of herbal products and herbal medicines that are reported are attributable either to poor product quality or to improper use. Inadequate regulatory measures, weak quality control systems and largely uncontrolled distribution channels (including mail order and Internet sales) may have been contributing to the occurrence of such events. In order to expand knowledge about genuine adverse reactions to herbal medicines, and to avoid wasting scare resources for identifying and analysing adverse events, events resulting from such situations will need to be reduced or eliminated. Member States are therefore encouraged to strengthen national regulation, registration and quality assurance and control of herbal medicines. In addition, national health authorities should give greater attention to consumer education and to qualified practice in the provision of herbal medicines.

WHO has welcomed the active participation of drug regulatory authorities and national pharmacovigilance centres, among others, in the development of these guidelines. This has provided a useful starting point for strengthening communication between these authorities, which will be needed to ensure progress towards the common goal – the safety of herbal medicines.

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Part I

WHO Guidelines on safety monitoring of herbal medicines in pharmacovigilance systems

1. General introduction

1.1 Introduction

Safety is a fundamental principle in the provision of herbal medicines and herbal products for health care, and a critical component of quality control. These guidelines provide practical technical guidance for monitoring the safety of herbal medicines within pharmacovigilance systems. The safety monitoring of herbal medicines is compared and contrasted with that of other medicines currently undertaken in the context of the WHO International Drug Monitoring Programme. While there are regulatory and cultural differences in the preparation and use of different types of medicines, they are all equally important from a pharmacovigilance perspective.

The guidelines were developed with the view that, within current pharmacovigilance systems, monitoring of the safety of medicines should be enhanced and broadened in ways that will allow the successful monitoring of herbal medicines. It is not the intention to suggest that different systems should be instituted for this purpose. The guidelines should therefore be considered in conjunction with the publication entitled, *Safety monitoring of medicinal products: guidelines for setting up and running a pharmacovigilance centre (1)*, which is reproduced as Part II of this publication.

The inclusion of herbal medicines in pharmacovigilance systems is becoming increasingly important given the growing use of herbal products and herbal medicines globally. For example, in the United States of America, some US$ 17 billion was spent by more than 158 million Americans in 2000 *(2)*. Further, a recent study indicated that more than 70% of the German population reported using "natural medicines" and that, for most of them, herbal medicinal products were the first choice in the treatment of minor diseases or disorders *(3)*. The worldwide consumption of herbal medicines is enormous, so that, in terms of population exposure alone, it is essential to identify the risks associated with their use. Safety of herbal medicines is therefore an important public health issue. Herbal medicines are frequently used in conjunction with other medicines, and it is essential to understand the consequences of such combined use and monitor whether any adverse effects are arising. This can be achieved most readily within existing pharmacovigilance systems.

1.2 Background

Problems

Among consumers, there is a widespread misconception that "natural" always means "safe", and a common belief that remedies from natural origin are harmless and carry no risk. However, some medicinal plants are inherently toxic.

Further, as with all medicines, herbal medicines are expected to have side effects, which may be of an adverse nature. Some adverse events reported in association with herbal products are attributable to problems of quality. Major causes of such events are adulteration of herbal products with undeclared other medicines and potent pharmaceutical substances, such as corticosteroids and non-steroidal anti-inflammatory agents. Adverse events may also arise from the mistaken use of the wrong species of medicinal plants, incorrect dosing, errors in the use of herbal medicines both by health-care providers and consumers, interactions with other medicines, and use of products contaminated with potentially hazardous substances, such as toxic metals, pathogenic microorganisms and agrochemical residues.

The following examples demonstrate the range of problems encountered with the use of herbal medicines and products.

- Some herbal products were found to contain 0.1–0.3 mg of betamethasone per capsule after some patients developed corticosteroid-like side effects.
- Owing to misidentification of the medicinal plant species, plant materials containing aristolochic acid were used for manufacturing herbal products, which caused severe kidney failure in patients in several countries.
- Reports have been received by drug safety monitoring agencies of prolonged prothrombin times, increased coagulation time, subcutaneous haematomas and intracranial haemorrhage associated with the use of *Ginkgo biloba*.
- One of the most well known traditionally used herbal medicines caused severe, sometimes fatal cases of interstitial pneumonia when used in conjunction with interferon.

Adverse events thus far reported in relation to herbal products are frequently attributable either to poor quality or to improper use, and it is therefore difficult to distinguish genuine adverse reactions to herbal medicines and herbal products until the cause of such events has been identified.

Current situation

Despite the growing interest in the safety of herbal medicines, national surveillance systems to monitor and evaluate adverse reactions associated with herbal medicines are rare, even among the more than 70 Member States participating in the WHO International Drug Monitoring Programme. Moreover, there is a lack of effective communication on this subject at all levels, from international to local. A recent WHO survey showed that around 90 countries, less than half of WHO's Member States, currently regulate herbal medicines, and an even smaller proportion has systems in place for the regulation/qualification of providers of herbal medicines. Moreover, there are disparities in regulation between countries, and this has serious implications for international access to and distribution of such products.

National pharmacovigilance systems should be closely linked to national drug regulatory systems. To function properly, a national safety monitoring programme for herbal medicines should be operated alongside an effective national drug regulatory system with the will and the potential to react to signals emanating from reports of adverse effects of herbal medicines and to take proper regulatory measures.

At the national level, the capacity for reporting adverse events on herbal medicines, analysing their causes and learning from past experience is seriously hampered in many Member States by the lack of methodological uniformity in identification and measurement, the lack of information on adverse effects of herbal medicines, inadequate reporting schemes, fear of professional liability, and inadequate information systems relating to the use of herbal medicines. Current knowledge of the epidemiology of adverse reactions to herbal medicines, such as frequency of occurrence and causes, is very limited.

Action required
For the safety of those using herbal medicines, four complementary actions are needed:

- clear identification of the nature of adverse events
- management of the risks
- institution of measure to prevent adverse events
- good communication of the risks and benefits of herbal medicines.

These require:

- increased ability to learn from identified adverse events through better reporting systems, skilful technical investigation of incidents and responsible sharing of data
- greater capacity to anticipate adverse events and to probe systemic weaknesses that might lead to problems
- identification of existing knowledge resources, within and outside the health sector
- improvements in the health-care delivery system, so that structures are reconfigured, incentives are realigned, and safety and quality are placed at the core of the system

In 2000 and 2001, the annual meetings of national pharmacovigilance centres participating in the WHO International Drug Monitoring Programme requested WHO to provide urgent support to Member States in developing national systems for the safety monitoring of herbal and traditional medicines. This was echoed by a recommendation made at the Third WHO Consultation on Selected Medicinal Plants, and at the WHO Informal Meeting on Methodologies for Quality Control of Finished Herbal Products, both held in Ottawa, Canada, in July 2001. The International Conference of Drug Regulatory Authorities (ICDRA) also made recommendations to WHO in 1999 and 2002 that it should support Member States in strengthening their capacity in these areas. In addition, resolution WHA56.31 on traditional medicine, adopted at the Fifty-Sixth World Health Assembly in May 2003, urged Member States to set up or expand and strengthen existing national drug safety monitoring systems to monitor herbal medicines and other traditional practices.

Action needed by WHO to respond to these requests includes:

- provision of technical guidance to facilitate the expansion of existing systems to monitor and report adverse drug reactions to herbal medicines or the establishment of comprehensive national drug safety monitoring systems that

incorporate the safety monitoring of herbal medicines, where these do not yet exist
- support to countries in strengthening their pharmacovigilance system for herbal medicines, allowing for the involvement of health-care providers, consumers and manufacturers.

WHO has taken the lead in tackling the need for drug safety monitoring since 1970 (resolution WHA23.13 on international monitoring of adverse reactions to drugs, 1970). The WHO International Drug Monitoring Programme, together with the WHO Collaborating Centre in Sweden, the Uppsala Monitoring Centre (UMC), has instituted a coherent programme of action for pharmacovigilance, which includes the establishment of a programme for exchange of safety information, maintenance of the global WHO database of adverse drug reaction (ADR) reports (hereafter referred to as the global WHO database), and the provision of numerous guidelines on monitoring drug safety. It also seeks to bridge the gap between industry and regulatory authorities. As an immediate response to the need for pharmacovigilance for herbal medicines, WHO has increased its efforts to promote their safety monitoring within the context of the WHO International Drug Monitoring Programme.

Where there is a national drug safety monitoring system in place, there is a clear need to expand its scope to include herbal medicines. If no such system exists, there is an urgent need to establish such a system, which should include monitoring of herbal medicines. However, adding herbal medicines to a list of target substances for a national drug safety monitoring activities is not enough in itself. Because of the particular nature of the distribution and use of herbal medicines, adequate and effective monitoring demands special requirements, including:

- expanding the source of case reports, for example by:
  - involving all providers of herbal medicines, including providers of traditional medicine and complementary/alternative medicine, according to national circumstances
  - strengthening the role of providers, such as pharmacists and health-care professionals, working in the community
  - involving manufacturers of herbal medicines
  - facilitating consumer reporting
  - developing systems of information exchange involving drug information centres, poisons centres, consumer organizations and manufacturers
- establishing a system for the exchange of regulatory and quality information on herbal medicines among national pharmacovigilance centres and national drug regulatory authorities
- strengthening capacity to carry out monitoring of herbal medicines at national pharmacovigilance centres by:
  - training staff in relevant technical areas
  - ensuring access to facilities for analysing products suspected of causing adverse reactions
  - providing access to reliable information
- developing a standard classification and/or coding system for herbal medicines, with standardized terms and definitions
- strengthening communication and awareness at all levels (global, regional, national, local and community) and among key players (international bodies,

regulatory authorities, national pharmacovigilance centres, health-care providers and consumers).

In response to these needs, WHO has developed these guidelines. It also plans to organize a series of training workshops to strengthen national capacity in safety monitoring of herbal medicines within pharmacovigilance systems in Member States.

1.3 Objectives

The objectives of these guidelines are to:

- support Member States, in the context of the WHO International Drug Monitoring Programme, to strengthen national pharmacovigilance capacity in order to carry out effective safety monitoring of herbal medicines
- provide technical guidance on the principles of good pharmacovigilance and the inclusion of herbal medicines in existing national drug safety monitoring systems; and where these systems are not in place, to facilitate the establishment of an inclusive national drug safety monitoring system
- provide standard definitions of terms relating to pharmacovigilance, and safety monitoring of herbal medicines
- promote and strengthen internationally coordinated information exchange on pharmacovigilance, and safety monitoring of herbal medicines among Member States
- promote the safe and proper use of herbal medicines.

The regulation of herbal medicines and their place in national health-care systems differs from country to country, and these guidelines will therefore need to be adapted to meet the needs of the local situation.

1.4 Glossary

The terms used in Part I of these guidelines are defined below.

Terms relating to herbal medicines
These terms and their definitions have been selected and adapted from other WHO documents and guidelines that are widely used by the WHO Member States, such as the *General guidelines for methodologies on research and evaluation of traditional medicine (4)*. These definitions may differ from those included in national regulations, and are therefore, for reference only.

Herbal medicines include *herbs, herbal materials, herbal preparations* and *finished herbal products*. In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials).

Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stir-baking with honey, alcoholic beverages or other materials.

Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.

Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term "mixture herbal product" can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal.

Traditional use of herbal medicines refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities.

Therapeutic activity refers to the successful prevention, diagnosis and treatment of physical and mental illnesses. Treatment includes beneficial alteration or regulation of the physical and mental status of the body and development of a sense of general well-being as well as improvement of symptoms.

Active ingredients refer to ingredients of herbal medicines with therapeutic activity. Where the active ingredients have been identified, the preparation of the finished herbal product should be standardized to ensure that it always contains a defined amount of the active ingredients, providing adequate analytical methods are available. In cases where it is not possible to identify the active ingredients, the whole herbal medicine may be considered as one active ingredient.

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. The terms "complementary medicine", "alternative medicine" and "non-conventional medicine" are used interchangeably with "traditional medicine" in some countries.

Terms relating to safety monitoring of medicinal products

The terms and definitions below have been adopted by the national pharmacovigilance centres participating in the WHO International Drug

Monitoring Programme. Different medical paradigms may view clinical events differently in their relationship to herbal medicines, whether they are expected therapeutic outcomes or adverse reactions.

Side effect. Any unintended effect of a pharmaceutical product occurring at doses normally used in humans that is related to the pharmacological properties of the drug.

Adverse event/experience. Any untoward medical occurrence that may present during treatment with a pharmaceutical product but that does not necessarily have a causal relationship with this treatment.

Serious adverse event. Any untoward medical occurrence that, at any dose:
- ♦ results in death
- ♦ requires inpatient hospitalization or prolongation of existing hospitalization
- ♦ results in persistent or significant disability/incapacity
- ♦ is life-threatening.

Adverse reaction. A response to a drug that is noxious and unintended, and that occurs at doses normally used in humans for the prophylaxis, diagnosis or therapy of disease, or for the modification of physiological function.

Unexpected adverse reaction. An adverse reaction, the nature or severity of which is not consistent with domestic labelling or market authorization, or expected from the characteristics of the drug.

Signal. Reported information on a possible causal relationship between an adverse event and a drug, the relationship being unknown or incompletely documented previously. Usually, more than a single report is required to generate a signal, depending upon the seriousness of the event and the quality of the information.

Spontaneous reporting. A system whereby case reports of adverse drug events are voluntarily submitted by health professionals and pharmaceutical manufacturers to the national regulatory authority.

Pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems (see also section 2).

