



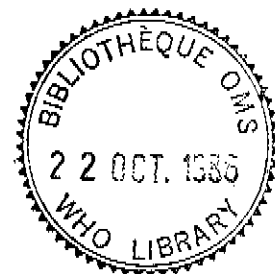
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BASIC TESTS FOR PHARMACEUTICAL DOSAGE FORMS

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1. INTRODUCTION

This consultative document is the second in a planned series on basic tests for verification of the identity of pharmaceutical substances in dosage forms. It contains identity tests for 23 drug substances drawn from the current revision of the WHO Model List of Essential Drugs. Other tests are currently being developed for other drugs. These will be included in the next selection that will be issued early next year.

Each of the tests described has been verified in at least four laboratories in different countries. Further validation of the tests (see Annex) on locally available samples is invited. These results or any other relevant comments should be forwarded to:

Pharmaceuticals Unit
 World Health Organization
 1211 Geneva 27
 Switzerland

2. DRUG INDEX

- | | |
|------------------------------------|-------------------------------------|
| amiloride hydrochloride tablets | lithium carbonate capsules |
| benzylpenicillin potassium tablets | lithium carbonate tablets |
| charcoal, activated tablets | norethisterone tablets |
| chlorpromazine hydrochloride syrup | pethidine hydrochloride injection |
| digitoxin tablets | pyrazinamide tablets |
| doxycycline hydrochloride capsules | rifampicin capsules |
| fluphenazine hydrochloride tablets | salbutamol aerosol |
| glibenclamide tablets | sodium nitroprusside soluble powder |
| haloperidol injection | sodium valproate tablets |
| haloperidol tablets | sulfadiazine tablets |
| hydrochlorothiazide tablets | tolbutamide tablets |
| lidocaine ointment | |

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3. TEST PROCEDURES

AMILORIDE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 5 mg of amiloride hydrochloride.

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 0.07 g of amiloride hydrochloride.
2. Grind the tablets, weigh out the above calculated equivalent amount to amiloride hydrochloride as powdered material, shake it with 50 ml of boiling water, filter while hot and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. Add 2.0 ml of hydrochloric acid (~70 g/l)TS and 0.5 ml of sodium nitrite (10 g/l)TS to 15 ml of the test solution, and shake for 2-3 minutes. Then add 0.5 ml of 2-naphthol TS; a reddish brown precipitate is produced.
2. To 15 ml of the test solution add 0.5 ml of nitric acid (~130 g/l)TS and a few drops of silver nitrate (40 g/l)TS; a white precipitate is formed. Add a few drops of ammonia (~100 g/l)TS; the precipitate dissolves.

BENZYLPENICILLIN POTASSIUM TABLETS

Description. Each tablet usually contains 100-500 mg (100'000-800'000 IU) of benzylpenicillin potassium.

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 40 mg, about 1 mg and 0.10 g of benzylpenicillin potassium.
2. Grind the tablets, weigh out the above calculated equivalent amounts to benzylpenicillin potassium as powdered material and use them directly: 40 mg for test substance 1 and divide it into four equal parts; about 1 mg for test substance 2 and 0.10 g for test substance 3.
3. Suspend test substance 3 in 5 ml of ethanol (~750 g/l)TS. Place a strip of filter-paper into the suspension and allow the solution to ascend for 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 and dry the remaining part of the strip in air at room temperature (test-paper).

IDENTITY TESTS

Colour and other reactions

1. To 1 part of test substance 1 add 3 ml of water, shake and filter. To the filtrate add 0.10 g of hydroxylamine hydrochloride R, 1.0 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 about 0.5 ml of ferric chloride (25 g/l)TS; a violet-red colour is produced.

Alternate test by filter-paper technique:

Place onto the test-paper 1 drop of hydroxylamine hydrochloride (10 g/l)TS, followed by 1 drop of sodium hydroxide (~80 g/l)TS and allow to react for 5 minutes. Following this apply at the same place on the test-paper 1 drop of hydrochloric acid (~70 g/l)TS and 1 drop of ferric chloride (25 g/l)TS; a violet-red ring is produced.

2. To 1 part of test substance 1 add a few drops of ethanol (~750 g/l)TS, 1.0 ml of water, shake and filter. To the filtrate add 1-2 drops of ferric chloride (25 g/l)TS; a yellowish precipitate is produced.

3. To 10 mg of paraformaldehyde R dissolved in about 1 ml of sulfuric acid (~1760 g/l)TS add test substance 2; a colourless solution is produced. Heat the solution in a water-bath for 2 minutes and cool; the colour changes to yellow-brown.

4. To 2 parts of test substance 1 add 2.0 ml of water, shake and filter. To the filtrate add 2-3 drops of glacial acetic acid R and 1.0 ml of sodium cobaltinitrite (100 g/l)TS; an orange-yellow precipitate is produced.

CHARCOAL, ACTIVATED TABLETS

Description. Each tablet usually contains 100-350 mg of activated charcoal.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 1.5 g of activated charcoal.
2. Grind the tablets, weigh out the above calculated equivalent amount to activated charcoal as powdered material and use it directly as the test substance.

IDENTITY TESTS

Colour and other reactions

1. Heat a small quantity of the test substance to redness; it burns slowly without a flame.
2. Shake the remaining test substance with 30 ml of methylthioninium chloride (1 g/l)TS for 5 minutes and filter; a colourless solution is produced.
Note. Some pharmaceutical aids e.g. carmellose, present in the formulation form a more or less gelatinous solution after shaking, and may cause difficulties to filter. Separate by centrifugation or use a sintered glass filter; only observe the first few ml of the filtrate.

CHLORPROMAZINE HYDROCHLORIDE SYRUP

Description. The syrup usually contains 25 mg of chlorpromazine hydrochloride in 5 ml of a suitable vehicle.

Preparation of the sample

1. To a stoppered flask transfer a volume equivalent to 50 mg of chlorpromazine hydrochloride, add 10 g of anhydrous sodium sulfate R and 25 ml of chloroform R. Shake for 3 minutes, filter the chloroform layer and use the filtrate as the test solution.
2. Evaporate 15 ml of the test solution to dryness on a water-bath and use the residue as the test substance.

IDENTITY TESTS

Colour and other reactions

1. To 1-2 mg of the test substance add a few drops of sulfuric acid (~1760 g/l)TS; a cherry red colour is produced which gradually changes to deep red (distinction from promazine).
2. To 5 ml of the test solution add 1.0 ml of sodium metaperiodate (60 g/l)TS and 1.0 ml of sulfuric acid (~100 g/l)TS. Shake vigorously and allow the layers to separate; the aqueous layer shows a red colour that fades slowly on standing and the chloroform layer acquires a pink colour (distinction from promethazine).
3. Dissolve 10 mg of the test substance in 5 ml of water and add about 2 ml of nitric acid (~1000 g/l)TS; a dark red colour is produced which suddenly fades to almost colourless. Add 2.0 ml of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced.

DIGITOXIN TABLETS

Description. Each tablet usually contains 0.05-0.20 mg of digitoxin.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.15 mg of digitoxin.
2. Grind the tablets, weigh out the above calculated equivalent amount to digitoxin as powdered material and use it directly as the test substance. Divide the test substance into three equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 2 parts of the test substance add 2.0 ml of a solution prepared by mixing 0.5 ml of ferric chloride (25 g/l)TS with 100 ml of glacial acetic acid R and shake. Superimpose this solution onto 1 ml of sulfuric acid (~1760 g/l)TS; a brown ring, but no red colour is produced at the junction of the two liquids and after some time the acetic acid layer acquires a blue colour.
2. To 1 part of the test substance add 0.5 ml of ethanol (~750 g/l)TS and 3 ml of ethanol (~750 g/l)TS and 3 ml of alkaline trinitrophenol TS; an orange-yellow colour is slowly produced.

DOXYCYCLINE HYDROCHLORIDE CAPSULES

Description. Each capsule usually contains 100 mg of doxycycline hydrochloride.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amounts equivalent to 5 mg and 0.10 g of doxycycline hydrochloride.
2. Empty the capsules, weigh out the above calculated equivalent amounts to doxycycline hydrochloride and use them directly: 5 mg for test substance 1 and 0.10 g for test substance 2.
3. Shake test substance 2 with 10 ml of water, filter and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To test substance 1 add about 2 ml of sulfuric acid (~1760 g/l)TS; an intense yellow colour is produced.
2. Heat carefully 2.0 ml of zinc chloride (500 g/l)TS in a porcelain dish on a hot plate or 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; the yellow colour imparted by the test solution becomes more intense.
3. To 2.0 ml of the test solution add 1 drop of ferric chloride (25 g/l)TS; a dark red-brown colour is produced.
4. To 2.0 ml of the test solution add 1 drop of alkaline potassio-mercuric iodide TS; a light yellow, fine crystalline precipitate is formed. In an excess of the reagent and on shaking the precipitate dissolves.
5. To 1.0 ml of the test solution add 5 drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is formed which dissolves on addition of 1.0 ml of ammonia (~100 g/l)TS.

