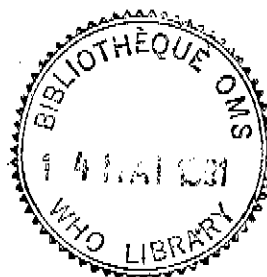




**BASIC TESTS FOR PHARMACEUTICAL SUBSTANCES
AND
MEDICINAL PLANT MATERIALS (2)**

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1. Introduction

Further to the manual *Basic Tests for Pharmaceutical Substances*, published by WHO in 1986, analogue tests for 27 pharmaceutical substances and medicinal plant materials contained in the WHO Model List of Essential Drugs are described in this document. They should not be used to replace the monographs contained in The International Pharmacopoeias since they are intended only to verify the identity of pharmaceutical substances. Initial validation of these tests was carried out in at least 4 different laboratories. They are now sent to you for final approval. Any additional comments which you may have should be forwarded to:

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2. Test Procedures

2.1 Pharmaceutical substances

AMIKACIN SULFATE

Identity tests

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

Colour and other reactions

1. Dissolve 0.05 g in 5 ml of water, add 1.0 ml of sodium hydroxide (~80 g/l) TS and mix, then add 20 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 TS₁; a bluish violet colour is produced.
3. Dissolve 20 mg in 1.0 ml of water and add 1.0 ml of barium chloride (50 g/l) TS; a white precipitate is formed, which is practically insoluble in hydrochloric acid (~250 g/l) TS.

BACITRACIN ZINC

Composition. Bacitracin zinc is a zinc complex of bacitracin, a polypeptide produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis*. The main components are Bacitracin 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 1.0 ml of water, add 1.0 ml of triketohydrindene/butanol TS and 0.5 ml of pyridine R and heat at 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 two volumes.
 - a) To 1 volume add 1.0 ml of potassium ferrocyanide (45 g/l) TS; a white precipitate is produced which is insoluble on the addition of hydrochloric acid (~250 g/l) TS.
 - b) To 1 volume add 1 drop of copper(II) sulfate (1 g/l) TS and 1.0 ml of ammonium mercurithiocyanate TS; a violet precipitate is formed.

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, faint.

Colour and other reactions

1. Dissolve 10 mg in 2.0 ml of hydrochloric acid (0.1 mol/l) VS and add 1.0 ml of iodine TS; the colour of the iodine disappears immediately and a white, turbid solution is produced.
2. Dissolve 10 mg in 2.0 ml of water and add 10 drops 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 trinitrophenyltetrazolium 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 4 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, 1.0 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 1.0 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 but omitting the zinc R powder; no red colour is produced.
3. Heat carefully 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 is produced, which disappears on the addition of 1 ml of hydrochloric acid (~250 g/l) TS.
4. Introduce the test solution from a nichrome or platinum wire sealed to a glass rod into a nonluminous flame; a strong yellow colour can be observed.

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 add a few crystals of potassium iodide R; a brownish yellow colour is produced which on standing turns to reddish brown. Keep the remaining solution for test 2.
2. To the remaining solution from test 1 add a few crystals of tin(II) chloride R; a reddish orange colour is produced which on standing turns to reddish brown

DOXORUBICIN HYDROCHLORIDE

Identity tests

Description. A red-orange, crystalline powder.

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

Colour and other reactions

1. Dissolve 2 mg in 2.0 ml of methanol R, add 2.0 ml of water and 1 drop of sodium hydroxide (~80 g/l) TS; the orange-red colour of the solution turns to blue-violet.
2. Place a small quantity of the test substance on a white tile, add 1 drop of formaldehyde/sulfuric acid TS; the orange-red colour of the substance turns to violet.
3. Dissolve 0.05 g in 1.0 ml of water, add a few drops of ammonia (~100 g/l) TS and filter. Acidify the filtrate with nitric acid (~130 g/l) TS and add 1.0 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, oily solid; odour, faint, ester-like.