National pharmacovigilance centre. A single, governmentally recognized centre (or part of an integrated system) within a country with the clinical and scientific expertise to collect, collate, analyse and give advice on all information related to drug safety.

2. Pharmacovigilance and the WHO International Drug Monitoring Programme

2.1 What is pharmacovigilance?

The WHO publication entitled *The importance of pharmacovigilance: safety monitoring of medicinal products (5)*, describes pharmacovigilance as follows.

> Pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects of drugs or any other possible drug-related problems.
> Recently, its concerns have been widened to include:
> - herbals
> - traditional and complementary medicines
> - blood products
> - biologicals
> - medical devices
> - vaccines.
>
> Many other issues are also of relevance to the science:
> - substandard medicines
> - medication errors
> - lack of efficacy reports
> - use of medicines for indications that are not approved and for which there is inadequate scientific basis
> - case reports of acute and chronic poisoning
> - assessment of drug-related mortality
> - abuse and misuse of medicines
> - adverse interactions of medicines with chemicals, other medicines and food.
>
> The specific aims of pharmacovigilance are to:
> - improve patient care and safety in relation to the use of medicines and all medical and paramedical interventions
> - improve public health and safety in relation to the use of medicines
> - contribute to the assessment of benefit, harm, effectiveness and risk of medicines, encouraging their safe, rational and more effective (including cost-effective) use
> - promote understanding, education and clinical training in pharmacovigilance and its effective communication to the public.

These aims of pharmacovigilance can be achieved *(1)* by:

- early detection of hitherto unknown adverse reactions and interactions
- detection of increases in frequency of (known) adverse reactions

- identification of risk factors and possible mechanisms underlying adverse reactions
- estimation of the quantitative aspects of benefit/risk, and analysis and dissemination of the information needed to improve the prescription, dispensing, provision and regulation of medicines.

The ultimate goal of pharmacovigilance is the safe and proper use of effective medicines of all types.

2.2 How does pharmacovigilance operate?

It must be emphasized that there is no difference in principle between the safety monitoring of herbal medicines and that of other medicines.

The WHO International Drug Monitoring Programme

Under the WHO International Drug Monitoring Programme, national pharmacovigilance centres designated by the competent health authorities are responsible for the collection, processing and evaluation of case reports of suspected adverse reactions supplied by health-care professionals (mainly spontaneous reporting by physicians of reactions associated with the use of prescribed medicines). The Programme is described in two publications: *Safety monitoring of medicinal products: guidelines for setting up and running a pharmacovigilance centre (1)*, chapters 7 and 8; and *The importance of pharmacovigilance: safety monitoring of medicinal products (5)*, especially chapters 3 and 4.

The Programme currently comprises a network of more than 70 national pharmacovigilance centres that operate independently, but whose functions are coordinated and facilitated by WHO and UMC. UMC manages the global WHO database to which all case reports received by the national pharmacovigilance centres are sent. UMC uses the global WHO database to identify/detect signals of new adverse reactions from the cumulative data and to communicate risk assessments back to the national pharmacovigilance centres and to others concerned with drug safety.

The core functions in this collaborative international programme can be summarized as follows.

Functions of national pharmacovigilance centres

- Continuous collection of reports of suspected adverse reactions for medicines on the market
- Assessment of case reports in respect of:
  - quality of documentation
  - causality assessment
  - coding to international standards using the appropriate medicine classification (the anatomical-therapeutic-chemical (ATC) classification), adverse reaction classification (WHO Adverse Reaction Terminology (WHO-ART)) and the Medical Dictionary for Drug Regulatory Activities (MedDRA)
  - clinical relevance
  - quality control, in particular identification of duplicate reports

- Transmission in suitable format of the assessed reports to UMC
- Generation of hypotheses or the identification of signals. These activities may be strengthened by a search of the global WHO database (managed by UMC) for similar reports
- Communication of relevant safety information to the national and regional regulatory authorities, health professionals, pharmaceutical companies and other players as appropriate
- Further investigation of signals, risk factors or pharmacological mechanisms
- Receipt and communication as appropriate of safety information resulting from analyses by UMC and from regulatory agencies, case reports and the literature
- Provision of feedback to reporters
- Timely advice to health-care professionals and consumers on drug safety issues
- Education and training
- Information sharing at regional and global levels.

Functions of UMC

- Receipt and storage of reports from national pharmacovigilance centres
- Provision of facilities to enable national pharmacovigilance centres to search the global WHO database
- Generation of signals from the global WHO database
- Communication of signal analyses to national pharmacovigilance centres and clinical review of the analyses by experts
- Provision of technical assistance to national pharmacovigilance centres
- Facilitation of communication between countries
- Maintenance and development of WHO-ART and the use of MedDRA within the WHO International Drug Monitoring Programme
- Training of national pharmacovigilance centre personnel
- Standardization of procedures relating to pharmacovigilance activities
- Publication of relevant documents
- Provision of data as appropriate to other parties.

Among the many players that need to be involved in pharmacovigilance systems at different levels are: qualified health professionals such as providers of medicines (physicians, dentists, pharmacists) and nurses, researchers and academics, media writers, the pharmaceutical industry, national and regional drug regulatory authorities, patients/consumers, lawyers, poisons centres, drug information centres, and international and regional health organizations *(6)*.
In collaboration with UMC, national pharmacovigilance centres have already achieved a great deal in the following areas *(5)*:

- collecting and analysing case reports of adverse drug reactions
- distinguishing signals from "background noise"
- making regulatory decisions based on strengthened signals
- alerting prescribers, manufacturers and the public to new risks of adverse reactions.

The framework of values and practice for the collection, analysis and subsequent communication of drug safety issues is provided by the Erice Declaration (Annex 2). Issues of privacy and confidentiality in relation to personal health data are covered in Annex 3.

3. Challenges in monitoring the safety of herbal medicines

3.1 Regulation, quality assurance and control

Regulation

National regulation and registration of herbal medicines vary from country to country. Where herbal medicines are regulated, they may be categorized as either prescription or non-prescription medicines. Herbal products may also be categorized other than as medicines. Moreover, the regulatory status of a particular herbal product may differ in different countries. The national regulatory framework usually also includes involved qualified providers and distributors of respective substances. Regulatory status consequently determines the access to or distribution route of these products.

If trade in a particular herbal product is made between countries where different regulatory status is given, reclassification of the regulatory status in the importing country depends not on the nature or characteristics (medical or therapeutic value) of the product itself, but on the regulatory framework of the importing country. Further, herbal products categorized other than as medicines and foods are becoming increasingly popular, and there is potential for adverse reactions due to lack of regulation, weaker quality control systems and loose distribution channels (including mail order and Internet sales).

National regulatory information on herbal medicines is not fully shared among national regulatory authorities, and is often not shared between national regulatory authorities and national safety monitoring/pharmacovigilance centres.

Almost all new medicines are introduced to the market as prescription medicines, and a significant volume of post-marketing safety data from spontaneous reporting will have been realized over time. At some stage, some of these medicines will subsequently be reclassified as non-prescription medicines and will become major sources of self-medication. However, in many countries, a significant proportion of herbal products enters directly into the non-prescription medicines category rather than by reclassification from the prescription medicines category.

Quality assurance and control

Quality assurance and control measures, such as national quality specification and standards for herbal materials, good manufacturing practices (GMP) for herbal medicines, labelling, and licensing schemes for manufacturing, imports and marketing, should be in place in every country where herbal medicines are regulated. These measures are vital for ensuring the safety and efficacy of herbal

medicines. Weak regulation and quality control may result in a high incidence of adverse reactions attributable to poor quality of herbal medicines, in particular resulting from adulteration with undeclared potent substances and/or contamination with potentially hazardous substances and residues.

Requirements and methods for quality control of finished herbal products, particularly for mixture herbal products, are far more complex than for other pharmaceuticals. The quality of such products is influenced by the quality of the raw material used. Good agricultural and good collection practices (GACP) for medicinal plants, including plant selection and cultivation, are therefore important measures *(7)*.

National/regional pharmacopoeias
National and regional pharmacopoeias define quality specifications and standards for herbal materials and some herbal preparations, such as essential oils and powdered herbal materials. Use and inclusion of herbal materials in such pharmacopoeias are based on local availability of these products. Availability is dependent on the original medicinal plants, which have ecologically and environmentally specific habitats. Therefore, even if the same pharmacopoeial monograph name is given to a herbal material, its listing in one pharmacopoeia may refer to a different original medicinal plant and/or processing method from that defined in another (see also section 4.3, Reporting of suspected adverse reactions, under Recording and coding the identity of herbal medicines).

Action required
As with other medicines for human use, herbal medicines should be covered by a drug regulatory framework to ensure that they conform to required standards of safety, quality and efficacy.

3.2 Appropriate use

Providers of herbal medicines
A variety of health-care professionals serve as qualified providers of herbal medicines, according to each country's national health-care delivery system and legislative framework. In those countries where herbal medicines are classified as prescription medicines, prescribers and dispensers other than physicians, dentists and pharmacists are sometimes excluded from current reporting systems.

In many countries, prescriptions are not required to obtain herbal medicines since these are categorized as non-prescription medicines or products suitable for self-care. Providers of herbal medicines in this category are not normally physicians. They include providers of traditional and complementary or alternative medicine as well as community pharmacists and nurses.

Action required
All providers of herbal medicines should play a role in monitoring the safety of non-prescription herbal medicines. Nurses are becoming increasingly involved in this area and are making a valuable contribution to safety monitoring. For

providers of herbal medicines to be effectively involved, it is essential to create an atmosphere of trust to enable the sharing of knowledge about the use and safety of herbal medicines.

Lack of proper knowledge of herbal medicines
Providers of medicines, such as physicians, nurses and pharmacists, may have little training in and understanding of how herbal medicines affect the health of their patients, who are often also taking other medicines, prescription or non-prescription. An appropriate knowledge base is also relevant to diagnostic and treatment decision-making. Other health-care professionals who are not providers of herbal medicines are also likely to be poorly informed about these products and how they are being used. If they see patients who are taking herbal medicines, they should ask about their use. Health professionals who work in poisons centres and health information services also need to be informed about herbal medicines.

The use of medicinal plants is the most common form of traditional medication, worldwide. Herbal medicines are used within many different healing traditions with different knowledge bases and so there is still a question as to the suitability of the categories defined in section 4.2.

Traditional medicines are increasingly being used outside the confines of traditional cultures and far beyond traditional geographical areas without proper knowledge of their use and the underlying principles. They are also being used in different doses, extracted in different ways and used for non-traditional indications. The concomitant use of traditional medicines with other medicines, which is now quite frequent, is quite outside the traditional context and has become a particular safety concern.

Patient/consumer attitudes to herbal medicines
As mentioned in section 1, there is often a misconception that "natural" means "safe" and many consumers believe that remedies of natural origin carry no risk. Patients who use herbal medicines and other medicines together, as is often the case, will often not mention the use of herbal medicines to their physician. Likewise, patients commonly fail to mention the use of other medicines to their providers of herbal medicines.

Health-care professionals and providers of herbal medicines should ask patients directly, respectfully and persistently what other medicines they are taking, including prescription medicines, herbal medicines and other health products for self-care.

Action required
The education of health-care professionals, providers of herbal medicines and patients/consumers is vital for the prevention of potentially serious risks from misuse of herbal medicines.

4. Safety monitoring of herbal medicines

4.1 Sources of reports

The Council for International Organizations of Medical Sciences (CIOMS) Working Group V has recommended that, as a general guiding principle, emphasis should be placed on the quality of a report and not on its source. Thus, the value of a report lies not in who made it, but in the care and thoroughness with which it is prepared, documented, received, recorded, followed-up, clarified and analysed (8). However, the source of a report can be an important factor in evaluating the report as it may affect the quality and value of the information. The nature, degree and even feasibility of any follow-up will also be highly dependent on the source.

The most common sources of information on adverse events and reactions to medicines are clinical trials and spontaneous reports (voluntary, unsolicited communications on marketed medicinal products). The latter ordinarily far exceed the former in numbers and type, especially serious reports, over the lifetime of a product. In some countries, adverse reaction reporting by physicians is mandatory; such reports are regarded as spontaneous.

In many countries, providers of herbal medicines other than physicians, dentists, pharmacists and nurses are excluded from reporting systems. If adequate coverage of herbal medicines is to be achieved, national reporting schemes should be developed to include all providers of herbal medicines (both prescribers and dispensers), and providers of traditional, complementary and alternative medicine, according to national circumstances.

Reports from health-care professionals

Internationally, adverse drug reaction reporting systems in the post-marketing safety surveillance setting depend primarily on voluntary reporting by health-care professionals, preferably those directly associated with the care of the patient/consumer (i.e. the patient's primary health-care provider or specialist). This is appropriate, since the understanding of adverse drug reactions depends on medical knowledge and such professionals should be aware of the patient's medical history and attuned to the subtleties of clinical differential diagnosis.

A substantial proportion of herbal medicines are non-prescription medicines, and many come directly into this category without prior post-marketing safety monitoring as prescription medicines. It is therefore most important to take measures to strengthen pharmacovigilance activity in the non-prescription medicines setting. Community pharmacists and nurses can play a particularly useful role in monitoring the safety of non-prescription medicines, although many such products are sold outside pharmacies.

Reports from consumers
The involvement of consumers in the use of herbal medicines and herbal products in health care, and their concern regarding possible adverse effects should be valued positively. Consumer reports on adverse reactions should be accepted as a serious source of information, which can contribute to the identification of signals for unknown effects of herbal medicines.