FLUPHENAZINE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 0.25-10 mg of fluphenazine hydrochloride. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 20 mg of fluphenazine hydrochloride.
2. Grind the tablets or cores, weigh out the above calculated equivalent amount to fluphenazine hydrochloride as powdered material and use it directly as the test substance. Divide the test substance into four equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of the test substance add 5 ml of ethanol (~750 g/l)TS, shake and add cautiously about 2 ml of sulfuric acid (~1760 g/l)TS; at the junction of the two solutions a pink colour is observed which becomes yellow on mixing.
2. To 1 part of the test substance add 2 ml of a mixture of 3 ml of sulfuric acid (~1760 g/l)TS and 2 drops of formaldehyde TS; an orange colour is produced. Heat on a water-bath for 2 minutes; the colour turns to dark brown.
3. To 1 part of the test substance add 2.0 ml of water and filter. To the filtrate add 3 drops of potassium dichromate (100 g/l)TS and shake; a yellow precipitate is formed.
4. To 1 part of the test substance add 2.0 ml of water, shake and filter. To the filtrate add 3 drops of nitric acid (~130 g/l)TS; a white, curdy precipitate is produced. Add a few drops of ammonia (~100 g/l)TS; the precipitate dissolves.

GLIBENCLAMIDE TABLETS

Description. Each tablet usually contains 5 mg of glibenclamide.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 50 mg of glibenclamide.
2. Grind the tablets, weigh out the above calculated equivalent amount to glibenclamide as powdered material and use it directly as the test substance. Divide the test substance into five equal parts.

IDENTITY TESTS

Colour and other reactions

1. Mix 1 part of the test substance with 0.04 g of anhydrous sodium carbonate R and 0.04 g of potassium carbonate R. Ignite the mixture, cool, to the residue add 5 ml of hot water, stir well and filter. Acidify 2.0 ml of the filtrate with nitric acid (~130 g/l)TS, and add 2 drops of silver nitrate (40 g/l)TS; a white precipitate is produced. Acidify a further 2.0 ml of the filtrate with hydrochloric acid (~70 g/l)TS, and add 1.0 ml of barium chloride (50 g/l)TS; a white precipitate is formed.
2. Boil 1 part of the test substance with about 1 ml of sodium hydroxide (~200 g/l)TS; the fumes evolved change moistened red litmus paper R to blue.
3. Extract the remaining test substance with three successive portions, 10 ml each, of a mixture of 2 volumes of dichloromethane R and 1 volume of acetone R. Filter the extracts through the same dry filter-paper, evaporate the combined filtrate to dryness, recrystallize the residue using a mixture of equal volumes of acetone R and methanol R, separate the crystals and dry at 105 °C; melting temperature, about 169 °C. Mix a portion of the residue with an equal amount of tolbutamide R; eutectic temperature, about 114 °C.

HALOPERIDOL INJECTION

Description. The injection is a sterile solution usually containing 5.0 mg of haloperidol in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 10 mg of haloperidol in a platinum crucible, add 20 mg of anhydrous sodium carbonate R and evaporate to dryness on a water-bath. Heat until a white residue is obtained, dissolve it in 2.0 ml of water warming gently on a water-bath, cool, neutralize with hydrochloric acid (~70 g/l)TS and use it as the test solution.

IDENTITY TEST

Colour and other reactions

In a test-tube mix 1 drop of ferric chloride (25 g/l)TS with 1 drop of ammonium thiocyanate (75 g/l)TS, dilute with 10 ml of water and acidify with 1 drop of hydrochloric acid (~70 g/l)TS. To 1.0 ml of this solution, add drop by drop, the test solution; the red colour is discharged.

HALOPERIDOL TABLETS

Description. Each tablet usually contains 1-2 mg of haloperidol.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 10 mg of haloperidol.
2. Grind the tablets, weigh out the above equivalent amount to haloperidol as powdered material, shake it with 10 ml of chloroform R for 5 minutes, filter into a platinum crucible, add 20 mg of anhydrous sodium carbonate R and evaporate to dryness on a water-bath. Heat until a white residue is obtained, dissolve it in 2.0 ml of water warming gently on a water-bath, cool, neutralize with hydrochloric acid (~70 g/l)TS and use it as the test solution.

IDENTITY TEST

Colour and other reactions

In a test-tube mix 1 drop of ferric chloride (25 g/l)TS with 1 drop of ammonium thiocyanate (75 g/l)TS, dilute with 10 ml of water and acidify with 1 drop of hydrochloric acid (~70 g/l)TS. To 1.0 ml of this solution add, drop by drop, the test solution; the red colour is discharged.

HYDROCHLOROTHIAZIDE TABLETS

Description. Each tablet usually contains 25-50 mg of hydrochlorothiazide.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.15 g of hydrochlorothiazide.
2. Grind the tablets, weigh out the above calculated equivalent amount to hydrochlorothiazide as powdered material and use it directly as the test substance. Divide the test substance into three equal parts.
3. Suspend 1 part of the test substance in 5 ml of dehydrated ethanol R. Place a strip of filter-paper into the suspension and allow the solution to ascend for 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, and dry the remaining part of the strip in air at room temperature (test-paper).

IDENTITY TESTS

Colour and other reactions

1. To 1 part of the test substance add 5 ml of sodium carbonate (50 g/l)TS, shake and filter. To the filtrate add 1.5 ml of potassium permanganate (10 g/l)TS; the colour of the solution turns from violet to brown and on standing a colloidal precipitate is formed.

Alternate test by filter-paper technique:

Place onto the test-paper 1 drop of sodium carbonate (50 g/l)TS followed by 1 drop of potassium permanganate (10 g/l)TS; after a few minutes the colour of the spot turns from violet to brown.

2. Using a test-tube fuse carefully 1 part of the test substance with about 0.1 g of quickly ground sodium hydroxide R, avoiding carbonization; ammonia is evolved. Insert moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range. Dissolve the melt in 2.0 ml of water. Filter and divide the filtrate into two equal volumes:
 - a) Acidify 1 volume with 1.0 ml of nitric acid (~130 g/l)TS and add a few drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced, which dissolves in ammonia (~100 g/l)TS and reprecipitates upon addition of nitric acid (~130 g/l)TS.
 - b) To the second volume add, drop by drop, iodine TS until a pale yellow colour appears. Add a few drops of barium chloride (50 g/l)TS; a white, crystalline precipitate is produced.

LIDOCAINE OINTMENT

Description. The ointment usually contains 20-50 mg of lidocaine per g of a suitable ointment base.

Preparation of the sample

Withdraw and weigh an amount equivalent to 0.20 g of lidocaine and use it directly as the test substance. Divide the test substance into two equal parts.

IDENTITY TESTS

Colour and other reactions

1. Dissolve 1 part of the test substance in 2.0 ml of ethanol (~750 g/l)TS, add 2.0 ml of copper(II) sulfate (160 g/l)TS and 1.0 ml of sodium hydroxide (~200 g/l)TS, and mix; a strong blue colour is produced.
2. Dissolve 1 part of the test substance in 1.0 ml of ethanol (~750 g/l)TS, add 10 drops of cobalt(II) chloride (30 g/l)TS and shake; a bright green coloured solution with a precipitate is produced.

LITHIUM CARBONATE CAPSULES

Description. Each capsule usually contains 150-300 mg of lithium carbonate.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 0.30 g of lithium carbonate.
2. Empty the capsules, weigh out the above calculated equivalent amount to lithium carbonate and use it directly as the test substance. Divide the test substance into six equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 2 parts of the test substance add 1.0 ml of hydrochloric acid (~70 g/l)TS, shake and filter. Neutralize the filtrate with a few drops of sodium hydroxide (0.1 mol/l)VS, add 2.0 ml of disodium hydrogen phosphate (100 g/l)TS, and heat to boiling; a white precipitate is produced.
2. To 2 parts of the test substance add 1.0 ml of sulfuric acid (~100 g/l)TS; a gas evolves which is colourless and odourless.
3. To 1 part of the test substance add 5 ml of water, shake and filter. To the filtrate add 10 drops of magnesium sulfate (50 g/l)TS; a fine, white precipitate is slowly formed.
4. Moisten a small amount of the remaining test substance with a few drops of hydrochloric acid (~70 g/l)TS and introduce the mixture from a nichrome or platinum wire sealed to a glass rod into a nonluminous flame; a carmine-red colour is observed.

LITHIUM CARBONATE TABLETS

Description. Each tablet usually contains 250-300 mg of lithium carbonate.