Colour and other reactions

1. Dissolve 5 mg in about 2 ml of sulfuric acid (~1760 g/l) TS and allow to stand for 5 minutes; an orange-brown colour is produced.
2. Dissolve 5 mg in about 2 ml of formaldehyde/sulfuric acid TS; an orange colour is produced. Heat on a water-bath for 2 minutes; the colour turns to dark brown.
3. Dissolve 5 mg in 2.0 ml of water and add 3 drops of potassium dichromate (100 g/l) TS; a yellow precipitate is formed.
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 in the acid layer.

Degradation tests

Discoloration and a change in the physical aspect 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 1.0 ml of potassio-mercuric iodide TS; a yellowish precipitate is immediately formed.
2. Dissolve 0.05 g in 5 ml of water, add 1.0 ml of sulfuric acid (~100 g/l) TS and 1.0 ml of potassium nitrite (100 g/l) TS; a brownish coloration is produced. Shake the solution with 2.0 ml of chloroform R; the aqueous phase turns colourless and the chloroform phase to violet.
3. Dissolve 0.05 g in 5 ml of water, add 1 drop of nitric acid (~1000 g/l) TS and 1.0 ml of silver nitrate (40 g/l) TS; a yellow precipitate is formed 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 1.0 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 is produced immediately which turns to brownish yellow with a greenish fluorescence. Dilute the solution with 10 ml of water; the colour of the solution turns to yellow with a greenish fluorescence.
3. Dissolve a small quantity in about 1 ml of phosphoric acid (~1440 g/l) TS, and heat cautiously; a yellow solution is obtained with a pale greenish fluorescence.
4. Dissolve about 2 mg in 1.0 ml of water and introduce the solution into a nonluminous flame using a magnesia stick, a nichrome or platinum wire sealed to a glass rod; a strong yellow colour is produced in the flame.
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 2.0 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 test substance and non-compliance with the following test usually indicate gross degradation:

Dissolve 0.2 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 an ice-bath. 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. Filter the solution, and dry the crystalline precipitate in vacuum over phosphorus pentoxide R; melting temperature, about 92 °C.
2. Dissolve 10 mg in 4 ml of water, 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 0.06 g in 20 ml of water. Add 1.0 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 chloroform R, discard the aqueous layer and wash the chloroform layer with 10 ml of water. Discard the aqueous layer, shake with about 1 g of anhydrous sodium sulfate R and filter. Evaporate the filtrate at room temperature and dry in vacuum at a temperature not exceeding 40 °C; the residue melts at about 59 °C.
2. Dissolve 0.06 g in 20 ml of water. Add 1.0 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. Dissolve 0.06 g in 20 ml of water, add 1.0 ml of nitric acid (~130 g/l) TS and a few drops of silver nitrate (40 g/l) TS; a white precipitate is produced. Filter, wash with water and add a few drops ammonia (~100 g/l) TS; the precipitate dissolves.

MAGNESIUM SULFATE

Identity tests

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

Colour and other reactions

1. Dissolve 0.5 g in 1.0 ml of water and add 1.0 ml of ammonia (~100 g/l) TS; a white precipitate is produced which dissolves after addition of 1.0 ml of ammonium chloride (100 g/l) TS. Add 1.0 ml of disodium hydrogen phosphate (100 g/l) TS; a white, fine crystalline precipitate is produced.
2. Dissolve 10 mg in 2.0 ml of water, add 3 drops of titan yellow TS and 2.0 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 10 ml of hydrochloric acid (~70 g/l) TS and 1.0 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 forms at the interface of the two liquids.
2. Heat 0.05 g with 2.0 ml of potassium hydroxide/ethanol TS in a water-bath for 5 minutes. Cool, add 1.0 ml of water, about 1 ml of sulfuric acid (~1760 g/l) TS and 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 using a small test-tube, add about 3 mg of sodium nitroprusside R, 0.05 g of sodium carbonate R, 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 small quantity in about 1 ml of sulfuric acid (~1760 g/l) TS. Incline the tube and carefully add, without mixing, 1.0 ml of water; a green colour forms at the interface of the two liquids. Allow to stand; the colour turns to bluish violet (distinction from hydroxyprogesterone caproate).