For non-prescription medicines, often taken without health professional involvement, reports received directly from consumers may provide the only source of signals. With herbal medicines in the non-prescription medicines setting, there is clearly an essential role for consumer reporting.

Consumer reporting, in one form or another, is therefore an essential development if adequate information on risk is to be obtained. However, only a few national regulatory authorities currently explicitly require collection of direct reports from consumers. The CIOMS Working Group proposes several policy approaches and practices aimed at ensuring that consumer reports are treated with appropriate respect and that there is a rational approach for handling them (Annex 4).

Manufacturers[1]
Manufacturers of herbal medicines could be a source of information on adverse events associated with their products. Some countries include reporting of adverse events by manufacturers as part of their regulatory framework.

Consumers may report directly to companies or their representatives. However, there are reasons other than concern about an adverse effect that might prompt a consumer to contact a company. These include legal concerns and, most frequently, requests for further information about the product. Another source of consumer reports derives from a variety of industry programmes in which adverse reaction information may be solicited; such cases are not regarded as spontaneous reports.

Reports from other sources
Problems associated with herbal medicines may be reported as toxicity to the following.

- *National poisons centres*. Where resources are very limited in the national situation and where no pharmacovigilance centre has been established, a poisons centre could play a core role in pharmacovigilance for and safety monitoring of herbal medicines.
- *Drug information centres* may also be a first point of contact and may provide a wealth of clinical information. National pharmacovigilance centres should have a good level of communication with such centres.
- *Consumer organizations* receive complaints about any type of product in the marketplace and may obtain relevant information about herbal medicines
- *Clinical trials and studies* can also be a source of information (see section 4.2).

[1] For the purpose of these guidelines, the term "manufacturer" refers to the producer, importer, distributor or marketer of a finished herbal product and, where applicable, to the holder of the marketing authorization or registration for that product in the country in question.

4.2 Herbal products targeted for safety monitoring

In order to obtain comprehensive coverage, it is useful to think of herbal products in the following categories:

- according to their regulatory status
  - herbal medicines in the prescription medicines category
  - herbal medicines in the non-prescription medicines category
  - other herbal products intended for use in health care
- according to their registration/marketing status
  - herbal medicines undergoing the new drug development process: in clinical trials prior to national drug regulatory approval
  - herbal medicines undergoing the new drug development process: under post-marketing safety surveillance
  - herbal medicines undergoing re-evaluation under the current protocol: in clinical trials
  - herbal medicines undergoing re-evaluation under the current protocol: under post-marketing safety surveillance
  - herbal medicines on the market: under post-marketing safety surveillance
  - other herbal products marketed for health care, such as dietary supplements.

Recommendations on how to record and report adverse events occurring during clinical trials should be covered by national guidelines on good clinical practice for trials on pharmaceutical products (GCP) *(9)*.

4.3 Reporting of suspected adverse reactions

Who should report and to whom?

The setting (see section 4.2) in which an adverse reaction is noted and the status of the person noting the reaction will determine the most appropriate means of reporting. Although the term "national pharmacovigilance centre" has been used in these guidelines, it is recognized that in some countries the national pharmacovigilance system consists of a network of national and regional centres. Reports should be sent to the appropriate centre in accordance with the particular national reporting scheme. The following should provide reports.

- *Health professionals who are providers of herbal medicines*, including physicians, pharmacists and nurses, should report to the national pharmacovigilance centre.
- *Patients/consumers* should normally report to their physicians or providers of herbal medicines. They may also report directly to the national pharmacovigilance centre, consumer organizations or manufacturers.
- *Manufacturers* should report directly to the national pharmacovigilance centre or national regulatory authority.

What information should be requested?

Any suspected adverse reaction associated with the use of a herbal medicines should be reported. A case report should contain information on the following elements:

- where it is permitted by the country health information privacy code, and with appropriate confidentiality, some form of identification of the patient/consumer in order to avoid duplications and facilitate follow-up
- age, sex and a brief medical history of the consumer/patient (when relevant); in some countries, ethnicity may need to be specified
- details of suspected herbal product(s) if known: species name (Latin binomial name and common vernacular name of medicinal plant) and/or brand or ingredient name(s), including the part of medicinal plant used, preparation methods; manufacturer, country of origin, batch number, expiry date and provider
- administration details: dose and quantity supplied, dosage form, route, start/stop dates
- indication or reason for use
- adverse reaction data: date of onset (or duration from first administration to onset of event), description with symptoms and signs, severity and seriousness, results of clinical investigations and tests, course and outcome, and dechallenge/rechallenge with the same product, where appropriate
- all other medicines used (including self-medication), with administration details
- risk factors, e.g. age, impaired renal function, previous exposure to the herbal medicine(s) concerned, previous allergies, drug misuse or abuse, the social use of drugs
- name and address of reporter (to be considered confidential and to be used only for data verification, completion and case follow-up).

Details of the factors to consider when developing a report are provided in Annex 5, together with an example of a reporting form.

How to report

A single reporting form covering all medicines, including herbal medicines, should be used. For health-care providers already included in a national pharmacovigilance system, a familiar form will facilitate reporting; the introduction of a second type of reporting form may cause confusion. It is desirable to use a standard printed or electronic reporting form and to ensure that forms are widely available. It should also be acceptable to receive reports by telephone, letter or e-mail. If possible, a sample of the herbal product and its packaging should be submitted with the report.

Consideration should be given to the distribution of reporting forms to those involved in the provision of herbal medicines, such as providers of traditional medicine and of complementary/alternative medicine, who may not previously have been part of the national pharmacovigilance system. It may be necessary to design a special reporting form for those not familiar with the reporting of suspect reactions to medicines. Educational materials, including a list of simple terminology that can be understood by all parties, should be developed to inform and assist those not familiar with reporting.

Recording and coding the identity of herbal medicines

Use of a standardized classification and identification for transmitting reports to UMC is desirable. Coding of adverse events/adverse reactions to herbal medicines should be compatible with that for other medicines. UMC therefore proposes the use of the WHO Drug Dictionary (WHO-DD) *(10)*, as it has been developed to store structured, classified information on the names of herbal products and their ingredients in the same way as similar information on other medicines. For the therapeutic classification of herbal products, UMC proposes the herbal anatomical-therapeutic-chemical (HATC) classification, which is structurally equivalent to the anatomical-therapeutic-chemical (ATC) classification used for chemical substances in other medicines. HATC is being implemented within the WHO-DD structure as part of the global WHO database. A combination of the use of the HATC classification and the expanded global WHO database structure can manage all levels of data input, however imprecise (Annex 6). In addition, UMC also proposes a system checklist for cross-referencing of botanical and vernacular names used as names of ingredients. UMC suggests that the WHO-DD, the HATC classification and the checklist should prove useful tools for national pharmacovigilance centres when asking questions of the reporter to increase the clarity and accuracy of reports.

Herbal medicines usually contain multiple ingredients and it is not always possible to identify them all. In such cases, the product name should be recorded and referred to UMC, which will assist with identification. If the product is not already in the global WHO database, it will be added, together with the available information. A particular herbal product may have a number of indications and therefore appear in several places in the HATC classification.

Local input by the reporter as to the precision or otherwise of the information on the product is most useful. This can be provided in free text, as a commentary on the report, or by the submission of manufacturer's information or the original packaging. A national inventory or catalogue of medicinal plants may also serve as a reference on medicinal plants and their use in the community. In many countries, however, knowledge of medicinal plants and their medicinal use has not been documented. The establishment of a national inventory or catalogue should therefore be encouraged.

If the finished herbal product concerned or its raw materials were imported from other countries, the drug regulatory authority of the exporting country may be able to provide helpful information.

The precise Latin binomial botanical name (genus, species, author; as well as name of family) of the medicinal plants concerned should be used whenever possible, together with information about the plant parts used and the extraction and preparation methods employed. This information allows accurate comparison with other reports. A common vernacular name may be used in order not to delay or cancel submission of a report. National pharmacovigilance centres should collaborate with the pharmacognosy departments of universities and with botanists, zoologists and botanical garden staff regarding taxonomic (botanical and chemical) identification and botanical and vernacular nomenclature.

Further classification systems may need to be specially developed in order to cover additional products used in traditional medicine.

Other reporting issues

Under no circumstances should information obtained during pharmacovigilance activities be divulged for commercial purposes. The identity of both the patient and the reporter should remain confidential unless their written permission to reveal this information is obtained (Annex 3).

Reporting on herbal medicines should be as accurate and complete as possible. On the other hand, that fact that information is less than optimal should not deter reporting.

4.4 Assessment of case reports

Individual case reports

Assessment of reports on adverse reactions to herbal medicines should be undertaken by national pharmacovigilance centres in the same way as for other medicines. Each data element in the report should be considered and a causality assessment made using a standard approach. The assessment is usually based on:

- the association in time between administration of the herbal product and the event
- the outcome of dechallenge and rechallenge
- known pharmacology (including current knowledge of the nature and frequency of adverse reactions)
- medical or pharmacological plausibility (the sequence of symptoms, signs and laboratory tests and also pathological findings and knowledge of mechanisms)
- likelihood of other causes or their exclusion
- testing for adulterants or contaminants that could be the source of adverse events.
- inappropriate use.

The WHO causality categories benefit from long and extensive use and have the advantage of being internationally agreed and easy to use. The causality categories are listed in Table 1 *(1)*.

It is most important to determine whether a reaction is caused by the way a herbal medicine has been used or prepared. Particular attention to these factors should be given when an adverse reaction is suspected in connection with the use of herbal medicines usually employed in a traditional medicine. Misdiagnosis and use outside an established tradition by poorly trained providers and practitioners can be unsafe and may lead to overdose and adverse reactions. A change in the procurement sources of herbal materials, misidentification of the medicinal plant(s) and/or herbal material(s) used, or a change in the mode of preparation may lead to entirely preventable and sometimes serious adverse reactions. This should be taken into account when assessing individual cases. The best evidence should be sought to determine the established standards of practices.

Table 1. Causality categories

The causality categories described by the Uppsala Monitoring Centre		
1	***Certain:***	a clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drugs (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.
2	***Probably/Likely:***	a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.
3	***Possible:***	a clinical event, including laboratory test abnormality, with a reasonable time sequence to administrations of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
4	***Unlikely:***	a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
5	***Conditional/Unclassified:***	a clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data is essential for a proper assessment, or the additional data is under examination.
6	***Unassessable/Unclassifiable:***	a report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

As a step towards harmonization in drug regulation in the countries of the European Union (EU), three causality categories were proposed by the EU pharmacovigilance working parties	
Category A:	"Reports including good reasons and sufficient documentation to assume a causal relationship, in the sense of plausible, conceivable, likely, but not necessarily highly probable".
Category B:	"Reports containing sufficient information to accept the possibility of a causal relationship, in the sense of not impossible and not unlikely, although the connection is uncertain and may be even doubtful, e.g. because of missing data, insufficient evidence or the possibility of another explanation".
Category C:	"Reports where causality is, for one or another reason, not assessable, e.g. because of missing or conflicting data".

Feedback to reporters

The receipt of each report should be acknowledged and a new reporting form supplied to the reporter. The reporter will also appreciate receiving further information about the reaction concerned, for example, on experience held at the national pharmacolovigilance centre or that may be helpful in further use of the medicines, unless the provision of such information is in conflict with regulatory policy. Such feedback will motivate the reporter to send in further reports.

Detection of signals at national level

The national pharmacovigilance centre should, at regular intervals, analyse the case reports in its database by class of organ system and in smaller groups of clinically related events. This may reveal case series of similar events that could constitute a signal and/or indicate the need for further study or regulatory action. Such signals should be communicated to UMC. Weak signals may be

strengthened by examination of reports from other countries held in the global WHO database.

Detection of signals at international level

The major aim of pharmacovigilance is the early detection of signals of previously unrecognized adverse reactions. Early signals may be strengthened by combining the experiences reported in various countries. Regional studies may be of particular value in the monitoring of herbal medicines. Data-mining techniques can be helpful in individual countries, but are most effective in the global WHO database managed by UMC.

Use of an advisory committee

Each national pharmacovigilance centre should have an advisory committee composed of experts to give advice on:

- maintaining quality standards in data collection and assessment procedures
- data interpretation
- publication of information
- follow-up action required.

The committee should be selected according to the expertise available but it should not be too large, so that it may not be possible to have all of the relevant disciplines represented. A committee might be selected from the following disciplines: general medicine, pharmacy, pharmaceutics, clinical pharmacology, clinical toxicology, pharmacogenetics, epidemiology, pharmacoepidemiology, pathology, drug regulation and quality assurance, drug information, information science, medical anthropology, communications, ethnopharmacology, pharmacognosy, phytochemistry, traditional medicine and/or complementary/alternative medicine.

Investigation and analysis of the cause of suspected adverse reactions

Some adverse reactions, particularly serious ones should be further investigated scientifically. The investigations may include the following:

- medical investigation of the adverse reactions: pathology, clinical pharmacology, clinical toxicology, pharmacogenetic studies
- pharmaceutical investigation of the adverse reactions: pharmacokinetics, pharmaco-dynamics and pharmaceutical, pharmacological and toxicological analysis
- pharmacognosical/phytochemical investigation (including authentification) of the herbal medicines
- physicochemical analysis to identify the constituents of the herbal medicines
- pharmacoepidemiology.

Technical expertise and basic equipment

Where possible, national pharmacovigilance centres should have the necessary technical expertise to handle herbal medicines. This might include:

- access to reliable information support on herbal medicines
- trained personnel in relevant technical areas (e.g. pharmacognosy, phytochemistry, ethnobotany, ethnopharmacology) and in the use and provision of herbal medicines

- access to facilities for analysis of potentially causative products about which there is often insufficient information.