Preparation of the sample

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

2. Grind the tablets, weigh out the above calculated equivalent amount to lithium carbonate as powdered material and use it directly as the test substance. Divide the test substance into six equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 2 parts of the test substance add 1.0 ml of hydrochloric acid (~70 g/l)TS, shake and filter. Neutralize the filtrate with a few drops of sodium hydroxide (0.1 mol/l)VS, add 2.0 ml of disodium hydrogen phosphate (100 g/l)TS, and heat to boiling; a white precipitate is produced.
2. To 2 parts of the test substance add 1.0 ml of sulfuric acid (~100 g/l)TS; a gas evolves which is colourless and odourless.
3. To 1 part of the test substance add 5 ml of water, shake and filter. To the filtrate add 10 drops of magnesium sulfate (50 g/l)TS; a fine, white precipitate is slowly formed.
4. Moisten a small amount of the remaining test substance with a few drops of hydrochloric acid (~70 g/l)TS and introduce the mixture from a nichrome or platinum wire sealed to a glass rod into a nonluminous flame; a carmine-red colour is observed.

NORETHISTERONE TABLETS

Description. Each tablet usually contains 5 mg of norethisterone.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.050 g of norethisterone.
2. Grind the tablets, weigh out the above calculated equivalent amount to norethisterone as powdered material and use it directly as the test substance. Divide the test substance into five equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of the test substance add about 2 ml of sulfuric acid (~1760 g/l)TS; a reddish brown solution is produced. Very cautiously dilute the solution with 10 ml of water; the colour changes to yellow and a brownish yellow precipitate is formed.
2. To 1 part of the test substance add about 1 ml of phosphoric acid (~1440 g/l)TS and heat cautiously; a yellow colour is produced which changes after a while to green and to cherry-red.
3. Transfer 3 parts of the test substance to a test-tube, add a mixture of 0.5 ml of potassium hydroxide/ethanol TS and 1.5 ml of ethanol (~750 g/l)TS, and heat in a water-bath for 5 minutes. Cool, add cautiously 1.0 ml of water, about 1 ml of sulfuric acid (~1760 g/l)TS, and boil gently for about 1 minute; no odour of ethyl acetate is perceptible.

PETHIDINE HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 50 mg of pethidine hydrochloride in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the contents of the ampoules equivalent to 0.50 g of pethidine hydrochloride, evaporate it to dryness on a water-bath, and use the residue as the test substance.
2. Pool the contents of the ampoules equivalent to 50 mg of pethidine hydrochloride and use it directly as the test solution.

IDENTITY TESTS

Colour and other reactions

1. Dissolve about 1 mg of the test substance in 1 ml of formaldehyde/sulfuric acid TS and heat cautiously; the colour of the solution turns pink changing to violet-red and showing a red fluorescence in translucent light.
2. Dissolve the remaining test substance in 5 ml of ethanol (~750 g/l)TS, add 5 ml of trinitrophenol/ethanol TS and shake; a yellow, crystalline precipitate is produced. Filter, wash with water, and dry the crystals at 105 °C for 2 hours; melting point, about 190 °C.
3. To the test solution add 5 ml of water, acidify with 1.0 ml of nitric acid (~130 g/l)TS and add a few drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced.

PYRAZINAMIDE TABLETS

Description. Each tablet usually contains 500 mg of pyrazinamide.

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 180 mg and 10 mg of pyrazinamide.
2. Grind the tablets, weigh out the above calculated equivalent amounts to pyrazinamide as powdered material and use them directly: 180 mg for test substance 1 and divide it into 3 equal parts; 10 mg for test substance 2.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of test substance 1 add 5 ml of sodium hydroxide (~80 g/l)TS and heat in a water-bath; vapours are evolved. Insert moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range, and an odour of ammonia is perceptible.
2. To 2 parts of test substance 1 add 5 ml of water, heat gently, and add 1.0 ml of ferrous sulfate (15 g/l)TS; an orange colour is produced. Add a few drops of sodium hydroxide (~80 g/l)TS; the colour changes to dark blue.
3. To test substance 2 add 1.0 ml of 4-dimethylaminobenzaldehyde TS and heat on a water-bath; a bright yellow colour is produced.

RIFAMPICIN CAPSULES

Description. Each capsule usually contains 150-450 mg of rifampicin.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 20 mg of rifampicin.
2. Empty the capsule, weigh out the above calculated equivalent amount to rifampicin, shake it with 10 ml of dehydrated ethanol R, filter, evaporate the filtrate to dryness on a water-bath and use the residue as the test substance.

IDENTITY TESTS

Colour and other reactions

1. To about 1 mg of the test substance add 3 ml of water, 3 drops of copper(II) sulfate (160 g/l)TS, shake, and heat to boiling; a violet colour is produced.
2. To about 1 mg of the test substance add about 2 ml of sulfuric acid (~1760 g/l)TS; an orange colour is produced. Heat on a water-bath for 2 minutes; the colour turns to dark red.
3. To 5 mg of the test substance add about 1 ml of pyridine R, 1.0 ml of sodium hydroxide (~80 g/l)TS, 2 drops of benzenesulfonyl chloride R, and shake well; a dark red-violet colour is observed.

SALBUTAMOL AEROSOL

Description. The aerosol spray contained in a pressurized canister usually contains a fine suspension of 1-2 mg of salbutamol in 1.0 ml of a suitable mixture of aerosol propellents.

Preparation of the sample

From the labelled amount calculate the volume of suspension necessary equivalent to 10 mg of salbutamol. Expell and pool it, shake with 8 ml of water, filter and use the filtrate as the test solution. Divide the test solution into three equal volumes.

IDENTITY TESTS

Colour and other reactions

1. To 2 volumes of the test solution add about 0.1 ml of ferric chloride (25 g/l)TS; a reddish violet colour develops. Add 10 mg of sodium hydrogen carbonate R; a fleshy precipitate is produced with an evolution of gas. Add 1-2 drops of sulfuric acid (~1760 g/l)TS; the solution becomes colourless.
2. To 1 volume of the test solution add 2-3 drops of sulfuric acid (~100 g/l)TS and 2-3 drops of potassium permanganate (10 g/l)TS; the colour of permanganate is discharged.

SODIUM NITROPRUSSIDE SOLUBLE POWDER

Description. Each vial contains a sterile powder usually equivalent to 50 mg of sodium nitroprusside.

Preparation of the sample

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

IDENTITY TESTS

Colour and other reactions

1. Dissolve 1 part of the test substance in 4 ml of water, add 4 drops of acetone R and 1.0 ml of sodium hydroxide (~80 g/l)TS; an orange colour is produced. Then add 4 ml of acetic acid (~300 g/l)TS; the colour turns to purple.
2. Dissolve 1 part of the test substance in 10 ml of water, add 1.0 ml of nitric acid (~130 g/l)TS and 1.0 ml of silver nitrate (40 g/l)TS; a light pink, flocculant precipitate is produced.
3. Introduce a small quantity of the test substance moistened with hydrochloric acid (~250 g/l)TS from a nichrome or platinum wire sealed to a glass rod into a nonluminous flame; a strong yellow colour is observed.

SODIUM VALPROATE TABLETS

Description. Each tablet usually contains 200 mg of sodium valproate. The tablets may be sugar-coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 0.40 g of sodium valproate.
2. Grind the tablets or cores, weigh out the above calculated equivalent amount to sodium valproate as powdered material, add 5 ml of water, stir well, filter, and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. Dip a magnesia stick or a nichrome or platinum wire sealed to a glass rod first into hydrochloric acid (~420 g/l)TS, then into the test solution and introduce it into a nonluminous flame; a bright yellow colour is observed.
2. Add about 0.5 ml of cobalt(II) chloride (30 g/l)TS to 1.0 ml of the test solution; a violet precipitate is produced which is soluble in carbon tetrachloride R.

SULFADIAZINE TABLETS

Description. Each tablet usually contains 300-500 mg of sulfadiazine.

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to 0.14 g and 15 mg of sulfadiazine.
2. Grind the tablets, weigh out the above calculated equivalent amounts to sulfadiazine as powdered material and use them directly: 0.14 g for test substance 1 and divide it into two equal parts; 15 mg for test substance 2.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of test substance 1 add 2.0 ml of sodium nitrite (10 g/l)TS, 1.0 ml of hydrochloric acid (~70 g/l)TS, shake and allow to stand for 1 minute. Using pH-indicator paper R add sufficient sodium hydroxide (~80 g/l)TS for the solution to become alkaline, and 5 mg of 2-naphthol R; a deep red colour is produced.
2. To test substance 2 add 5 ml of water, 1.0 ml of sodium hydroxide (~80 g/l)TS and 2 drops of copper(II) sulfate (160 g/l)TS. Heat to boiling; an olive-green precipitate is formed. Allow to stand; the colour of the precipitate turns to grey.
3. To 1 part of test substance 1 add 1.0 ml of water and 1.0 ml of 4-dimethylaminobenzaldehyde TS; a yellow-orange colour is produced.