METHIONINE

Identity tests

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

Colour and other reactions

1. Dissolve 0.10 g in 5 ml of hydrochloric acid (0.1 mol/l) VS, add 0.20 ml of triketohydrindene/ethanol VS and heat; a violet colour is produced.
2. Dissolve 10 mg in 1.0 ml of water, 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 0.10 g in 5 ml of potassium hydroxide (~110 g/l) TS, add 0.3 ml of sodium nitroprusside (45 g/l) TS with shaking. Heat the solution in a water-bath at a temperature between 35 and 40 °C for 10 minutes. Cool in an ice-bath for 2 minutes, add about 2 ml of hydrochloric acid (~250 g/l) TS and shake well; a red colour is produced.

METHYLOSANILINIUM CHLORIDE

Identity tests

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

Colour and other reactions

1. Add a small quantity 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. Place on a filter-paper 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 1.0 g add 10 ml of water and heat to 80 °C to dissolve. Add 20 ml of sodium hydroxide (~8 g/l) TS, cool in ice and filter. To 2.0 ml of the filtrate (keep the remaining filtrate for tests 2 and 3) add about 0.2 ml of nitric acid (~1000 g/l) TS followed by about 0.2 ml of ceric ammonium nitrate TS; a red-orange colour is produced.
2. Neutralize 5 ml of the 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 formed.
3. Transfer a further 10 ml of the filtrate from test 1 to a crucible, add 2.5 ml of hydrogen peroxide (~60 g/l) TS, mix and evaporate to dryness on a water-bath. Dissolve the residue in 1.0 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 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 formed 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 1.0 g add 10 ml of water and heat to 80 °C to dissolve. Add 20 ml of sodium hydroxide (~80 g/l) TS, cool in ice, and filter. To 2.0 ml of the filtrate (keep the remaining filtrate for tests 3 and 4) add about 0.2 ml of nitric acid (~1000 g/l) TS followed by about 0.2 ml of ceric ammonium nitrate TS; a yellow colour is produced.
2. Heat 0.5 g with about 1 ml of sodium hydroxide (~400 g/l) TS in a test-tube; ammonia, perceptible by its odour evolves.
3. Neutralize 5 ml of the 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 formed.
4. Transfer a further 10 ml of the filtrate from test 1 to a crucible, add 2.5 ml of hydrogen peroxide (~60 g/l) TS, mix and evaporate to dryness on a water-bath. Dissolve the residue in 1.0 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 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 formed 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 turns from colourless to yellow, to orange and later to reddish brown.
3. Dissolve 10 mg in 1.0 ml of methanol R, add 1.0 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 nonluminous flame using a magnesia stick, a nichrome or platinum wire sealed to a glass rod; the flame acquires a bright 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 potassio-mercuric iodide TS; a creamy white precipitate is produced.
2. Shake 1.0 ml of methyl orange/ethanol TS and 5 ml of water with 2 ml of chloroform R; the chloroform 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; a yellow colour is produced in the chloroform layer.
3. Ignite about 0.1 g with 0.5 g of anhydrous sodium carbonate R, extract the residue with water and filter. Neutralize 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.

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 2.0 ml of phosphomolybdic acid/ethanol TS; within a few minutes the colour of the solution turns to light blue.
2. To 10 mg add 4 ml of pyridine R, about 2 ml of acetic anhydride R and shake; a yellow colour is immediately produced. Heat gently on a water-bath; a rose-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, 4 drops of glacial acetic acid R and 4 drops 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 2.0 ml of water. Add 1.0 ml of hydrochloric acid (~70 g/l) TS and 1.0 ml of barium chloride (50 g/l) TS; a white precipitate is produced which is practically insoluble in hydrochloric acid (~250 g/l) TS.

2.2 Medicinal plant materials

COAL TAR

Composition. Coal tar is a by-product usually obtained during the destructive distillation of coal. It is a complex and an 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 it hardens, burns in air with a luminous sooty flame, and when ignited 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 1 g vigorously with 9 ml of water for 10 minutes and filter; the filtrate gives a neutral or only a slightly acid reaction when tested with litmus paper R (unlike wood tar) and an odour of naphthalene is discernable (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 tests

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

Dissolve 0.1 g in 10 ml of nitrobenzene R; a clear or almost clear solution is produced.