Not all countries have access to suitable analytical laboratories. The establishment of regional laboratories specializing in the analysis of herbal products should be considered.

4.5 Data management

- *Data quality*. Strenuous efforts should be made to ensure that there are quality controls on data processing and that the data elements of reports are as complete and accurate as possible. Mechanisms to check for duplications should be instituted.
- *Data storage*. Computer databases should be managed to as high a standard as possible to facilitate access to and use of the data. Software should be selected with expert advice so that analytical needs can be met.
- *Data analysis*. Programmes should be developed to provide for regular analyses and data output appropriate for local needs.
- *Analysis of the global WHO database*. The global WHO database managed by UMC is being improved on the basis of the proposed "Database management and classification for coding of herbal medicines", of which the previously mentioned HATC is one part (Annex 6). Data-mining techniques that have proved effective on the very large numbers of reports for other medicines will be used for signal detection on reports for herbal medicines. The success of these techniques depends on the volume and quality of data submitted by national pharmacovigilance centres.
- *Support on technical and data management* is available from the WHO Collaborating Centre for International Drug Monitoring, UMC (http://www.who-umc.org/).

5. Communication

5.1 General

The successful safety monitoring of herbal medicines depends on good communication (Annex 2). There are many barriers to be broken down if all the players in this field are to be involved. There is distrust between some and ignorance of the work and function of different groups. Transparent communication is essential to overcome these problems and ensure that all players collaborate to meet the goal of the safe and effective use of herbal medicines.

National pharmacovigilance centres should ensure that manufacturers receive timely information so that they can take appropriate action regarding their products. Effective communication of the results of monitoring is also essential so that pharmacovigilance activities can have a positive impact on the health of the people.

If there is no national pharmacovigilance centre, consideration should be given to designating other relevant organizations, such as the national regulatory authority, poisons centres, drug information centres and consumer complaints authorities as the focal point.

Communication should be established at many different levels, for example, between:

- the national pharmacovigilance centre and health professionals
- the national pharmacovigilance centre and providers of herbal medicines
- health professionals and providers of herbal medicines, and consumers and patients
- providers of herbal medicines and those for other medicines
- the national pharmacovigilance centre and consumers
- the national pharmacovigilance centre and the regulatory authority
- the national pharmacovigilance centre and such centres in other countries, within the region or in other regions
- the national pharmacovigilance centre and UMC
- the national pharmacovigilance centre and the mass media.

The development of effective communication needs to be adequately resourced. It is likely that this most important part of the safety monitoring programme for herbal medicines will require proportionately greater resources than is the case for other medicines.

5.2 Risk communication

Communication strategies should be established to effectively reach all relevant target audiences, such as providers of herbal medicines, other health professionals, manufacturers and patients/consumers.

Communication of safety information is a shared responsibility between national pharmacovigilance centres, national regulatory agencies, manufacturers and health professionals. Different risk communication vehicles can be considered, including:

- adverse reaction bulletins or articles distributed in reputable journals
- public advisories or warnings
- “Dear Health Professional” letters.

Various methods of information dissemination can be considered, such as:

- Internet posting
- direct mass mailing to providers of herbal medicines and health professionals
- briefings to the mass media
- briefings to patient/consumer associations
- education sessions at health professional society meetings.

In order to reach consumers and the wide range of providers of herbal medicines successfully, messages should be tailored to suit the recipients, including translation into local languages where appropriate.

6. References

1. *Safety monitoring of medicinal products: guidelines for setting up and running a pharmacovigilance centre*. Uppsala, Uppsala Monitoring Centre, 2000 (reproduced in Part II of this publication).
2. US report calls for tighter controls on complementary medicine. *British Medical Journal*, 2002, 324:870.
3. Three out of four Germans have used complementary or natural remedies. *British Medical Journal*, 2002, 325:990.
4. *General guidelines for methodologies on research and evaluation of traditional medicine*. Geneva, World Health Organization, 2000 (WHO/EDM/TRM/2000.1).
5. *The importance of pharmacovigilance: safety monitoring of medicinal products*. Geneva, World Health Organization, 2002.
6. Bowdler J. *Effective communications in pharmacovigilance: the Erice report*. Birmingham, W Lake, 1997.
7. *WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants*. Geneva, World Health Organization, 2003.
8. *Current challenges in pharmacovigilance: pragmatic approaches*. Report of CIOMS Working Group V. Geneva, The Council for International Organizations of Medical Sciences, 2001.
9. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: *The use of essential drugs. Sixth report of the WHO Expert Committee*. Geneva, World Health Organization, 1995, Annex 3 (WHO Technical Report Series, No. 850).
10. *WHO Drug Dictionary*. Uppsala, Uppsala Monitoring Centre (electronic database, updated quarterly; information available at http//www.umc-products.com/).

7. Selected bibliography

Safety monitoring and pharmacovigilance

- Bowdler J. *Effective communications in pharmacovigilance: the Erice report*. Birmingham, W Lake, 1997.
- *Current challenges in pharmacovigilance: pragmatic approaches. Report of CIOMS Working Group V*. Geneva, The Council for International Organizations of Medical Sciences, 2001.
- Edwards IR, Hugman B. The challenge of effectively communicating risk-benefit information. *Drug Safety*, 1999, 17:216–227.
- *Effective communications in pharmacovigilance*. Uppsala, Uppsala Monitoring Centre, 1998. (Report of the International Conference on Developing Effective Communications in Pharmacovigilance, Erice, Sicily, 24–27 September 1997).
- Elvin-Lewis M. Should we be concerned about herbal remedies? *Journal of Ethnopharmacology*, 2001, 75:141–164.
- *The importance of pharmacovigilance: safety monitoring of medicinal products*. Geneva, World Health Organization, 2002.
- *Viewpoint, watching for safer medicines, Part 1: issues, controversies and science in the search for safer and more rational use of medicines*. Uppsala, The Uppsala Monitoring Centre, 2002.

Regulation of herbal medicines/traditional medicines

Global review of regulation of herbal medicines

- *Legal status of traditional medicines and complementary/alternative medicines: a worldwide review*. Geneva, World Health Organization, 2001 (WHO/EDM/TRM/2001.2).
- *Regulatory situation of herbal medicines: a worldwide review*. Geneva, World Health Organization, 1998 (WHO/TRM/98.1).
- *WHO global survey on national policy on traditional medicine and complementary/alternative medicine and regulation of herbal medicines*. Geneva, World Health Organization (in preparation).

Assessment of herbal medicines

- *General guidelines for methodologies on research and evaluation of traditional medicines*. Geneva, World Health Organization, 2000 (WHO/EDM/TRM/2000.1).
- Guidelines for assessment of herbal medicines. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-fourth report*. Geneva, World Health Organization, 1996, Annex 11 (WHO Technical Report Series, No. 863). (These guidelines are also included in: *Quality assurance of Pharmaceuticals: a compendium of guidelines and related materials, Vol. 1*. Geneva, World Health Organization, 1997.)

Quality assurance/control of herbal medicines

- Good Manufacturing Practices for pharmaceutical products: main principles. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-seventh report*. Geneva, World Health Organization, 2003, Annex 4 (WHO Technical Report Series, No. 908).
- Good Manufacturing Practices: supplementary guidelines for manufacture of herbal medicinal products. In: *WHO Expert Committee on Specification for Pharmaceutical Preparations. Thirty-fourth report*. Geneva, World Health Organization, 1996, Annex 8 (WHO Technical Report Series, No. 863). (These supplementary guidelines are also included in *Quality assurance of pharmaceuticals: a compendium of guidelines and related materials, Vol. 2. Good manufacturing practices and inspection*. Geneva, World Health Organization, 1999. They are currently being updated).
- Good trade and distribution practices (GTDP) for pharmaceutical starting materials. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-seventh report*. Geneva, World Health Organization, 2003, Annex 2 (WHO Technical Report Series, No. 908).
- Guide to good storage practices for pharmaceuticals. In: *WHO Expert Committee on Specifications for Pharmaceutical Preparations. Thirty-seventh report*. Geneva, World Health Organization, 2003, Annex 9 (WHO Technical Report Series, No. 908).
- *Quality control methods for medicinal plant materials*. Geneva, World Health Organization, 1998.
- *WHO guidelines for assessing safety and quality of herbal medicines with reference to contaminants and residues*. Geneva, World Health Organization (in preparation).
- *WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants*. Geneva, World Health Organization, 2003.

Rational use of herbal medicines

- *WHO guidelines for developing consumer information on proper use of traditional medicines and complementary/alternative medicine*. Geneva, World Health Organization, 2004.
- *WHO monographs on selected medicinal plants, Vol. 1*. Geneva, World Health Organization, 1999.
- *WHO monographs on selected medicinal plants, Vol. 2*. Geneva, World Health Organization, 2002.
- *WHO monographs on selected medicinal plants, Vol. 3*. Geneva, World Health Organization (in preparation).

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Annex 2: The Erice Declaration on Communicating Drug Safety Information, 1997

Monitoring, evaluating and communicating drug safety is a public-health activity with profound implications that depend on the integrity and collective responsibility of all parties – consumers, health professionals, researchers, academia, media, pharmaceutical industry, drug regulators, governments and international organizations – working together. High scientific, ethical and professional standards and a moral code should govern this activity. The inherent uncertainty of the risks and benefits of drugs needs to be acknowledged and explained. Decisions and actions that are based on this uncertainty should be informed by scientific and clinical considerations and should take into account social realities and circumstances.

Flaws in drug safety communication at all levels of society can lead to mistrust, misinformation and misguided actions resulting in harm and the creation of a climate where drug safety data may be hidden, withheld, or ignored.

Fact should be distinguished from speculation and hypothesis, and actions taken should reflect the needs of those affected and the care they require. These actions call for systems and legislation, nationally and internationally, that ensure full and open exchange of information, and effective standards of evaluation. These standards will ensure that risks and benefits can be assessed, explained and acted upon openly and in a spirit that promotes general confidence and trust.

The following statements set forth the basic requirements for this to happen, and were agreed upon by all participants, from 30 countries at Erice:

1. Drug safety information must serve the health of the public. Such information should be ethically and effectively communicated in terms of both content and method. Facts, hypotheses and conclusions should be distinguished, uncertainty acknowledged, and information provided in ways that meet both general and individual needs.
2. Education in the appropriate use of drugs, including interpretation of safety information, is essential for the public at large, as well as for patients and health-care providers. Such education requires special commitment and resources. Drug information directed to the public in whatever form should be balanced with respect to risks and benefits.
3. All the evidence needed to assess and understand risks and benefits must be openly available. Constraints on communication parties, which hinder their ability to meet with this goal, must be recognized and overcome.
4. Every country needs a system with independent expertise to ensure that safety information on all available drugs is adequately collected, impartially evaluated, and made accessible to all. Adequate nonpartisan financing must be available to support the system. Exchange of data and evaluation among countries must be encouraged and supported.

5. A strong basis for drug safety monitoring has been laid over a long period, although sometimes in response to disasters. Innovation in this field now needs to ensure that emergent problems are promptly recognized and efficiently dealt with, and that information and solutions are effectively communicated.

These ideals are achievable and the participants at the conference commit themselves accordingly. Details of what might be done to give effect to this declaration have been considered at the conference and form the substance of the conference report.

Erice September 29, 1997

Annex 3: Privacy and the confidentiality of personal health data[2]

Legislation or rules recently enacted or in progress in the EU, US and elsewhere in many countries have introduced new data subject rights and the need for strong safeguards in the collection, processing and transfer (especially across country borders) of personally identifiable data handled via any media, electronic or physical (paper, film, etc.). It has particular relevance to health information, among the more sensitive types of data, and certainly applies to adverse events reports, which often include data that directly identify the subject and/or the reporter with name, address, national health number, or other overt identifiers. Within some legal systems, *indirect* information that might allow determination of an individual's identity must also be protected (i.e. reference to one or more factors specific to a person's physical, physiological, mental, economic, cultural or other characteristics that could facilitate determination of his/her identity).

Although current practices throughout the pharmaceutical industry and by regulatory authorities reflect a commitment to protection of personal data, new laws in many countries necessitate some changes in personal-data handling practices. Increased rights for data subjects include notification on who is processing their data, for what purpose, and with whom the data may be shared, as well as the ability to access their own data and make corrections. Under appropriate circumstances, this may require enhancement of the ordinary informed consent process for activities, such as clinical trials. The use of secondary databases, so important to pharmacoepidemiology and retrospective studies in general, may also be affected.

There is no intention to cover this complicated topic here in more detail and those working in pharmacovigilance, and clinical research generally, should familiarize themselves with applicable data protection laws and regulations. However, it is important to explain that the term "identifiability" does not have the same meaning under one of the CIOMS V topics, "Assessing Patient and Report Idntifiability", as it does within the context of data protection legal regimes. For adverse event reporting, an identifiable patient of reporter relates to the existence of a real person that can be verified/vaiadated in some way. Under data protection schemes, the term refers to an ability to associate a data set with a particular person ("trace" a person from the data available).

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[2] Reproduced, by permission, from *Current challenges in pharmacovigilance: pragmatic approach. Report of CIOMS Working Group V.* Geneva, The Council for International Organizations of Medical Sciences, 2001.