TOLBUTAMIDE TABLETS

Description. Each tablet usually contains 500 mg of tolbutamide.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 1.5 g of tolbutamide.
2. Grind the tablets, weigh out the above calculated equivalent amount to tolbutamide as powdered material and use it directly as test substance 1. Divide test substance 1 into three equal parts.
3. Extract 2 parts of test substance 1 with five volumes, each of 4 ml, of chloroform R, filter and carefully evaporate to dryness on a water-bath. Use the residue as test substance 2.

IDENTITY TESTS

Melting point. Test substance 2 melts at about 128 °C.

Colour and other reactions

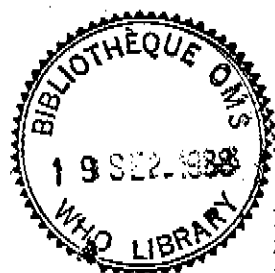
Fuse 1 part of test substance 1 with 0.5 g of sodium hydroxide R and 2 drops of water using a porcelain crucible; an odour of butylamine is perceptible. Insert moistured pH-indicator paper R into the vapours; its coloration is changed to an alkaline range.

4. REAGENTS

The preparation of the required reagents is described in "Basic tests for pharmaceutical substances." An additional reagent is listed below:

Dichloromethane R

* * *



BASIC TESTS FOR PHARMACEUTICAL DOSAGE FORMS

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1. INTRODUCTION

This consultative document is the third in a planned series on basic tests for verification of the identity of pharmaceutical substances in dosage forms. It contains identity tests for 49 drug substances drawn from the current revision of the WHO Model List of Essential Drugs. Other tests are currently being developed for other drugs. These will be included in the next selection that will be issued at a later date.

Each of the tests described has been verified in at least four laboratories in different countries. Further validation of the tests (see Annexes) on locally available samples is invited. These results or any other relevant comments should be forwarded to:

Pharmaceuticals Unit
World Health Organization
1211 Geneva 27, Switzerland

2. DRUG INDEX

aminocaproic acid injection	dopamine hydrochloride injection
aminocaproic acid tablets	ergometrine hydrogen maleate injection
ampicillin powder for oral suspension	ergometrine hydrogen maleate tablets
betamethasone tablets	erythromycin estolate capsules
betamethasone valerate ointment	erythromycin stearate tablets
bupivacaine hydrochloride injection	fluorouracil injection
caffeine tablets	glyceryl trinitrate tablets
cefalexin capsules	haloperidol solution
chloramphenicol sodium succinate powder for injection	imipramine hydrochloride tablets
chloroquine phosphate syrup	isosorbide dinitrate tablets
cimetidine tablets	meprobamate tablets
cloxacillin sodium capsules	metronidazole injection
dexamethasone sodium phosphate injection	nicotinic acid tablets
diazepam injection	nikethamide injection
digoxin injection	nitrazepam tablets
digoxin oral solution	oxytetracycline hydrochloride capsules
	penicillamine capsules
	phenoxymethylpenicillin potassium tablets
	phenylbutazone tablets

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phenytoin sodium tablets
procainamide hydrochloride
injection
propylthiouracil tablets
pyrantel embonate tablets
quinine sulfate tablets
salbutamol sulfate pessaries
salbutamol sulfate syrup

sodium fluoride tablets
spironolactone tablets
streptomycin sulfate injection
tetracycline hydrochloride ophthalmic
ointment
tetracycline hydrochloride tablets
thiopental sodium powder for
injection
verapamil hydrochloride tablets
vincristine sulfate powder for
injection

3. TEST PROCEDURES

AMINOCAPROIC ACID INJECTION

Description. The injection is a sterile solution usually containing 200-400 mg of aminocaproic acid in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the contents of the ampoules equivalent to 0.5 g of aminocaproic acid and use it directly as the test solution.
2. Place 3 strips of filter-paper into the test solution and allow the solution to ascend for about 4 cm. Take out the strips, cut away the lower dipped portion as well as the part that has not been wetted by the solution and dry the remaining part of the strips in air at room temperature (test-papers).

IDENTITY TESTS

Colour and other reactions

1. Place onto 1 test-paper 1 drop of copper(II) sulfate (160 g/l)TS; a blue green spot is produced.
2. Place onto 1 test-paper 1 drop of triketohydrindene/ethanol TS, and allow to react for a few minutes at room temperature; a purple spot is produced.
3. Place onto 1 test-paper 1 drop of ferric chloride (25 g/l)TS; an orange red spot is produced.

AMINOCAPROIC ACID TABLETS

Description. Each tablet usually contains 500 mg of aminocaproic acid.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.5 g of aminocaproic acid.
2. Grind the tablets, weigh out the above equivalent amount to aminocaproic acid as powdered material and suspend it in 5 ml of water. Place 3 strips of filter-paper into the suspension and allow the solution to ascend for about 4 cm. Take out the strips, cut away the lower dipped portion as well as the part that has not been wetted by the solution and dry the remaining part of the strips in air at room temperature (test-papers).

IDENTITY TESTS

Colour and other reactions

1. Place onto 1 test-paper 1 drop of copper(II) sulfate (160 g/l)TS; a blue green spot is produced.
2. Place onto 1 test-paper 1 drop of triketohydrindene/ethanol TS, and allow to react for a few minutes at room temperature; a purple spot is produced.
3. Place onto 1 test-paper 1 drop of ferric chloride (25 g/l)TS; an orange-red spot is produced.

AMPICILLIN POWDER FOR ORAL SUSPENSION

Description. The reconstituted suspension usually contains 25 mg of anhydrous ampicillin in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amounts equivalent to 10 mg and 0.10 g of anhydrous ampicillin.
2. Empty the vials, weigh out the above calculated equivalent amounts to anhydrous ampicillin and use them directly: 10 mg for test substance 1; 0.10 g for test substance 2.
3. Suspend test substance 2 in 5 ml of ethanol (~750 g/l)TS. Place 2 strips of filter-paper into the suspension and allow the solution to ascend for about 4 cm. Take out the strips, cut away the lower dipped portion as well as the part that has not been wetted by the solution and dry the remaining part of the strips in air at room temperature (test-papers).

IDENTITY TESTS

Colour and other reactions

1. Place onto 1 test-paper 1 drop of hydroxylamine hydrochloride (10 g/l)TS, followed by a small drop of sodium hydroxide (~80 g/l)TS and allow to react for 5 minutes. At the place of application of the reagents on the test-paper superimpose 1 drop of hydrochloric acid (~70 g/l)TS and 1 drop of ferric chloride (25 g/l)TS; a violet ring is produced.
2. Place onto 1 test-paper 1 drop of a solution composed of 1.0 ml of potassio-cupric tartrate TS and 6 ml of water; a faint violet spot is produced.
3. To test substance 1 add 2.0 ml of hydrochloric acid (~70 g/l)TS and boil the mixture for 2 minutes. Cool and add a few drops of triketohydrindene/ethanol TS; an intense orange-red colour is produced (the presence of colouring agents in certain formulations may interfere with the reaction to varying degrees).

BETAMETHASONE TABLETS

Description. Each tablet usually contains 250-600 µg of betamethasone.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 30 mg of betamethasone.
2. Grind the tablets, weigh out the above calculated amount to betamethasone as powdered material, shake it with 10 ml of chloroform R, filter, evaporate the filtrate to dryness on a water-bath, and use the residue as the test substance.

IDENTITY TESTS

Colour and other reactions

1. To 5 mg of the test substance add 1 drop of a mixture composed of 2 drops of formaldehyde TS in about 1 ml of sulfuric acid (~1760 g/l)TS; an orange colour is produced. Heat on a water-bath for 1 minute; the colour changes to brown.
2. Dissolve 5 mg of the test substance in 0.5 ml of methanol R, add 1.0 ml of hot potassio-cupric tartrate TS, and filter. Wash the filter with water; a red precipitate remains on the filter.
3. 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 15 mg of the test substance, shake well and heat again in a water-bath for 5 minutes; a violet-black colour is obtained and the solution no longer wets the sides of the tube.

BETAMETHASONE VALERATE OINTMENT

Description. The ointment contains betamethasone valerate usually equivalent to 1 mg of betamethasone in 1.0 g of a suitable ointment base.

Preparation of the sample

Withdraw an amount equivalent to 10 mg of betamethasone, add 30 ml of methanol R and 20 ml of cyclohexane R, and shake well. Separate the upper methanol layer and wash it with two 10-ml portions of cyclohexane R. Filter the methanol layer, evaporate the filtrate on a water-bath to a volume of 10 ml and use it as the test solution.

IDENTITY TESTS

Colour and other reactions

1. Evaporate 2-3 drops of the test solution from a porcelain dish to dryness on a water-bath and add 1 drop of a mixture composed of 2 drops of formaldehyde TS in about 1 ml of sulfuric acid (~1760 g/l)TS; an orange colour is produced. Heat on a water-bath for 1 minute; the colour changes to brown.