IPECACUANHA ROOT

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

Identity tests

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

Macroscopic characteristics.

- *Cephaelis ipecacuanha.* Dark brick-red to very dark brown. A somewhat tortuous root, seldom more than 15 cm long or 6 mm thick; externally closely annulated, completely encircled with rounded ridges. The fracture in the bark is short and in the wood splintery; a transversely cut surface shows wide greyish bark and uniformly dense wood. The rhizomes are short lengths attached to roots, cylindrical, up to 2 mm in diameter, finely wrinkled longitudinally, and with pith occupying approximately one-sixth of the whole diameter.
- *Cephaelis acuminata.* In general, resembles the root of *Cephaelis ipecacuanha*, but has the following characteristics: thickness, often up to 9 mm of the external surface, greyish brown or reddish brown with transverse ridges at intervals of about 1-3 mm and having a width of about

0.5-1 mm, extending about half-way round the circumference and diminishing at the extremities into the general surface level.

Colour and other reactions

1. Mix 0.05 g of the coarsely powdered material 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. Mix about 0.2 g of the coarsely powdered material with 2 drops of ammonia (~260 g/l) TS and 2 ml of chloroform R. Shake, filter the mixture, evaporate to dryness about 1 ml of the filtrate (keep the remaining filtrate for test 3), dissolve the residue in 0.2 ml of water and add 3 drops of potassium iodobismuthate/acetic acid TS; an orange precipitate is produced.
3. Mix the remaining filtrate from test 2 with 0.5 ml of ethanol (~750 g/l) TS and transfer the mixture to a small test-tube, 100 x 10 mm. Dip vertically into the tube a strip of filter-paper, 100 x 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 *Podophyllum hexandrum* Royle (*P. emodi* Wall.) or *Podophyllum peltatum* L. after percolation with ethanol and precipitation from water or very diluted acids.

Identity tests

Description. Light brown to greenish yellow or brownish grey masses or an amorphous powder. It 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. 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 from the solution.
2. Dissolve 0.4 g in a mixture of 1.5 ml of water and 2.0 ml of ethanol (~750 g/l) TS, and add 0.5 ml of potassium hydroxide (~55 g/l) TS. Shake; if the solution does not gelatinize, it indicates the presence of *Podophyllum peltatum* resin and if it does gelatinize, it indicates the presence of *Podophyllum hexandrum* resin.
3. Dissolve 10 mg in 2.0 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 about 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; *Podophyllum peltatum* resin forms a yellowish green solution, whereas *Podophyllum hexandrum* resin forms an orange to brownish red solution.

SENNA FRUIT

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

Identity tests

Description. Leaflike, flat and thin pods, yellowish green to yellowish brown with a dark brown centre, oblong or reniform; odour, slight; taste, first mucilaginous and sweet, then slightly bitter.

Macroscopic examination.

- *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 longitudinal prominent 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.

Colour and other reactions

1. Mix about 0.2 g of the powdered material 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 to dryness 1 ml of the yellowish coloured toluene extract. 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 material onto 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. *Cassia auriculata* L., *Cassia goratensis* Fres.).

Degradation test

Discoloration of the test material usually indicates gross degradation.

SENNA LEAF

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

Identity tests

Description. Pale greyish green, thin, fragile leaflets or yellowish green leaflets; odour, slight; taste, first mucilaginous and sweet, then slightly bitter.

Colour and other reactions

Prior to 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 material add 10 ml of ethanol (~375 g/l) TS, warm on a water-bath for 5 minutes and filter while hot. To the filtrate add about 1 ml of hydrochloric acid (~420 g/l) TS and 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 2.0 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 material with 10 ml of water on a water-bath for 30 minutes and filter. To the filtrate add 1 drop of hydrochloric acid (~420 g/l) TS, shake with two quantities, each of 5 ml of chloroform R, and discard the chloroform 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 mixture composed of 4 ml of ferric chloride (25 g/l) TS and 6 ml of water, mix and heat on 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 chloroform

R, evaporate the chloroform extract to dryness on a water-bath and dissolve the residue in 2.0 ml of potassium hydroxide (~55 g/l) TS; a red-orange colour is produced.