Annex 4: Spontaneous reports from persons other than health-care professionals[3]

The CIOMS Working group proposes several policy approaches and practices which aim to ensure that consumer reports are treated with appropriate respect and that there is a rational approach for handling them. In general, because the treating healthcare professionals remain vital partners in understanding and managing treatment emergent adverse events, their involvement in the confirmation process should take place whenever possible. Because much time and effort are expended on the management of consumer reports, international alignment of expectations regarding the handling of consumer-cases is also needed to assure proper focus on efforts likely to add public health value. Therefore the following principles and practices are recommended:

Definition of medical confirmation

A situation in which a healthcare professional, preferably one directly involved in the care of the patient (primary healthcare provider), confirms (i.e., agrees) that the circumstances as reported by or on behalf of the patient occurred and that the facts, as amended or updated in the confirmation process, constitute an adverse event case for which there is a suspicion by that healthcare professional of drug causality (thus, it should be considered an adverse drug reaction).

The important point in this context is to distinguish between verification of the facts by the healthcare professional (things did or did not happen as described by the patient) and the professional's confirmation that a drug-related adverse event (i.e., an adverse drug reaction) occurred.

General policy issues

- Consumers should be encouraged to report personal adverse experiences to healthcare providers, but primarily to their treating physician. Companies and regulators should convey this message through educational materials or in the course of responding to consumer inquiries or complaints. Consumer advocacy groups and disease-specific patient support groups should also be encouraged to foster this practice among their constituents.
- Neither a company nor a regulator should refer a consumer/patient to a specific healthcare professional.
- Physicians and other healthcare professionals, as part of any medical education, should be sensitized to the importance of listening to their patients for circumstances which might constitute a reportable adverse drug reaction. When reports about consumers are received from a third party who is not a healthcare professional (e.g., a relative or other patient advocate, traditional healer, lawyer), that party should be encouraged to have the patient contact his/her physician and request that the physician report the case, if appropriate, or alternatively (or in addition) to encourage the consumer to authorize the sponsor/authority to contact the doctor directly.

[3] Reproduced, by permission, from *Current challenges in pharmacovigilance: pragmatic approach. Report of CIOMS Working Group V*. Geneva, The Council for International Organizations of Medical Sciences, 2001.

Case management practices for companies and regulators:

- Regarding all reports directly from consumers or from their non-healthcare-professional representatives:

  During all contacts, attempts should be made to obtain information sufficient to ascertain the nature and seriousness of the complaint. Based upon this understanding, the strategy for documentation and follow-up will be determined (see below).

  Permission should be sought to contact the consumer's primary healthcare provider in order to obtain additional medical details when relevant; such permission should be documented. If the patient prefers to obtain and forward supporting/confirmatory medical records, attempts should still be made to obtain physician-contact permission.

  All such reports should be documented as for any other types of cases and should be taken into consideration when overall safety assessments are conducted.

  As with the handling of all other individual case reports, patient-specific information (personal data) should be treated confidentially[4]. Identification of the case should be sufficient to permit recall and cross-linkage with any subsequently obtained medical information, with all requisite steps to assure protection of patient privacy.

In addition to these general practices, some special considerations apply that depend on the perceived serious or non-serious nature of the case. The information provided in the initial consumer report will usually permit a judgement as to whether the case is "apparently" serious or on-serious; this may be the only judgement possible in the absence of subsequent medical confirmations.

- When the event is apparently non-serious and already labeled/expected:

  No additional effort (follow-up or medical confirmation) is required by the company or regulatory recipient as long as the minimum criteria for a case are satisfied[5].

- When the event is apparently serious, or is non-serious unlabeled/ unexpected:

  Special effort should be made to obtain permission to contact the consumer's physician. If the patient refuses, attempts should be made to encourage the consumer to provide relevant medical records on his/her own.

  If permission is obtained to contact the patient's physician or other healthcare professional, who in turn is unwilling to respond to company

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[4] See Annex 3, Privacy and the confidentiality of personal health data.

[5] An identifiable reporter; an identifiable patient; a reaction/event; a suspected medicinal product.

attempts at follow-up for confirmation, it is possible that regulators in some countries may be in a better position to obtain the requisite follow-up or confirmatory data.

Even in the absence of medical confirmation, any report containing suspected adverse drug reactions with possible implications for the medicine's benefit-risk relationship should be submitted to regulators on an expedited and/or periodic basis.

Although the US and Canadian regulatory authorities appear to be the only ones currently requiring submission of consumer reports, consideration should be given to submitting such important cases to regulators.

Considerations on periodic safety reporting

- To satisfy current European, Japan and other countries' requirements, medically unconfirmed consumer reports should not be routinely included in official international summary reports, such as ICH Periodic Safety Update Reports (PSURs). It should be recognized, however, that others (such as the US and Canadian regulators) may require that a listing or summary of such reports be provided as an appendix to a PSUR.
- Nevertheless, all consumer reports regarded as adverse drug reactions should be regularly scrutinized for new "signals" or to confirm or extend the safety experience derived from all other sources. A statement should be made in the PSUR that such unconfirmed reports have been reviewed and either add no important new information or, conversely, suggest new findings.
- It is possible that unconfirmed consumer reports could contribute new, important information; if so, a separate tabulation and comment within the formal PSUR should be included.

Annex 5: Model reporting form

General considerations

- The reporting form for herbal medicines should be the same as that used for other medicines.
- Countries should modify their national reporting forms to facilitate the reporting of suspect reactions to herbal medicines or interactions between herbal medicines and other medicines.
- Only basic and important information should be requested. A request for great detail will result in fewer reports.
- The form should have a simple format.
- The form should look simple; a design with plenty of "white space" is helpful.
- The form should be designed in such a way that it is self-evident how it should be filled in. Any instructions for use should be simple; detailed instructions may discourage those with little training. Simplicity will encourage the recording of the best information available in the circumstances.
- The form should include instructions on how it should be completed and where it should be sent (i.e. a return address).
- The use of reporting forms should be seen as a screening process designed to reveal evidence of problems that need further investigation. The information supplied should be perfectly adequate, in most cases, to permit adequate identification and evaluation of a problem.
- Follow-up forms should be available for use when further details are required. These should be designed for use by district investigation teams or hospitals.
- Several types of follow-up forms may be needed for the investigation of specific problems, e.g. liver toxicity, death, inefficacy.
- Accurate identification of the patient/consumer is important for follow-up purposes and to avoid duplication. Essential information includes: name (or unique health facility number), date of birth (if unknown, then approximate age) and sex.

An example of a reporting form on suspected adverse reactions to medicines, including herbal medicines and vaccines, is shown on the following page. It is designed to support Member States in establishing a national drug monitoring system for the first time or in revising their reporting form to include herbal medicines.

Example of Reporting form for suspected adverse reaction to medicines, including herbal medicines and vaccines

PLEASE NOTE: all consumer/patient and reporter information will remain confidential.

Patient/consumer identification (please complete or tick boxes below as appropriate)

Last name	**First name(s)**	**Patient/record number**
Ethnicity		
Address (place and region, or health facility may be used)		**Date of birth**
		Sex □ M □ F

List of all medicines/vaccines/herbal medicines used by the patient. Please indicate suspected medicines with an asterisk (*) (please complete boxes below)

Medicine(s)Vaccine(s) + batch no.	**Daily dose**	**Route of administration**	**Date started**	**Date stopped**	**Reason for use**

For herbal medicines please give detailed information on the product
Product name:

How was the product obtained?

List of product ingredients; attach product label if available:

Name and address of the manufacturer;

Name and address of the distributor:

Other relevant information:

Description of the suspected adverse reaction (please complete boxes below)

Date of onset of reaction (dd/mm/yy):
Description of reaction (please include results of laboratory tests if available):

Outcome of the suspected adverse reaction (please tick boxes as appropriate)

Recovered □	**Not yet recovered** □	**Unknown** □	**Fatal** □	**Date of death**
Severe? Yes □ No □		**Rechallenge?** Yes □ No □ **Result:**		
Was the patient admitted to hospital? **If yes, give name and address of hospital:**		Yes □ No □		

Other factors (please tick box or describe as appropriate)

Kidney disease □	**Liver disease** □	**Allergy** (please describe) ....
Other illnesses (please describe):		**Malnutrition** □

Reporter identification

Type (please circle): nurse/doctor/pharmacist/other health worker /manufacturer/ distributor/supplier
Name:
Address: **Telephone:**
E-mail address:

Signature of reporter: .... **Date:** ....

Please send completed form to: ....

Annex 6: Proposed database management and classification for coding herbal products (the Uppsala Monitoring Centre, Uppsala, Sweden)

Database management structure

With the aim of capturing data about adverse drug reactions (ADRs) to herbal products in the same system as ADRs to other medicines, the WHO Collaborating Centre for International Drug Monitoring in Uppsala (the Uppsala Monitoring Centre; UMC) has restructured the management of data relating to herbal products. This has mainly involved the structure of information held in the substance register of the WHO Drug Dictionary (WHO-DD). The register identifies the "preferred names" of ingredients of products mentioned on all ADR reports in the global WHO database. The logic for identifying "preferred names" for herbal substances follows, as far as possible, that for identifying preferred chemical substance names in the WHO-DD.

The validity of any scientific name (botanical names) that may be used as a "preferred name" for herbal products is problematic, since such names may be revised during taxonomic review. It is important to stress that the use of valid Latin binomial (scientific) names in the substance register of the WHO-DD is not for the purpose of providing a botanical reference work. They are the names that UMC has decided to use in order to provide unique names for herbal ingredients equivalent to international non-proprietary names (INN) for chemical ingredients in the global WHO database. The Royal Botanic Gardens, Kew, United Kingdom, has collaborated in ensuring that these names represent unique species. If there are other scientific names, they are regarded as synonyms. The scientific names comprise the Latin binomial (a genus name and a species epithet), the name of the author who described the specific species, and the publication source.

To determine which botanical names were synonyms, and to find further information on each medicinal plant, its major chemical constituents/entities and medical uses, the major reference publications that UMC considers relevant were examined. For information on ingredients of reported herbal products, a variety of sources was consulted, including the scientific literature, summaries of product characteristics (SPCs) from national pharmacovigilance centres, and direct input from national pharmacovigilance centres.

In addition to the preferred name, the list of ingredients should identify which part of the plant is used and give an indication of how the "active substances" have been extracted. This provides a more complete identification of the "active herbal ingredient". For conventional drugs, all preferred names of single ingredient medicines are either bases or salts. For all salts there must be a link to a base, e.g. omeprazole sodium is linked to omeprazole. Herbal products are treated in a similar way, in that the "mother herbs" (medicinal plants) will be the equivalent of bases and the different plant parts (herbal materials) and/or types of extract/herbal preparations are equivalent to the salts.

As shown in the herbal substance data links (Fig. 1), the herbal ingredients given as valid scientific names of medicinal plants are linked to common names of the plants and also to plant parts and the extracts or other herbal preparations used. So when retrieving information about a specified medicinal plant, starting from any scientific botanical name, vernacular, or common name, it will be easy to find all related substances (chemical

entities) including those where different parts/herbal materials and/or extracts or other herbal preparations are specified, and vice versa.

Herbal anatomical-therapeutic-chemical classification

The herbal anatomical-therapeutic-chemical classification (HATC) is a classification primarily based on those herbal products that have adverse drug reactions reported in the global WHO database and therefore appear in the WHO-DD. They are not necessarily categorized in a medicines category in any particular country. The HATC classification, unlike the regular ATC system, is based on botanical science, pharmacognosy, phytochemistry, literature search and documented traditional use rather than chemistry and evidence-based medicine. It is linked to botanical synonyms and vernacular names via the substance register of the WHO-DD, which contains all ingredients, herbal and chemical, of medicinal products mentioned on reports in the global WHO database.

In addition to the identification by preferred name and information on the plant parts used and methods of preparation, the HATC classification as used in the global WHO database, indicates:

- the suggested anatomical site of pharmaceutical action
- the range of intended medical uses including traditional therapeutic uses.

The HATC classification is mainly used as an administrative tool for placing groups of herbal products in the coding systems, and to group-related products in signal work and other congregated searches

The storage and management of safety monitoring information on herbal medicines

Principles of the system

It is often impossible to obtain information on both the traditional product composition and the use of such products by patients. No data management system can capture more than is known, but the combination of the HATC classification and the current global WHO database structure allows any and all information to be entered.

The basic philosophy behind the data management of herbal products and traditional medicines is to achieve a system that is capable of handling all levels of information, at the same time being utterly transparent to users over any imprecision, missing data and the links that are built into the hierarchies in the system. Precision versus uncertainty, for example, can be considered as occurring along several axes:

- identification of the medicinal plant
- itemization of the medicinal plant part (herbal material)
- stipulation of preparation methods (processing including extraction procedures)
- definition of the major chemical constituents extracted
- definition of the major ingredients/herbal materials (name and proportion) in mixture herbal products (complex preparations and products)
- note of any variations in product composition (and dosage form)
- intended medical use, indicating diseases or symptoms that can be treated.

Detailed descriptions of the HATC classification and the global WHO database system are available from UMC.

Adverse drug reaction terms, and indications, used in ADR reports on herbal products and traditional medicines will often be those recommended for other medicines. However, additions to WHO-ART and MedDRA may be needed to capture differences in expressing ADRs caused by the use of herbal products, especially in the case of traditional medicines, in accordance with particular treatment concepts and/or principles. For example, "increased/decreased Yin" are possible states of diseases within the Chinese medicine system, a concept unique to this particular type of medicine. Such details will be added to WHO-ART, as required.