2. Add 0.5 ml of hot potassio-cupric tartrate TS to 2.0 ml of the test solution; the slightly yellowish colour of the initial test solution turns to blue. Heat the solution for 10-15 minutes; the colour of the solution changes to greenish and a reddish precipitate is produced.

3. 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. Cool and add carefully, drop by drop, 3 ml of the test solution; a green coloration appears. Shake well and heat again for 15 minutes; the colour of the solution turns to violet-black and it no longer wets the sides of the tube.

BUPIVACAINE HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 2.5-5.0 mg of bupivacaine hydrochloride in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 20 mg of bupivacaine hydrochloride and use it directly as the test solution. Divide the test solution into four equal volumes.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of the test solution add 1.0 ml of pyridine R, 0.5 ml of sodium hydroxide (~80 g/l)TS and 5 drops of benzenesulfonyl chloride R; a cherry red colour is produced.
2. Evaporate 1 part of the test solution to dryness on a water-bath. To the residue add a mixture of 1.0 ml of sulfuric acid (~1760 g/l)TS and 2 drops of formaldehyde TS, and warm on a water-bath for 1 minute; a reddish brown to red colour is produced.
3. To 1 part of the test solution add about 0.5 ml of sulfuric acid (~1760 g/l)TS, boil for 1 minute, cool and add 0.5 ml of sodium nitrite (10 g/l)TS. Allow to stand for 1 minute, then add 2.5 ml of sodium hydroxide (~80 g/l)TS and 1-2 drops of 2-naphthol TS; an orange to red colour is produced.
4. To 1 part of the test solution add 0.5 ml of nitric acid (~130 g/l)TS and 5 drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced, which is soluble in an excess of ammonia (~100 g/l)TS.

CAFFEINE TABLETS

Description. Each tablet usually contains 50-200 mg of anhydrous caffeine.

Preparation of the sample

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

2. Grind the tablets, weigh out the above calculated equivalent amount to anhydrous caffeine as powdered material, shake it twice with 10 ml of chloroform R and filter, evaporate the combined filtrate to dryness on a water-bath, and use the residue as the test substance.

IDENTITY TESTS

Melting point. The test substance melts at about 236 °C.

Colour and other reactions

1. Place 10 mg of the test substance in a porcelain dish, add 1 drop of hydrogen peroxide (~330 g/l)TS, 5 drops of hydrochloric acid (~250 g/l)TS, and evaporate to dryness on a water-bath. To the yellow-red coloured residue add 1 drop of ammonia (~100 g/l)TS and 1 drop of sodium hydroxide (~80 g/l)TS; a violet-red colour is produced.
2. Dissolve about 2 mg of the test substance in 1.0 ml of hot water and add a few drops of silver nitrate (40 g/l)TS; no turbidity is produced.
3. Shake 0.20 g of the test substance with 2.0 ml of ammonia (~100 g/l)TS; the test substance remains undissolved.

CEFALEXIN CAPSULES

Description. Each capsule usually contains 250-500 mg of cefalexin.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amounts equivalent to 0.10 g and 2.0 mg of cefalexin.
2. Empty the capsules, weigh out the above calculated equivalent amounts to cefalexin and use them directly: 2.0 mg as the test substance; for the test solution, shake 0.10 g with 10 ml of water, filter and use the filtrate.

IDENTITY TESTS

Colour and other reactions

1. To 2.0 ml of the test solution add 1 ml of water, 0.10 g of hydroxylamine hydrochloride R, 1.0 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 violet-red colour is produced which fades to light yellow within seconds.
2. To 2.0 ml of the test solution add 2.0 ml of a mixture composed of 2.0 ml of potassio-cupric tartrate TS and 6 ml of water; an olive-green colour is immediately produced which changes to yellow-brown after 30 seconds.
3. To 10 mg of paraformaldehyde R dissolved in 1.0 ml of sulfuric acid (~1760 g/l)TS add the test substance; a yellow colour is produced. Heat the mixture in a water-bath for 2 minutes, cool and dilute with 10 ml of water; the yellow colour of the solution remains.

CHLORAMPHENICOL SODIUM SUCCINATE POWDER FOR INJECTION

Description. Each vial contains a sterile powder of chloramphenicol sodium succinate usually equivalent to 1.0 g of chloramphenicol.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amount equivalent to 1.0 g of chloramphenicol.
2. Empty the vial, weigh out the above calculated equivalent amount to chloramphenicol, dissolve it in 5 ml of water and use it directly as the test solution.

IDENTITY TESTS

Colour and other reactions

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. Following this 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 1 ml of sodium hydroxide (~400 g/l)TS; a yellow-green fluorescence appears, which disappears on the addition of 1.0 ml of hydrochloric acid (~250 g/l)TS.
4. Introduce the test solution from a nicrome or platinum wire sealed to a glass rod into a nonluminous flame; a strong yellow colour is observed.

CHLOROQUINE PHOSPHATE SYRUP

Description. The syrup contains chloroquine phosphate usually equivalent to 10 mg of chloroquine in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the well-homogenized contents of the containers equivalent to 50 mg of chloroquine, and use it directly as the test solution.
2. Transfer the test solution to a separating funnel, add 25 ml of water and 2.0 ml of ammonia (~100 g/l)TS. Extract twice with 25 ml volumes of chloroform R. Separate the chloroform layers, evaporate to reduce the volume to about 5 ml and use it as test solution 1, dividing it into two equal volumes. Filter the aqueous layer through a filter-paper and use the filtrate as test solution 2.

IDENTITY TESTS

Colour and other reactions

1. To 1 volume of test solution 1 add 2.0 ml of hydrochloric acid (~70 g/l)TS and 5 drops of potassio-mercuric iodide TS; a white or light yellow precipitate is produced.
2. Evaporate 1 volume of test solution to dryness, use the residue with 1 pellet of potassium hydroxide R. Dissolve the fused mass in 2.0 ml of water, filter, acidify the filtrate with 1.0 ml of nitric acid (~130 g/l)TS and add 5 drops of silver nitrate (40 g/l)TS; an off-white curdy precipitate is produced. Add an excess of ammonia (~100 g/l)TS; the precipitate dissolves.
3. To 2.0 ml of test solution 2 add 1.0 ml of silver nitrate (40 g/l)TS; a yellow precipitate is produced. To a portion of the precipitate add a few drops of nitric acid (~130 g/l)TS; a clear solution is obtained. To another portion of the precipitate add a few drops of ammonia (~100 g/l)TS and shake; the precipitate dissolves.
4. To 2.0 ml of test solution 2 add 1.0 ml of nitric acid (~130 g/l)TS and 1.0 ml of ammonium molybdate (95 g/l)TS; a yellow precipitate is obtained.

CIMETIDINE TABLETS

Description. Each tablet usually contains 200 mg of cimetidine.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 60 mg of cimetidine.
2. Grind the tablets, weigh out the above calculated equivalent amount to cimetidine as powdered material and use it directly as the test substance. Divide the test substance into three equal parts.

IDENTITY TESTS

Colour and other reactions

1. Ignite a small quantity of the test substance; the vapours evolved darken lead nitrate paper R.
2. To 1 part of the test substance add 10 ml of water, shake well and filter. To the filtrate add 1 drop of ammonia (~100 g/l)TS and 1 drop of copper(II) sulfate (160 g/l)TS; a green precipitate is produced which turns greyish on shaking and is soluble in an excess of ammonia (~100 g/l)TS.
3. To 1 part of the test substance add 10 ml of water, 2 ml of diazobenzene-sulfonic acid TS and 2-3 drops of sodium hydroxide (~80 g/l)TS; a yellowish orange colour is produced.

CLOXACILLIN SODIUM CAPSULES

Description. Each capsule contains cloxacillin sodium usually equivalent to 500 mg of cloxacillin.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 25 mg of cloxacillin.
2. Empty the capsules, weigh out the above calculated equivalent amount to cloxacillin and use it directly as the test substance. Divide the test substance into five equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of the test substance add 3 ml of water, shake and filter. To the filtrate add 0.10 g of hydroxylamine hydrochloride R, 1.0 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 violet-red colour is produced.
2. To 2 parts of the test substance add 5 ml of ethanol (~750 g/l)TS, shake and filter. Evaporate the filtrate using a stream of air at room temperature. Dissolve the residue in 2.0 ml of water and add 2.0 ml of a mixture composed of 2.0 ml of potassium-cupric tartrate TS and 6 ml of water; a light blue solution is immediately produced.
3. To 1 part of the test substance add 1.0 ml of water, shake and filter. To the filtrate add 1 drop of ferric chloride (25 g/l)TS; a yellow-greenish precipitate is produced.
4. To 10 mg of paraformaldehyde R dissolved in about 1 ml of sulfuric acid (~1760 g/l)TS add a trace 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. Moisten a small amount of the test substance with a few drops of hydrochloric acid (~250 g/l)TS and introduce it from a nichrome or platinum wire sealed to a glass rod into a nonluminous flame; a bright yellow colour appears in the flame.