3. Sprinkle 10 mg of the powdered material onto 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. *Cassia auriculata* L., *Cassia goratensis* Fres.).

Degradation test

Discoloration of the test material usually indicates gross degradation.

3. Reagents

The reagents, test solutions and volumetric solutions mentioned are described in the manual *Basic Tests for Pharmaceutical Dosage Forms*¹ and additional reagents are given below. Reagents are denoted by the abbreviation R, test solutions by the abbreviation TS, and volumetric solutions by the abbreviation VS. The concentration of the reagent solutions is expressed in g/l, that is, grams of anhydrous substance per litre of water or solvent, as indicated. Where no solvent is indicated, demineralized water should be used. The procedures for the preparation of test solutions that require special attention are given in detail.

Ammonium acetate R.

Ammonium chloride (100 g/l) TS.

Ammonium mercurithiocyanate TS.

Procedure. Dissolve 30 g of ammonium thiocyanate R and 27 g of mercuric chloride R in sufficient water to produce 1000 ml.

Anthrone R.

Anthrone TS1.

Procedure. Dissolve 35 mg of anthrone R in 100 ml of sulfuric acid (~1760 g/l).

Ceric ammonium nitrate R.

Ceric ammonium nitrate TS.

Procedure. Dissolve 6.25 g of ceric ammonium nitrate R in 10 ml of nitric acid (15 g/l) TS.

Shelf life. Use the solution within 3 days.

Cobalt(II) nitrate R.

Cobalt(II) nitrate (10 g/l) TS.

Copper(II) nitrate (10 g/l) TS.

Copper(II) sulfate (1 g/l) TS.

Ethanol (~150 g/l) TS.

Ethanol (~375 g/l) TS.

Hydrochloric acid (0.1 mol/l) VS.

Hydrogen Peroxide (~60 g/l) TS.

¹ *Basic tests for pharmaceutical dosage forms*, World Health Organization, Geneva 1991.

Methyl orange R.

Methyl orange/ethanol TS.

Procedure. Dissolve 0.04 g of methyl orange R in sufficient ethanol (~150 g/l) TS to produce 100 ml.

Nitric Acid (15 g/l) TS.

Nitrobenzene R.

Phosphomolybdic acid R.

Phosphomolybdic acid/ethanol TS.

Procedure. Dissolve 5 g of phosphomolybdic acid R in sufficient dehydrated ethanol R to produce 100 ml.

Potassium carbonate (100 g/l) TS.

Potassium hydroxide (~55 g/l) TS.

Potassium hydroxide (~110 g/l) TS.

Potassium nitrite R.

Potassium nitrite (100 g/l) TS.

Sodium nitroprusside R.

Sodium nitroprusside (45 g/l) TS.

Note: Sodium nitroprusside (45 g/l) TS must be freshly prepared.

Sucrose R.

Sucrose/hydrochloric acid TS.

Procedure. Dissolve about 0.1 g of sucrose R in 10 ml of hydrochloric acid (~420 g/l) TS.

Tetramethylammonium hydroxide/ethanol TS.

Procedure. Dilute 10 ml of tetramethylammonium hydroxide (~100 g/l) TS with sufficient ethanol (~750 g/l) TS to produce 100 ml.

Tin(II) chloride R.

Titan yellow R.

Titan Yellow TS.

Procedure. Dissolve 0.05 g of titan yellow R in sufficient water to produce 100 ml.

Toluene R.

Triketohydrindene/butanol TS.

Procedure. Dissolve 0.2 g of triketohydrindene hydrate R in sufficient 1-butanol R to produce 100 ml.

Triphenyltetrazolium chloride R.

Triphenyltetrazolium chloride/ethanol TS.

Procedure. Dissolve 0.5 g of triphenyltetrazolium chloride R in sufficient dehydrated ethanol R to produce 100 ml.

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