It is clear that other systems can be devised to accomplish the same ends. The purpose of the data management system described above is specifically for pharmacovigilance, and is specially designed to allow its use alongside pharmacovigilance activities for other medicines. This is an important consideration in view of the increasing likelihood that patients/consumers may use both forms of treatment concurrently. Other systems developed and used for pharmacovigilance should function in a similar way and it should be possible to link them with the global WHO database, to ensure that all international data are pooled for global benefit.

Data analysis

The new structure and classification of herbal substances (entities) within the global WHO database will facilitate finding information about finished herbal products containing a specified medicinal plant or just a specific part, herbal materials, or extract or other herbal preparation of the specified medicinal plant. This is crucial in finding and evaluating signals concerning herbal medicines and traditional medicines (more complex than for other medicines), as the following example shows.

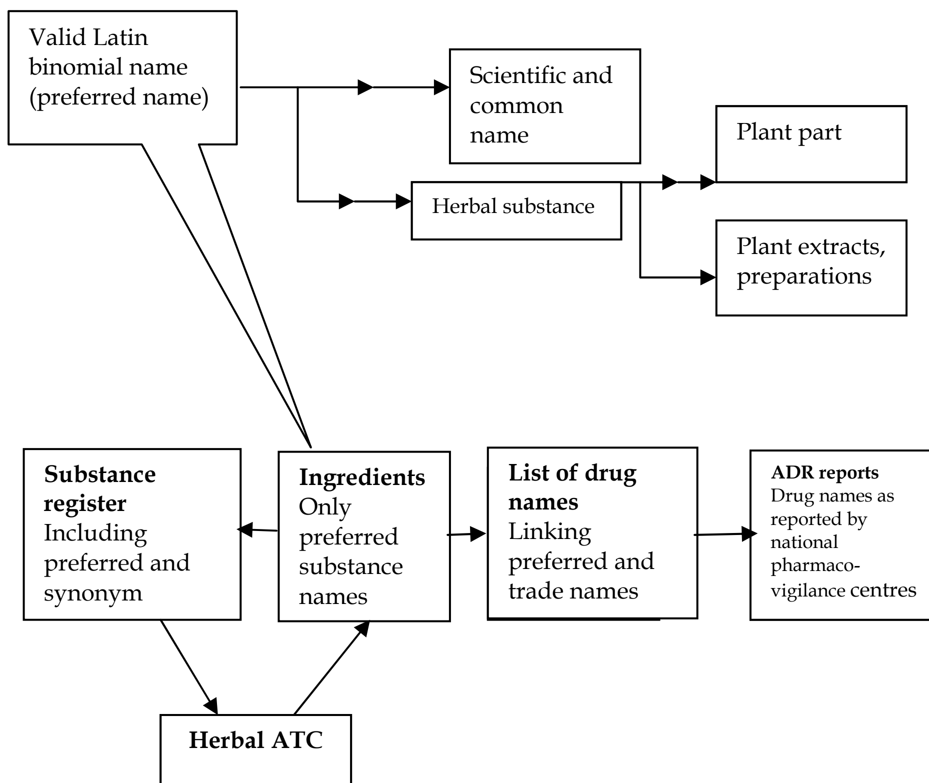
> A company has for years produced a product containing Senna alexandrina Miller, which in the labelling is called "Cassia". Another company markets a product that also lists "Cassia"' as active ingredient but the product is derived from Senna armata Wats, a different botanical species. Then reports of serious ADRs associated with "Cassia" appear and they are so serious that a withdrawal from the market is considered. It may be that only Senna armata Wats is causing these problems. In this case the other species, Senna alexandrina Miller, risks being wrongly accused because there is no distinction between the labelled names: "Cassia" is the suspected cause.

While the use of the global WHO database and, in particular, the proposed HATC classification does not solve the problem of missing or inaccurate information, it is hoped that it will facilitate proper classification of all herbal product information and, most importantly, show where there is a potential for confusion and/or error.

Another linked benefit is that it will be possible to produce a checklist of common and vernacular names covering several different languages, which will be valuable for all those seeking to identify the contents of herbal remedies used nationally. It may also prove useful at poisons centres and probably also for pharmaceutical companies in labelling their herbal products. In the end all involved with herbal products should use the valid scientific names, to avoid any confusion.

The use of data-mining tools on pooled international data will be particularly valuable in trying to find useful patterns within such a large volume of heterogeneous data. Much consideration will need to be given to the development of such tools and to the use of international expertise in the interpretation of information. As always, epidemiological studies, where they can be undertaken, will aid the quantification and validation of early signals. However, epidemiological studies on herbal products are difficult because of the problems of ascertaining precise information.

Fig. 1. Data links for herbal substances



Part II

Part II of these guidelines reproduces, in its entirety, the publication 'Safety monitoring of medicinal products: guidelines for setting up and running a pharmacovigilance centre', issued by the Uppsala Monitoring Centre, Uppsala, Sweden, in 2000, by kind permission of the Centre. WHO acknowledges the Uppsala Monitoring Centre (namely Dr David Coulter, Dr Ralph Edwards, Dr Jenny Ericsson and Dr Mohamed Farah) for their contribution in this respect.

Safety monitoring of medicinal products: guidelines for setting up and running a pharmacovigilance centre
(The Uppsala Monitoring Centre, Uppsala, Sweden, 2000)

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INTRODUCTION

This booklet aims to provide practical guidelines and information for the setting up of new Pharmacovigilance Centres.

The history of international pharmacovigilance goes back as much as thirty years, when the twentieth World Health Assembly adopted a resolution to start a project on the feasibility of an international system of monitoring adverse reactions to drugs. This resolution was the basis of WHO's Programme on International Drug Monitoring.

At this moment more than fifty countries participate in this Programme. The world of today no longer is as it was at the time the Programme was established. New developments challenge our attention, require adequate reaction, and raise new questions in adverse drug reaction monitoring.

A few examples may illustrate this: The current financial climate forces national authorities to find ways to contain the cost of pharmaceutical care. In some countries a strong tendency to self-medication can be seen, and many pharmaceuticals that used to be on prescription only are now available over the counter. The question arises: Does this have consequences for the safety of the patients?

Traditional medication is increasing in the Western world, but the use of herbal medicines risks escaping control. Nonetheless several herbal medicines are quite active, and may be associated with adverse effects. Continuing vigilance is needed.

A phenomenon that has received the attention it deserves, in only the last few years, is the prevalence of counterfeit drugs on the market. Instances of calamities, claiming the lives of numerous children due to the use of a toxic solvent have been documented. Drug monitoring programmes may well be instrumental in detecting such products.

The way drugs are being monitored has changed, both internationally as well as on the national level. The WHO Programme was established with ten countries, all of them highly developed. Gradually more countries showed interest and eventually joined the Programme, once they felt that their national systems were sufficiently developed.

Criteria for this development are not only the functioning of the centre in question itself, but also the presence of an effective drug regulatory body in the country that has the will and the potential to react to signals emanating from the centre and to take proper regulatory measures. WHO considers this point as vital: ***a pharmacovigilance system must be backed up by the regulatory body.***

In particular the last five years have seen an increasing number of countries expressing the wish to participate in the Programme, and several countries are in contact with WHO and the WHO Collaborating Centre, the Uppsala Monitoring Centre in Sweden, to receive support with the development of their national programmes. Practically all industrialised countries already participate; new countries now are all coming from the developing world. In several cases new countries have requested WHO's collaboration and assistance in setting up a monitoring system.

At national level also many changes have been taking place. In the original model a pharmacovigilance system is strongly centralised, and consists of one national centre collecting reports from health professionals in the country. Many countries, however, now prefer a more

decentralised system, with a national centre functioning as a focal point for some regional or local centres. Several countries are in the process of starting their systems (conforming to this model), and countries with a long-standing experience in drug monitoring are changing their programmes into a decentralised organisation. Both situations are similar in many aspects.

Monitoring Centres always start on a very small scale, often with only one enthusiastic (part-time) professional. These pioneers in their field need help and guidance. There is a need to provide such emerging centres with some information:

- the material and resources required,
- how to operate
- what kind of support is needed
- where to find adequate literature sources
- what kind of assistance can be expected
- what is the relationship to be sought with drug information centres and poison information systems, and so on.

WHO has reacted to this perceived need by holding a consultative meeting that was asked to share experience and competence through discussion of a draft guideline, prepared by Dr Ronald Meyboom. On the basis of this discussion this document has been produced, that is intended to be used by new monitoring centres, in order to prevent them from losing time and money as a consequence of the lack of experience. It discusses practical aspects of how to run a pharmacovigilance centre at the technical level, with down-to-earth recommendations. We hope that this guideline booklet helps people on the way to a well-organised and well-run pharmacovigilance centre.

This Guideline booklet is based on the proceedings of a Consultation on Setting up and Running of a Pharmacovigilance Centre, World Health Organization, Geneva, 26-27 June 1996.

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1. WHY PHARMACOVIGILANCE?

The information collected during the pre-marketing phase of a medical drug is inevitably incomplete with regard to possible adverse reactions (for definition see Glossary):

- tests in animals are insufficiently predictive of human safety
- in clinical trials patients are selected and limited in number, the conditions of use differ from those in clinical practice and the duration of trials is limited
- information about rare but serious adverse reactions, chronic toxicity, use in special groups (such as children, the elderly or pregnant women) or drug interactions is often incomplete or not available.

Pharmacovigilance is needed in every country, because there are differences between countries (and even regions within countries) in the occurrence of adverse drug reactions and other drug-related problems. This may be because of differences in:

- drug production
- distribution and use (e.g. indications, dose, availability)
- genetics, diet, traditions of the people
- pharmaceutical quality and composition (excipients) of locally produced pharmaceutical products
- the use of non-orthodox drugs (e.g. herbal remedies) which may pose special toxicological problems, when used alone or in combination with other drugs.

Data derived from within the country or region may have greater relevance and educational value and may encourage national regulatory decision-making. Information obtained in a certain country (e.g. the country of origin of the drug) may not be relevant to other parts of the world, where circumstances may be different. When information from a region itself is not available, it may take longer before a problem becomes known to drug regulatory authorities, physicians, pharmacists, patients and pharmaceutical companies.

On the other hand, international monitoring such as the WHO International Drug Monitoring Programme may provide information on possible safety issues which may not yet have emerged within the country's data. Pharmacovigilance is needed for the prevention of drug-induced human suffering and to avoid financial risks associated with unexpected adverse effects. In conclusion, medicines on the market need continuous monitoring in every country.

2. DEFINITION AND AIMS

Pharmacovigilance is concerned with the detection, assessment and prevention of adverse reactions to drugs. Major aims of pharmacovigilance are:

1. Early detection of hitherto unknown adverse reactions and interactions
2. Detection of increases in frequency of (known) adverse reactions
3. Identification of risk factors and possible mechanisms underlying adverse reactions
4. Estimation of quantitative aspects of benefit/risk analysis and dissemination of information needed to improve drug prescribing and regulation.

The ultimate goals of pharmacovigilance are:

- the rational and safe use of medical drugs
- the assessment and communication of the risks and benefits of drugs on the market
- educating and informing of patients.

Spontaneous reporting – a regional or country-wide system for the reporting of suspected adverse drug reactions – is the primary method in pharmacovigilance. In addition, other methods of data-collection exist or are under development (see § 8.5 and 10).

3. HOW TO START A PHARMACOVIGILANCE CENTRE

A new pharmacovigilance centre can start operating very quickly. The development of a pharmacovigilance system, however, from the first and uncertain stage to becoming an established and effective organisation, is a process that needs time, vision, dedication, expertise and continuity. The most promising location for a new pharmacovigilance centre may depend on the organisation and development of the healthcare system in the country and other local issues.

A governmental department (health authority, drug regulatory agency) can be a good host for a pharmacovigilance centre. However, any department in a hospital or academic environment, working in clinical pharmacology, clinical pharmacy, clinical toxicology or epidemiology, may be a suitable starting point for pharmacovigilance. The reporting of adverse drug reactions may start locally, perhaps in one hospital, then extend to other hospitals and family practices in the region, and progress step by step into a national activity. In some countries professional bodies such as the national medical association may be a good home for the centre.

When the centre is a country-wide organisation from the start, it should be remembered that much effort, especially in effective communications, will be needed before a substantial proportion of practitioners are contributing.

When a centre is part of a larger organisation (for example, a poison control unit, a clinical pharmacology department, or a hospital pharmacy) providing administrative continuity, it can get going as long as there is one professional (e.g. a physician or pharmacist) available who is primarily responsible for pharmacovigilance.

Whatever the location of the centre, pharmacovigilance is closely linked to drug regulation. Governmental support is needed for national co-ordination. Pharmacovigilance is nobody's individual privilege. Good collaboration, co-ordination, communications and public relations are needed for a coherent development and for the prevention of unnecessary competition or duplication.

3.1 Basic steps in setting up a Pharmacovigilance Centre

Prepare a plan according to the points below for the establishment of the pharmacovigilance system.

1. Make contacts with the health authorities and with local, regional or national institutions and groups, working in clinical medicine, pharmacology and toxicology, outlining the importance of the project and its purpose.
2. Design a reporting form (see § 4.1) and start collecting data by distributing it to hospital departments, family practitioners, etc.
3. Produce printed material to inform health professionals about definitions, aims and methods of the pharmacovigilance system.
4. Create the centre: staff, accommodation, phone, word processor, database management capability, bibliography, etc.
5. Take care of the education of pharmacovigilance staff with regard, for example, to:

    - data collection and verification
    - interpreting and coding of adverse reaction descriptions
    - coding of drugs
    - case causality assessment
    - signal detection
    - risk management.