DEXAMETHASONE SODIUM PHOSPHATE INJECTION

Description. The injection is a sterile solution of dexamethasone sodium phosphate usually containing the equivalent of 4.0 mg of dexamethasone in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 50 mg of dexamethasone, evaporate to dryness on a water-bath, and use the white to pale yellow, waxy residue as the test substance.

IDENTITY TESTS

Colour and other reactions

1. Dissolve 2.0 mg of the test substance in about 2 ml of sulfuric acid (~1760 g/l)TS, and allow to stand for a few minutes; a yellow or pale

orange coloured solution is produced. Pour the solution into 10 ml of water; the colour of the solution fades and a yellow flocculent precipitate may occur.

2. Place about 0.5 ml of chromic acid TS in a small test-tube and heat in a water-bath for 5 minutes; the solution wets the sides of the tube but there is no greasiness. Add about 3 mg of the test substance and again heat in a water-bath for 5 minutes; the solution no longer wets the sides of the tube and does not pour easily from the tube.

3. Heat carefully 0.04 g with about 2 ml of sulfuric acid (~1760 g/l)TS until white fumes are evolved; add nitric acid (~1000 g/l)TS drop by drop until oxidation is complete, and cool. Add 2.0 ml of water, heat until white fumes are again evolved, cool, add 10 ml of water, and neutralize with ammonia (~100 g/l)TS using pH-indicator paper R. Use this solution for reactions (a) and (b).

(a) Introduce the solution into a nonluminous flame using a magnesia stick, or a nichrome or platinum wire sealed to a glass rod; the flame acquires a bright yellow colour.

(b) 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 yellow-brown precipitate is produced which is readily soluble in ammonia (~100 g/l)TS (about 15 ml).

DIAZEPAM INJECTION

Description. The injection is a sterile solution usually containing 5.0 mg of diazepam in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 60 mg of diazepam and use it directly as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To one fifth of the volume of the test solution add about 1 ml of hydrochloric acid (~250 g/l)TS and heat on a water-bath for 30 minutes; a yellow solution is produced. Cool and dilute with about 10 ml of ice-water; a yellow, crystalline precipitate is formed.

2. To the remaining test solution add 5 ml of sodium carbonate (50 g/l)TS, 10 ml of water, and shake with 10 ml of chloroform R. Separate the chloroform-layer, and reduce it by evaporation on a water-bath to about 2 ml. Add 10 mg of triketohydrindene hydrate R and 5 drops of ethanol (~750 g/l)TS. Heat on a water-bath for 2 minutes and add 3.0 ml of ethanol (~750 g/l)TS; a blue colour is produced. To this solution add 2 drops of a mixture composed of 2 drops of copper(II) sulfate (160 g/l)TS, and 3.0 ml of water; an orange red colour is produced.

DIGOXIN INJECTION

Description. The injection is a sterile solution usually containing 250 µg of digoxin in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 1.5 mg of digoxin and use it directly as the test solution. Divide the test solution into three equal volumes.

IDENTITY TESTS

Colour and other reactions

1. Evaporate 2 volumes of the test solution to dryness on a water-bath. To the residue add 2.0 ml of a solution prepared by mixing 0.5 ml of ferric chloride (25 g/l)TS with 100 ml of glacial acetic acid R, and shake. Superimpose this solution onto 1 ml of sulfuric acid (~1760 g/l)TS; a brown ring, but no red colour is produced at the junction of the two liquids and after some time the acetic acid-layer acquires a bluish green colour.
2. To 1 volume of the test solution add 5 ml of ethanol (~750 g/l)TS and 3 ml of alkaline trinitrophenol TS; a yellow colour is produced, which darkens with time.

DIGOXIN ORAL SOLUTION

Description. The solution usually contains 50 µg of digoxin in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the containers equivalent to 0.25 mg of digoxin and use it directly as the test solution. Divide the test solution into two equal volumes.

IDENTITY TESTS

Colour and other reactions

1. To 1 volume of the test solution add 2.0 ml of a solution prepared by mixing 0.5 ml of ferric chloride (25 g/l)TS with 100 ml of glacial acetic acid R, and shake. Superimpose this solution onto 1 ml of sulfuric acid (~1760 g/l)TS; a brown ring, but no red colour is produced at the junction of the two liquids.
2. To 1 volume of the test solution add 5 ml of ethanol (~750 g/l)TS and 3 ml of alkaline trinitrophenol TS; a yellow colour is produced, which darkens with time.

DOPAMINE HYDROCHLORIDE INJECTION

Description. The injection is a sterile solution usually containing 40 mg of dopamine hydrochloride in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the contents of the ampoules equivalent to 100 mg of dopamine hydrochloride and use it directly as the test solution 1. Divide it into two equal volumes.

2. Dilute, if necessary, 1 volume of test solution 1 to 5 ml with water for test solution 2.
3. Evaporate 1.0 ml of test solution 2 to dryness on a water-bath and use the residue as the test substance. Divide the test substance into two equal parts.

IDENTITY TESTS

Colour and other reactions

1. Dilute 0.5 ml of test solution 2 with water to 2.0 ml and add 5 drops of ferric chloride (25 g/l)TS; a green colour is produced.
2. Add 1 part of the test substance to 1 ml of formaldehyde/sulfuric acid TS; an intense violet colour is produced immediately.
3. Dissolve 1 part of the test substance in 2.0 ml of sodium hydroxide (~80 g/l)TS; the solution turns orange. Heat to boiling; vapours are evolved. Insert moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range and the colour of the solution turns to dark brown.
4. Dilute 0.5 ml of test solution 2 with water to 2.0 ml, add 2 drops of nitric acid (~130 g/l)TS and 0.5 ml of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced.
5. To 1 volume of test solution 1 add 10 ml of a saturated solution of trinitrophenol R in water and mix. Filter the precipitate, wash first with water, then with a small quantity of ethanol (~750 g/l)TS, and dry at 105 °C; melting temperature, about 202 °C with decomposition.

ERGOMETRINE HYDROGEN MALEATE INJECTION

Description. The injection is a sterile solution usually containing 200 µg of ergometrine hydrogen maleate in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 1.0 mg of ergometrine hydrogen maleate and use it directly as the test solution. Divide the test solution into five equal volumes.

IDENTITY TESTS

Colour and other reactions

1. The test solution shows a blue fluorescence in ultraviolet light (365 nm).
2. To 1 volume of the test solution add slowly 2.0 ml of 4-dimethylamino-benzaldehyde TS; a blue colour is slowly produced.
3. To 2 volumes of the test solution add 3 ml of tartaric acid (10 g/l)TS, 5 drops of ammonia (~100 g/l)TS and extract three times with 5 ml of chloroform R. Evaporate the combined chloroform layers to dryness using a stream of air. Dissolve the residue in 5 ml of tartaric acid (10 g/l)TS and add 2 drops of potassium-mercuric iodide TS; no turbidity is produced (distinction from ergotamine tartrate).

ERGOMETRINE HYDROGEN MALEATE TABLETS

Description. Each tablet usually contains 200 µg of ergometrine hydrogen maleate. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 2.0 mg of ergometrine hydrogen maleate.
2. Grind the tablets or cores, weigh out the above calculated equivalent amount to ergometrine hydrogen maleate as powdered material and use it directly as the test substance. Divide the test substance into two equal parts.
3. Shake 1 part of the test substance with 5 ml of water, filter and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. The test solution shows a blue fluorescence in ultraviolet light (365 nm).
2. To 1.0 mg of the test solution add slowly 2.0 ml of 4-dimethylamino-benzaldehyde TS; a blue colour is slowly produced.
3. To 1 part of the test substance add 10 ml of tartaric acid (10 g/l)TS, shake for 5 minutes and filter. To 5 ml of the clear filtrate add 5 drops of ammonia (~100 g/l)TS and extract three times with 5 ml of chloroform R. Evaporate the combined chloroform layers to dryness using a stream of air. Dissolve the residue in 5 ml of tartaric acid (10 g/l)TS and add 2 drops of potassio-mercuric iodide TS; no turbidity is produced (distinction from ergotamine tartrate).

ERYTHROMYCIN ESTOLATE CAPSULES

Description. Each capsule contains erythromycin estolate usually equivalent to 125-250 mg of erythromycin.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 25 mg of erythromycin.
2. Empty the capsules, weigh out the above calculated equivalent amount to erythromycin and use it directly as the test substance. Divide the test substance into five equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 2 parts of the test substance add 2 drops of water and cautiously add 2 ml of sulfuric acid (~1760 g/l)TS; a dark red-brown colour is produced, which on dilution with water gives a dark greenish solution.