6. Establish a database (administrative system for the storage and retrieval of data; see also § 7.1).
7. Organise meetings in hospitals, academia and professional associations, explaining the principles and demands of pharmacovigilance and the importance of reporting.
8. Promote the importance of reporting adverse drug reactions through medical journals, other professional publications and communications activities.
9. Maintain contacts with international institutions working in pharmacovigilance, e.g. the WHO Department of Essential Drugs and Medicines Policy (Geneva), and the Uppsala Monitoring Centre, Sweden (see page 17 for all contact details).

4. REPORTING OF ADVERSE DRUG REACTIONS

Spontaneous reporting – a regional or country-wide system for the reporting of suspected adverse drug reactions – is currently the major source of information in pharmacovigilance.

4.1 Reporting form

A case report in pharmacovigilance can be defined as: ***A notification relating to a patient with an adverse medical event (or laboratory test abnormality) suspected to be induced by a medicine.***

A case report should (as a minimum to aim at) contain information on the following elements:

1. The patient: age, sex and brief medical history (when relevant). In some countries ethnic origin may need to be specified.
2. Adverse event: description (nature, localisation, severity, characteristics), results of investigations and tests, start date, course and outcome.
3. Suspected drug(s): name (brand or ingredient name + manufacturer), dose, route, start/stop dates, indication for use (with particular drugs, e.g. vaccines, a batch number is important).
4. All other drugs used (including self-medication): names, doses, routes, start/stop dates.

5. Risk factors (e.g. impaired renal function, previous exposure to suspected drug, previous allergies, social drug use).
6. Name and address of reporter (to be considered confidential and to be used only for data verification, completion and case follow-up).

Reporting should be as easy and cheap as possible. Special free-post or business reply reporting forms, containing questions 1-6 mentioned above, can be distributed throughout the target area to healthcare professionals at regular intervals (for example, four times a year).

It may take the yearly distribution of hundreds of thousands of forms to harvest only some hundreds of case reports. It may be effective to include reply-paid reporting forms in the national formulary, drug bulletin and professional journals. Also telephone, fax and electronic mail or internet may be easy means of reporting where reliable technology is available and accessible.

4.2 Reporting by whom?

Professionals working in healthcare are the preferred source of information in pharmacovigilance, for example family practitioners, medical specialists and pharmacists. Dentists, midwives, nurses and other health workers may also administer or prescribe drugs and should report relevant experiences.

In addition pharmacists and nurses can play an important role in the stimulation of reporting and in the provision of additional information (for example, on co-medication and previous drug use).

Pharmaceutical manufacturers, being primarily responsible for the safety of their products, have to ensure that suspected adverse reactions to their products are reported to the competent authority. If adverse reactions are reported directly by patients to the national or local centre, it is useful to consider the possibility of communication with their physicians for additional information and data verification.

4.3 What to report?

In the early stages of any pharmacovigilance system, reports on all suspected adverse reactions - known or not, serious or not - are welcome and useful, because it is necessary to create a *notification culture* in which the instinctive response to any suspected adverse drug reaction is to report it. Healthcare professionals need to learn how and what to notify, and the staff of the pharmacovigilance centre need to gain experience in assessment, coding and interpretation.

In established pharmacovigilance systems it is common practice to request the reporting of all suspected reactions, including minor ones for new drugs. For established drugs the reporting of serious or unusual suspected adverse reactions is of particular importance, whereas known and minor reactions are of less interest. (See Glossary for the definition of a *serious reaction*.) If an increased frequency of a given reaction is suspected this is also a reason for reporting.

Although pharmacovigilance is primarily concerned with pharmaceutical medicines (including radiologic contrast media, vaccines and diagnostics), adverse reactions associated with drugs used in traditional medicine (e.g. herbal remedies) should also be considered. Special fields of interest are drug abuse and drug use in pregnancy (teratogenicity) and lactation.

In addition, the reporting of lack of efficacy and suspected pharmaceutical defects is recommended, especially when there is the possibility of manufacturing problems, counterfeit pharmaceuticals or of the development of resistance (e.g. antibiotics). Pharmacovigilance and

poison control are closely related activities, since the problems encountered with accidental or intentional overdose may cast doubt on the safety of a medical drug.

Also adverse reactions to cosmetics may need to be reported, especially when cosmetics contain obsolete or toxic ingredients (e.g. mercury compounds or corticoids in bleaching creams). If there is no other organisation in the country dealing with the issues, a pharmacovigilance centre may also cover problems related to medical devices and equipment, although different expertise may be needed.

The reporting of adverse events occurring during clinical trials are not covered by these guidelines. Recommendations on how to record and report such events are included in guidelines on good clinical practice for trials on pharmaceutical products (GCP).

4.4 Mandatory or voluntary reporting?

In many countries the reporting of adverse drug reactions is voluntary, but in an increasing number of countries some legal reporting obligations on healthcare professionals have been established (although a penalty is not usually associated with failure to report). Little information is available regarding the advantages and disadvantages of such obligations. In addition, in many countries it is mandatory for pharmaceutical companies to report suspected adverse drug reactions to the health authorities.

5. SPECIAL ISSUES IN REPORTING

5.1 Central or decentralised reporting ?

As a rule spontaneous monitoring aims at country-wide reporting and the use of one central pharmacovigilance database to obtain a national overview. The collection of data may nevertheless be more successful in number and quality if reporting is organised regionally, especially when countries are large or have regional cultural differences. Regional centres with short lines of communication to healthcare professionals may improve communications and feedback. When regional centres are used, good collaboration and data-exchange with the national centre needs to be ensured. Regionalisation requires more staff and facilities and can therefore be more expensive.

5.2 Stimulation of reporting

The reporting of adverse reactions needs continuous stimulation. It is important to achieve the development of a positive attitude towards pharmacovigilance among healthcare professionals so that adverse reaction reporting becomes an accepted and understood routine. In summary, the following may stimulate reporting:

- easy access to pre-paid reporting forms and other means of reporting
- acknowledging the receipt of adverse drug reaction reports by personal letter or phone call
- providing feedback to reporters in the form of articles in journals, adverse drug reaction bulletins or newsletters
- participation of the centres staff in pre- and postgraduate education and scientific meetings
- collaboration with local drug or pharmacovigilance committees
- collaboration with professional associations
- integration of pharmacovigilance in the (further) development of clinical pharmacy and clinical pharmacology in a country.

5.3 Under-reporting

Under-reporting is a common phenomenon in all countries. Correcting for under-reporting is difficult, however, because its extent is unknown and very variable. Even at established centres the reported proportion of serious reactions may not be more than 10%. Several of the countries participating for many years in the WHO Drug Monitoring Programme receive 200 or more adverse reactions per million inhabitants annually from about 10% of physicians. In many other countries, however, the reporting rates are much lower.

Under-reporting may delay signal detection and cause underestimation of the size of a problem. However, in signal detection not only the quantity but also the relevance of case reports and the quality of data are important.

There are also a number of more elusive issues which require attention. Sometimes healthcare professionals fear that the acknowledgement of adverse reactions may reflect negatively on their competence or put them at risk of litigation. Some are reluctant to report adverse reactions because of doubts regarding the causal role of the drug (although, of course, it is essential that suspected reactions are reported). Under-reporting is both a technical and a psychological issue. Clarity of criteria for reporting, simple procedures and good motivational practice are all influential in addressing the problem.

6. PRACTICALITIES IN THE ORGANISATION OF A PHARMACOVIGILANCE CENTRE

6.1 Staff

The expertise desirable in the routines of a pharmacovigilance centre includes (see also § 7):

- clinical medicine
- pharmacology
- toxicology, and
- epidemiology.

However, a new pharmacovigilance centre often starts with only a part-time expert - usually a physician or a pharmacist - and some secretarial support. It may soon become necessary to have one expert who is responsible for pharmacovigilance for most of his/her time and for secretarial assistance to be expanded (see § 6.3, Continuity). When the reporting of adverse reactions increases, staff resource requirements may be calculated by assuming that the average assessment time per case report is about one hour.

6.2 Useful equipment (includes):

- multi-connection telephone
- computer (database, see § 7.1; word processor)
- printer (computer linked)
- fax
- e-mail
- photocopier.

6.3 Continuity

Continuity in accessibility and service is a basic feature of a successful pharmacovigilance centre. The centre therefore needs a permanent secretariat, for phone calls, mail, maintenance of the database, literature documentation, co-ordination of activities, etc. Secretarial continuity may be achieved through collaboration with related departments, provided there is sufficient capacity.

6.4 Advisory Committees

A multi disciplinary advisory committee is desirable, to support the pharmacovigilance centre with regard to the quality of the procedures in:

- data collection and assessment
- the interpretation of the data
- the publication of information.

An advisory committee may represent the following disciplines:

- general medicine
- pharmaceutics
- clinical pharmacology
- toxicology
- epidemiology
- pathology
- drug regulation and quality assurance
- drug information
- phytotherapy.

In addition a network of experienced advisors in various specialisations is helpful. When the centre is located in a hospital, specialised expertise is usually within easy reach.

6.5 Information service

The provision of a high quality information service to healthcare professionals is a basic task of a pharmacovigilance centre and a major instrument in the stimulation of reporting. For this purpose and for the assessment of case reports the centre should have access to a comprehensive and up-to-date literature information database (a list of relevant literature references may be obtained from *the* Uppsala Monitoring Centre).

Location of the centre in a large hospital usually has the advantage of a library within reach. National pharmacovigilance centres can have on-line access to the database of *the* UMC and be on the mailing lists of adverse drug reaction and drug bulletins produced by the World Health Organization and many national or regional centres throughout the world (ask *the* UMC for addresses or see WHO contacts on page 17).

6.6 Communications

A bulletin or newsletter distributed to all healthcare professionals or a regular column in reputed (medical and pharmaceutical) journals are good means for the dissemination of information. Prompt data-sheet amendments are important, but data-sheets may be printed infrequently and their educational impact may not be large. In urgent cases of sufficient importance "Dear Doctor" letters may alert the profession.

6.7 Poison control and drug information centres

Poison control and drug information centres have much in common with pharmacovigilance centres, both in organisation and from a scientific point of view. If pharmacovigilance is started in a country where a poison control or drug information centre is already in place it may be efficient to develop the pharmacovigilance system in conjunction with it. Expensive facilities such as secretariat, computer resources and library services can be shared.

In any case close collaboration between these organisations is desirable.

7. ASSESSMENT OF CASE REPORTS

The assessment of adverse reaction case reports needs combined expertise in clinical medicine, pharmacology and toxicology, and epidemiology. This expertise can be developed by training the centre's staff and by the use of specialised consultants. In the assessment of case reports the following elements can be recognised:

1. ***Quality of documentation*** (e.g. completeness and integrity of data, quality of diagnosis, follow-up). The basic elements of a case report are listed in § 4.1.
2. ***Coding***. Drug names should be registered in a systematic way, for example by using the WHO Drug Dictionary (which is based on the INN nomenclature and the ATC classification). For the coding of the adverse events the WHO Adverse Reaction Terminology (WHOART), or another internationally recognised terminology (e.g. MedDRA) should be used.
3. ***Relevance*** with regard to the detection of new reactions, drug regulation, or scientific or educational value. The following questions especially may be asked:
    - ***New drug?*** Products on the market less than five years are usually considered new drugs
    - ***Unknown reaction?*** (i.e. not included in the approved Summary of Product Characteristics or 'unlabelled'). Also important is whether the reaction is described in the literature, e.g. national drug formulary, Martindale, Meyler's Side Effects of Drugs. (Ask the Uppsala Monitoring Centre for books and other information sources)
    - ***Serious reaction?*** (See Glossary).

4. ***Identification of duplicate reports***. Certain characteristics of a case (sex, age or date of birth, dates of drug exposure, etc.) may be used to identify duplicate reporting.

5. Causality assessment or imputation. With few exceptions, case reports describe suspected adverse drug reactions. Various approaches have been developed for the structured determination of the likelihood of a causal relationship between drug exposure and adverse events, for example by the WHO Drug Monitoring Programme (see Glossary), the European Commission, and the French national pharmacovigilance programme. These systems are largely based on four considerations:

- the association in time (or place) between drug administration and event pharmacology (including current knowledge of nature and frequency of adverse reactions)
- medical or pharmacological plausibility (signs and symptoms, laboratory tests, pathological findings, mechanism)
- likelihood or exclusion of other causes.

The WHO causality categories have the advantages of being internationally agreed and easy to use. Definitions for selected adverse reactions have been worked out and reached by

international agreement. For some of these reactions special causality algorithms have also been developed (Bénichou, 1994).

7.1 Data-processing

In the early stages case-reports can be managed manually. When reporting increases, a computer system enabling the processing and retrieval of cases according to suspected drugs and adverse reactions is generally advisable.

The computer system used should include a hierarchical drug file allowing drugs to be recorded according to product name, generic name and therapeutic category. Similarly a hierarchical adverse reaction terminology should be employed. Hierarchical systems for the recording of drugs and adverse reactions are necessary to allow for specific recording of detailed case information while still permitting retrieval of information at higher levels.

As far as possible internationally recognised terminologies and classifications of drugs (ATC, INN) and adverse reactions (e.g. WHOART, MedDRA) should be used, to facilitate international comparisons of results and international transfer of data. Special care should be taken to attain compatibility with the reporting requirements of the WHO Drug Monitoring Programme. Detailed instructions on how to organise computerised data for submission to the WHO database are obtainable from the Uppsala Monitoring Centre. It may not be cost-effective to design a computer system for the management of adverse reaction reports from scratch. Commercial programmes are available which have been appropriately tested and can be customised according to local needs including local languages.