2. To 2 parts of the test substance add 2.0 ml of acetone R, 2 ml of hydrochloric acid (~250 g/l)TS and heat gently to boiling; a pale orange colour is produced that changes immediately to purple or deep violet. Add 2.0 ml of chloroform R and shake; the chloroform layer acquires a bluish green colour.

3. To 1 part of the test substance add 1.0 ml of ethanol (~750 g/l)TS and add 0.5 ml of potassium permanganate (10 g/l)TS; the purple colour is discharged leaving a brownish precipitate.

ERYTHROMYCIN STEARATE TABLETS

Description. Each tablet contains erythromycin stearate usually equivalent to 250 mg of erythromycin. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping or by dissolving in acetone R and drying the core in the air. Weigh 1 tablet or core and calculate the amounts equivalent to 20 mg and 0.10 g of erythromycin.
2. Grind the tablets or cores, weigh out the above calculated equivalent amounts to erythromycin and use them directly: 20 mg for test substance 1 and divide it into two equal parts; 0.10 g for test substance 2, shake it with 10 ml of chloroform R, filter, evaporate the filtrate to dryness on a water-bath, and use the residue.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of test substance 1 add 2 drops of water and cautiously add 2 ml of sulfuric acid (~1760 g/l)TS; a dark violet-brown colour is produced, which on dilution with water gives a brownish solution.
2. To 1 part of test substance 1 add 2.0 ml of acetone R, 2 ml of hydrochloric acid (~250 g/l)TS and shake; a pale orange colour is produced that changes immediately to red or red-purple. Add 2.0 ml of chloroform R and shake; the chloroform layer acquires a purple colour.
3. Heat gently test substance 2 with 10 ml of water and 5 ml of hydrochloric acid (~70 g/l)TS until the solution boils; oily globules rise to the surface. Cool, remove the fatty layer and heat it with 3.0 ml of sodium hydroxide (0.1 mol/l)VS. Allow to cool; the solution sets to a gel. Add 10 ml of hot water, shake and heat the mixture for 2-3 minutes; on shaking the solution froths. To 1.0 ml of the resulting solution add 1.0 ml of calcium chloride (100 g/l)TS; a granular precipitate is produced, which is insoluble in hydrochloric acid.

FLUOROURACIL INJECTION

Description. The injection is a sterile solution usually containing 50 mg of fluorouracil in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 250 mg of fluorouracil, add a few drops of hydrochloric acid (~70 g/l)TS until slightly acid to pH-indicator paper R. Filter, wash the precipitate with small amounts of water, dry it at 105 °C and use it as the test substance.

IDENTITY TESTS

Melting behaviour. The test substance melts at about 283 °C.

Colour and other reactions

1. To 0.05 g of the test substance add 5 ml of water and about 1 ml of bromine TS and shake; the colour is discharged immediately.
2. Transfer 0.5 ml of chromic acid TS to a small test-tube and heat in a water-bath for 5 minutes; the solution wets the sides of the tube, but there is no greasiness. Add 2-3 mg of the substance and again heat in a water-bath for 5 minutes; the solution does not wet the sides of the tube and does not pour easily from the tube.
3. Fuse 0.05 g of the test substance with 0.05 g of potassium carbonate R using a porcelain crucible. Heat the mixture until a white residue is obtained. Cool the melt to room temperature, carefully dissolve it in 2.0 ml of water and neutralize with hydrochloric acid (~70 g/l)TS (about 5-7 drops). In a separate test-tube mix 10 ml of water with 1 drop of ferric chloride (25 g/l)TS, 2 drops of hydrochloric acid (~70 g/l)TS and 2 drops of potassium thiocyanate (50 g/l)TS. Add 1.0 ml of this solution to the test solution above; the colour is immediately discharged.

GLYCERYL TRINITRATE TABLETS

Description. Each tablet usually contains 500 µg of glyceryl trinitrate.

Preparation of the sample

1. Weigh 1 tablet and calculate the amounts equivalent to about 1 mg and 5 mg of glyceryl trinitrate.
2. Grind the tablets, weigh out the above calculated equivalent amounts to glyceryl trinitrate as powdered material and use them directly: shake about 1 mg with 10 ml of dehydrated ethanol R, filter, evaporate the filtrate to dryness using a stream of air, and use the residue as test substance 1, dividing it into two equal parts; 5 mg for test substance 2.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of test substance 1 add 5 ml of water and a few drops of sulfuric acid (~100 g/l)TS. Then add 0.10 g of potassium iodide R, a few drops of starch TS and shake; no blue colour is observed. Add 1.0 ml of sodium hydroxide (~80 g/l)TS and heat gently to boiling. Cool and add 3 ml of sulfuric acid (~100 g/l)TS; a dark blue colour is immediately produced.

2. To 1 part of test substance 1 add 3-4 drops of sodium hydroxide (~80 g/l)TS, 3 ml of ferrous sulfate (15 g/l)TS and shake; a greenish brown precipitate is produced.
3. Shake test substance 2 with 3 ml of ethanol (~750 g/l)TS and filter. To the filtrate add carefully 1 ml of diphenylamine/sulfuric acid TS in a manner to form a lower layer; a dark blue colour is produced at the interface of the two layers.

HALOPERIDOL SOLUTION

Description. The solution usually contains 2.0 mg of haloperidol in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the containers equivalent to 10 mg of haloperidol in a platinum crucible, add 20 mg of anhydrous sodium carbonate R, and evaporate to dryness on a water-bath. Heat until a white residue is obtained, dissolve it in 2.0 ml of water warming gently on a water-bath. Cool, neutralize with hydrochloric acid (~70 g/l)TS, and use it as the test solution.

IDENTITY TESTS

Colour and other reactions

In a test-tube mix 1 drop of ferric chloride (25 g/l)TS with 1 drop of ammonium thiocyanate (75 g/l)TS, dilute with 10 ml of water and acidify with 1 drop of hydrochloric acid (~70 g/l)TS. To 1.0 ml of this solution add, drop by drop, the test solution; the red colour is discharged.

IMIPRAMINE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 10-25 mg of imipramine hydrochloride. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amounts equivalent to 5 mg and 100 mg of imipramine hydrochloride.
2. Grind the tablets or cores, weigh out the above calculated equivalent amounts to imipramine hydrochloride as powdered material and use them directly: two portions of 5 mg for test substance 1; 100 mg for test substance 2, dividing it into four equal parts.
3. Suspend 2 parts of test substance 2 in 10 ml of water, place 2 strips of filter-paper into the suspension and allow the solution to ascend for about 4 cm. Take out the strips, cut away the lower dipped portion as well as the part that has not been wetted by the solution and dry the remaining part of the strips in air at room temperature (test-papers).

IDENTITY TESTS

Colour and other reactions

1. Shake 1 portion of test substance 1 with 2.0 ml of water and filter. To the filtrate add about 0.5 ml of nitric acid (~1000 g/l)TS; an intense blue colour is produced, which turns yellow on standing.

Alternate test by filter-paper technique: Place onto 1 test-paper 1 drop of nitric acid (~1000 g/l)TS; an intense blue spot is produced.

2. To 1 portion of test substance 1 add 5 ml of hydrochloric acid (~70 g/l)TS, shake and filter. To the filtrate add 1.0 ml of sodium nitrite (10 g/l)TS; the solution acquires first a green-blue colour which after a few seconds turns green and yellow within 2 minutes.

Alternate test by filter-paper techniques: Place onto 1 test-paper 1 drop of hydrochloric acid (~70 g/l)TS, followed by 1 drop of sodium nitrite (10 g/l)TS applied at the same place; a green-blue spot is produced which after a few seconds turns green and yellow within 2 minutes.

3. To 1 part of test substance 2 add 2.0 ml of water, shake and filter. To the filtrate add a few drops of mercuric chloride (65 g/l)TS; a white turbidity is produced.

4. To 1 part of test substance 2 add 2.0 ml of water, shake and filter. To the filtrate add a few drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced.

ISOSORBIDE DINITRATE TABLETS

Description. Each tablet usually contains 5 mg of isosorbide dinitrate.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 60 mg of isosorbide dinitrate.
2. Grind the tablets, weigh out the above calculated equivalent amount to isosorbide dinitrate as powdered material, and use it directly as the test substance.

IDENTITY TESTS

Colour and other reactions

1. To the test substance add 6 ml of acetone R, shake and filter. Evaporate the filtrate to dryness on a water-bath and dissolve the residue in 8 ml of water. Keep half of this solution for test 2. To the remaining solution add 1.0 ml of sodium hydroxide (~80 g/l)TS, 0.10 g of zinc R powder and heat on a water-bath for 5 minutes. Cool, filter and to 2.0 ml of the filtrate add 1.0 mg of hydrochloric acid (~70 g/l)TS, 5 drops of sulfanilic acid TS and allow to stand for 5 minutes. Following this add 1.0 ml of sodium hydroxide (~80 g/l)TS and 3 drops of 2-naphthol TS; an orange colour is developed.