8. USE OF THE DATA

Data collected in pharmacovigilance can be used in a variety of ways.

8.1 Hypothesis generation and strengthening

A major aim of pharmacovigilance is the early detection of hypotheses or signals (see Glossary) with regard to possible adverse reactions. Early signals may be too uncertain, however, to justify firm conclusions and regulatory action, and may need further study (see § 8.5). A signal may be strengthened by combining the experiences reported in various countries. Therefore international collaboration is important.

8.2 Drug regulation

After approval of a medicinal product, all available domestic and international safety information is continuously monitored by the drug regulatory authority and the pharmaceutical company concerned. Often problems can be solved by adaptation of the approved product information (inclusion of new adverse effects, warnings, or indication changes). Sometimes stronger restrictive actions are needed, with withdrawal of the marketing authorisation as the extreme. For the approval of a given drug in a given country, it may be very helpful to have information on the experiences with the drug in countries where it is already in use (e.g. through collaboration with *the* Uppsala Monitoring Centre).

8.3 Information

For the dissemination of information of current importance or interest to healthcare practitioners, an adverse drug reactions bulletin or a column in medical and pharmaceutical journals may be very helpful. In the case of an emergency, a letter directly to all doctors and pharmacists may be needed. Usually such actions take place in collaboration with the regulatory authority and the pharmaceutical company's experts.

8.4 Education and feedback

Continuous pre- and postgraduate education of healthcare professionals is an important aspect of pharmacovigilance. Appropriate educational activities will improve knowledge and awareness of adverse drug reactions and stimulate reporting. Drug information officers and local or national Formulary Committees may benefit from close collaboration with the pharmacovigilance centre.

8.5 Limitations regarding the use of the data

Usually case reports of suspected adverse reactions may be influenced by all sorts of bias. The interpretation of pharmacovigilance data may be difficult. Often signals are unsubstantiated and require further study for confirmation or refutation (hypothesis testing) and for the assessment of the reaction frequency, for example, as needed for drug regulatory decision-making.

On the one hand a pharmacovigilance centre has the task to stimulate the use of the collected data by healthcare professionals, and on the other hand to ensure that the heterogeneous and largely unproven data are used in a careful and scientifically (and socially) responsible way.

The spontaneous reporting system is especially helpful in the detection of adverse reactions that are specific or occur in a suggestive time-relationship with drug use (e.g. anaphylactic shock), but may be less effective in studying other sorts of adverse reactions (e.g. cancer development). The potential of the spontaneous reporting system to determine the true frequency of adverse reactions is limited.

The detailed reporting of histories of patients with iatrogenic injury and the subsequent use of the reports are to a variable extent subject to rules regarding privacy and medical secrecy. Confidentiality of personal data is needed. The complex of details in a patient history may be as personal as a finger print and therefore a potential identifier. It is advisable for a pharmacovigilance centre to establish data-management protocols, identifying legitimate data-users and describing which data elements are available to whom and for which purpose and which uses are excluded. Confidentiality primarily concerns the secrecy of the identity of all individuals (patient, reporter, doctor) and institutions (hospital) involved. In many countries case report summaries are not considered confidential.

Besides legal obligations, the basis of spontaneous monitoring is the commitment of healthcare practitioners and patients together to make information available. If pharmacovigilance data were used against the wish of reporters, the system as a whole might collapse.

9. RELATIONS WITH OTHER PARTIES

9.1 The Drug Regulatory Authority

The Drug Regulatory Authority in the country needs to be informed about suspected adverse reactions without delay, especially when unusual (e.g. reactions not included in the approved Summary of Product Characteristics) or serious. In addition, a pharmacovigilance centre should inform the regulatory authority about any cluster of case reports that is of possible interest, or when an adverse reaction is reported in high or increasing frequency.

9.2 Pharmaceutical companies

Pharmaceutical companies need the same information as the regulatory authority. It will depend on the local situation whether companies are to be informed directly or via the regulatory authority.

9.3 Professional medical and pharmaceutical associations

A pharmacovigilance centre should seek the support of ***professional medical and pharmaceutical associations***. In the case of an emergency, these associations should be informed in good time.

9.4 World Health Organization and WHO Collaborating Centre for International Drug Monitoring

A new pharmacovigilance centre should make contact with the **World Health Organization** in Geneva and the **WHO Collaborating Centre for International Drug Monitoring** (*the* UMC) in Uppsala, Sweden.

9.5 National pharmacovigilance centres

In addition it may be helpful to make contacts with ***national pharmacovigilance centres*** in nearby countries. When more experienced, such centres may be helpful with staff training.

9.6 Academia

The need for pharmacovigilance and the nature of its procedures are a natural part of the curriculum of pre-graduate training. In addition a pharmacovigilance centre may contribute to and participate in postgraduate educational programs. Findings or hypotheses from the pharmacovigilance system may be of potential interest for further study with regard to mechanisms, reaction frequency, and so on, to academic pharmacological or epidemiological institutions.

9.7 Media and consumer organisation

Support from national associations of consumers and patients may add to the general acceptance of pharmacovigilance. Good relations with leading journalists may be helpful, e.g. for general public relations and as part of the *risk management* strategy whenever an acute drug problem arises. Special attention may be needed to explain to journalists the limitations of pharmacovigilance data (see § 8.5)

10. OTHER SOURCES OF INFORMATION

Spontaneous Reporting is especially useful in picking up signals of relatively rare, serious and unexpected adverse reactions. For less rare adverse reactions several other methods may be used, e.g. clinical trials or cohort studies. In addition to spontaneous reporting several other methods have become available to provide data relevant to pharmacovigilance. Examples are: Prescription Event Monitoring, Case-Control Surveillance and linkage of records from multipurpose databases. In addition, drug utilisation data is of value in safety assessment.

11. FUNDING

An estimation of the amount of money needed for pharmacovigilance can be calculated as a function of the rate of reporting required and the size of the population (see § 5.3 and 6.1). The collection of quantitatively and qualitatively good data and the careful assessment and distribution of such information obviously have a price. A pharmacovigilance centre should have some basic, regular source of funding in order to ensure continuity in its work. Such funding may be obtained as part of the drug registration fee, or through a special mandatory pharmacovigilance contribution. Both can be included in the budget of the drug regulatory authority.

Apart from the basic resources, the centre may try to get additional funding from various parties with an interest in pharmacovigilance. Institutions that may be approached include:

- health insurance companies and health insurance funds
- university departments
- professional associations
- governmental departments with an interest in drug safety.

In view of the great commercial and public health consequences of adverse reactions, the continuity of the funding of pharmacovigilance should be guaranteed and not be susceptible to possible pressure groups, political changes or economic factors.

REFERENCES

Stephens MDB. Detection of New Adverse Drug Reactions.
MacMillan Publishers, 1998, ISBN 0-333-693914.

Strom B (Ed). Pharmacoepidemiology (2nd ed).
Wiley, Chichester, 1994. ISBN 0 471 94058 5.

International Drug Monitoring: The Role of National Centres.
WHO Technical Report Series, No. 498, Geneva, 1972.

Rawlins MD. Spontaneous reporting of adverse drug reactions.
Br J Clin Pharmacol 1988;26:1-11.

Bégaud B, Chaslerie A, Fourrier A, Haramburu F, Miremont G (Eds). Methodological approaches in pharmacoepidemiology. Applications to spontaneous reporting.
Elsevier Science Publishers, Amsterdam, 1993. ISBN 0-444-81-577-5.

Griffin JP, Weber JCP. Voluntary systems of adverse reaction reporting. In: Griffin JP, D'Arcy PF, Harron DWG (Eds). Medicines: Regulation, Research and Risk.
Greystone Books, Antrim, 1989.

Bénichou C (Ed). Adverse drug reactions. A practical guide to diagnosis and management.
Wiley, Chichester, 1994. ISBN 0 471 94211.

Olsson S (Ed) National Pharmacovigilance Systems.
WHO Collaborating Centre for International Drug Monitoring, Uppsala, Sweden 2nd ed 1999, ISBN 91-630-7678-0.

GLOSSARY

A ***drug*** or ***medicine*** is 'a pharmaceutical product, used in or on the human body for the prevention, diagnosis or treatment of disease, or for the modification of physiological function'.

An ***unexpected adverse reaction*** is 'an adverse reaction, the nature or severity of which is not consistent with domestic labelling or market authorisation, or expected from characteristics of the drug'. Here the predominant element is that the phenomenon is unknown.

A ***side effect*** is 'any unintended effect of a pharmaceutical product occurring at doses normally used in man, which is related to the pharmacological proprieties of the drug'. Essential elements in this definition are the pharmacological nature of the effect, that the phenomenon is unintended, and that there is no overt overdose.

An ***adverse reaction*** is 'a response to a medicine which is noxious and unintended, and which occurs at doses normally used in man'. In this description it is of importance that it concerns the response of a patient, in which individual factors may play an important role, and that the phenomenon is noxious (an unexpected therapeutic response, for example, may be a side effect but not an adverse reaction).

A ***signal*** refers to 'reported information on a possible causal relationship between an adverse event and a drug, the relationship being unknown or incompletely documented previously'. Usually more than a single report is required to generate a signal, depending upon the seriousness of the event and the quality of the information.

In these definitions drug or drug food interactions are also included. It should be added that many patients have only suspected adverse reactions in which the causal role of the drug is unproven and may be doubtful, and that pharmacovigilance data usually refer to only suspected adverse reactions and side effects.

An ***adverse event*** or ***experience*** is defined as 'any untoward medical occurrence that may present during treatment with a medicine but which does not necessarily have a causal relationship with this treatment'. The basic point here is the coincidence in time without any suspicion of a causal relationship.

Serious adverse events can be defined as those that:

a. are life threatening or fatal
b. cause or prolong hospital admission
c. cause persistent incapacity or disability; or
d. concern misuse or dependence.

Type A effects ('drug actions') are those which are due to (exaggerated) pharmacological effects. Type A effects tend to be fairly common, dose related (i.e. more frequent or severe with higher doses) and may often be avoided by using doses which are appropriate to the individual patient. Such effects can usually be reproduced and studied experimentally and are often already identified before marketing.

Interactions between drugs, especially pharmacokinetic interactions, may often be classified as Type A effects, although they are restricted to a defined sub-population of patients (i.e. the users of the interacting drug).

Type B effects ('patient reactions') characteristically occur in only a minority of patients and display little or no dose relationship. They are generally rare and unpredictable, and may be serious and notoriously difficult to study. Type B effects are either immunological or non-immunological and occur only in patients, with - often unknown - predisposing conditions. Immunological reactions may range from rashes, anaphylaxis, vasculitis, inflammatory organ injury, to highly specific autoimmune syndromes. Also non-immunological Type B effects occur in a minority of predisposed, intolerant, patients, e.g. because of an inborn error of metabolism or acquired deficiency in a certain enzyme, resulting in an abnormal metabolic pathway or

accumulation of a toxic metabolite. Examples are chloramphenicol aplastic anaemia and isoniazid hepatitis.

Type C effects refer to situations where the use of a drug, often for unknown reasons, increases the frequency of a 'spontaneous' disease. Type C effects may be both serious and common (and include malignant tumours) and may have pronounced effects on public health. Type C effects may be coincidental and often concern long term effects; there is often no suggestive time relationship and the connection may be very difficult to prove.

Confidentiality: Maintenance of the privacy of patients, healthcare providers and institutes, including personal identities and all personal medical information.

Verification: The procedures carried out in pharmacovigilance to ensure that the data contained in a final report matches the original observations. These procedures may apply to medical records, data in case-report forms (in hard copy or electronic form), computer printouts, and statistical analyses and tables.

Validation: The action of proving that any procedure, process, equipment (including the software or hardware used), material, activity or system used in pharmacovigilance actually leads to the expected results.

CAUSALITY CATEGORIES

The causality categories described by the Uppsala Monitoring Centre are as follows:

1. ***Certain***: a clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.
2. ***Probable/Likely***: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.
3. ***Possible***: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administrations of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
4. ***Unlikely***: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
5. ***Conditional/Unclassified***: a clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data is essential for a proper assessment, or the additional data is under examination.
6. ***Unassessable/Unclassifiable***: a report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

As a step towards harmonisation in drug regulation in the countries of the European Union, the EU pharmacovigilance working parties proposed the following three causality categories:

- ***Category A***: 'Reports including good reasons and sufficient documentation to assume a causal relationship, in the sense of plausible, conceivable, likely, but not necessarily highly probable'.
- ***Category B***: 'Reports containing sufficient information to accept the possibility of a causal relationship, in the sense of not impossible and not unlikely, although the connection is uncertain and may be even doubtful, e.g. because of missing data, insufficient evidence or the possibility of another explanation'.
- ***Category O***: 'Reports where causality is, for one or another reason, not assessable, e.g. because of missing or conflicting data'.

SOURCES

Edwards IR, Biriell C. Harmonisation in pharmacovigilance. Drug Safety 1994,10:93-102.

Bégaud B, Evreux JC, Jouglard J, Lagier G. Unexpected or toxic drug reaction assessment (imputation). Actualisation of the method used in France. Thérapie 1985;40:111-8.

Meyboom RHB, Hekster YA, Egberts ACG, Gribnau FWJ, Edwards IR. Causal or casual? The role of causality assessment in pharmacovigilance. Drug Safety 1997,16:374-389.

Meyboom RHB, Egberts ACG, Edwards IR, Hekster YA, De Koning FHP, Gribnau FWJ. Principles of signal detection in pharmacovigilance. Drug Safety 1997;16:355-365.

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