2. To the solution kept in test 1 add about 0.5 ml of sulfuric acid (~1760 g/l) and a few crystals of ferrous sulfate R. Cautiously introduce about 2 ml of sulfuric acid (~1760 g/l)TS to form a lower layer; a brown colour is produced at the interface of the two liquids.

MEPROBAMATE TABLETS

Description. Each tablet usually contains 200-600 mg of meprobamate.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.8 g of meprobamate.
2. Grind the tablets, weigh out the above calculated equivalent amount to meprobamate as powdered material, shake it with 40 ml of ethanol (~750 g/l)TS, filter, wash the filter with 20 ml of ethanol (~750 g/l)TS, evaporate the combined filtrate cautiously on a water-bath, dry at 80 °C, and use the residue as the test substance.

IDENTITY TESTS

Melting point. The test substance melts at about 105 °C.

Colour and other reactions

1. Dissolve 20 mg of the test substance in 2.0 ml of 4-dimethylaminobenzaldehyde TS; a light yellow colour is produced. Heat in a water-bath for 2-5 minutes; the solution turns to a red colour. Cool and add, drop by drop, 5 ml of ice-water; the colour of the solution changes first to dark red and then to violet-grey.
2. Dissolve 0.10 g of the test substance in 3 ml of sodium hydroxide (~80 g/l)TS and heat in a water-bath for 5 minutes. Insert moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range.

METRONIDAZOLE INJECTION

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

Preparation of the sample

Pool the contents of the ampoules equivalent to 10 mg of metronidazole and use it directly as the test solution. Divide the test solution into two equal volumes.

IDENTITY TESTS

Colour and other reactions

1. To 1 volume of the test solution add 0.05 g of 4-dimethylaminobenzaldehyde R dissolved in 2.0 ml of hydrochloric acid (~70 g/l)TS; the solution is almost colourless. Add 0.05 g of zinc R powder; an orange colour is produced.
2. Boil 1 volume of the test solution 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, yellow.

NICOTINIC ACID TABLETS

Description. Each tablet usually contains 20-500 mg of nicotinic acid.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.20 g of nicotinic acid.
2. Grind the tablets, weigh out the above calculated equivalent amount to nicotinic acid as powdered material and use it directly as the test substance. Divide the test substance into four equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 2.0 ml of water add 2 drops of sodium hydroxide (~80 g/l)TS, 1 drop of phenolphthalein/ethanol TS and small amounts of the test substance until the solution becomes colourless, and filter. To the filtrate add 1 drop of copper(II) sulfate (160 g/l)TS; a light blue precipitate is produced.
2. To 2 parts of the test substance add 10 ml of carbon-dioxide-free water R, shake and filter. Insert a strip of pH-indicator paper R into the filtrate; its coloration indicates a pH of about 3.
3. Heat 1 part of the test substance with 1.0 g of anhydrous sodium carbonate R; pyridine, perceptible by its odour, is produced.

NIKETHAMIDE INJECTION

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

Preparation of the sample

Pool the contents of the ampoules equivalent to 0.5 g of nikethamide and use it directly as the test solution. Divide the test solution into two equal volumes.

IDENTITY TESTS

Colour and other reactions

1. Boil 2 drops of the test solution with 2-3 ml of sodium hydroxide (~80 g/l)TS; diethylamine, perceptible by its odour, is produced. Insert moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range.
2. Mix 2 drops of the test solution with 0.5 ml of water, add 1 drop of copper(II) sulfate (160 g/l)TS and 2 drops of ammonium thiocyanate (75 g/l)TS; a green precipitate is produced.
3. Heat 5 drops of the test solution with 1.0 g of anhydrous sodium carbonate R; pyridine, perceptible by its odour, is produced.
4. To 1 volume of the test solution add 2.0 ml of alkaline potassium-mercuric iodide TS; a yellowish white, voluminous precipitate is obtained.

NITRAZEPAM TABLETS

Description. Each tablet usually contains 5-10 mg of nitrazepam.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 10 mg of nitrazepam.
2. Grind the tablets, weigh out the above calculated equivalent amount to nitrazepam as powdered material and use it directly as the test substance. Divide the test substance into two equal parts.

IDENTITY TESTS

Colour and other reactions

1. Mix 1 part of the test substance with 15 ml of hydrochloric acid (~70 g/l)TS, heat on a water-bath for 15 minutes, cool and filter. To the filtrate add 0.20 ml of sodium nitrite (10 g/l)TS, allow to stand for 3 minutes and add 0.10 ml of sulfamic acid (100 g/l)TS. Allow to stand once more for 3 minutes, then add 0.20 ml of 2-naphthol TS; an orange-red colour is produced.
2. To 1 part of the test substance add 1.0 ml of methanol R and about 0.1 ml of sodium hydroxide (~80 g/l)TS; a bright yellow colour is produced.

OXYTETRACYCLINE HYDROCHLORIDE CAPSULES

Description. Each capsule usually contains 250 mg of oxytetracycline hydrochloride.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 0.10 g of oxytetracycline hydrochloride.
2. Empty the capsules, weigh out the above calculated equivalent amount to oxytetracycline hydrochloride, shake it with 10 ml of water, filter, and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. Add 2 drops of the test solution to about 2 ml of sulfuric acid (~1760 g/l)TS; a red-violet colour is produced which remains for more than 2 minutes. Allow to stand for 5 minutes then add cautiously 2.0 ml of water; a yellow colour is produced.
2. Warm 2.0 ml of zinc chloride (500 g/l)TS in a porcelain dish 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 grey-green to violet-brown colour is produced.
3. To 1.0 ml of the test solution add 5 drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced.

PENICILLAMINE CAPSULES

Description. Each capsule usually contains 250 mg of penicillamine.

Preparation of the sample

1. Weigh the contents of 1 capsule and calculate the amount equivalent to 40 mg of penicillamine.
2. Empty the capsules, weigh out the above calculated equivalent amount to penicillamine, and use it directly as the test substance. Divide the test substance into four equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 2 parts of the test substance add 10 ml of water, 5 drops of sodium hydroxide (~80 g/l)TS, 20 mg of triketohydrindene hydrate R, and shake; a dark violet-red colour is produced.
2. To 1 part of the test substance add 5 ml of water and 0.5 ml of ferric chloride (25 g/l)TS and shake; an intense blue colour is produced, which fades quickly and becomes colourless.
3. To 1 part of the test substance add 5 ml of water, 5 drops of copper(II) sulfate (160 g/l)TS and shake; a dark brown to black colour is produced. Add a few additional drops of copper(II) sulfate (160 g/l)TS and allow to stand for not less than 10 minutes; the colour of the solution turns to dark green.

PHENOXYMETHYLPENICILLIN POTASSIUM TABLETS

Description. Each tablet contains phenoxymethylpenicillin potassium usually equivalent to 250 mg of phenoxymethylpenicillin.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 30 mg of phenoxymethylpenicillin.
2. Grind the tablets, weigh out two portions of the above calculated equivalent amount to phenoxymethylpenicillin as powdered material and use them directly as the test substance. Divide 1 portion of the test substance into four equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of the test substance add 3 ml of water, shake and filter. To the filtrate add 0.10 g of hydroxylamine hydrochloride R, about 0.5 ml of sodium hydroxide (~80 g/l)TS, shake 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 violet-red colour is produced.

2. To 1 part of the test substance add a few drops of ethanol (~750 g/l)TS, 1.0 ml of water, shake and filter. To the filtrate add 1-2 drops of ferric chloride (25 g/l)TS; a light yellow precipitate is produced.
3. To 10 mg of paraformaldehyde R dissolved in about 1 ml of sulfuric acid (~1760 g/l)TS add a small amount of the test substance; a cherry-red colour is produced. Heat the solution in a water-bath for 2 minutes; the colour of the solution changes to dark red.
4. To 1 portion of the test substance add 2.0 ml of water, 2-3 drops of glacial acetic acid R, shake and filter. To the filtrate add 1.0 ml of sodium cobaltinitrite (100 g/l)TS; an orange-yellow precipitate is produced.

PHENYLBUTAZONE TABLETS

Description. Each tablet usually contains 100-200 mg of phenylbutazone. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 0.5 g of phenylbutazone.
2. Grind the tablets or cores, weigh out the above calculated equivalent amount to phenylbutazone as powdered material, shake it with 10 ml of acetone R, filter, evaporate the filtrate to dryness on a water-bath, and use the residue as the test substance.

IDENTITY TESTS

Melting point. The test substance melts at about 106 °C.

Colour and other reactions

1. To 0.3 g of the test substance add 1 ml of glacial acetic acid R and 2 ml of hydrochloric acid (~250 g/l)TS. Heat under reflux for 30 minutes (a conical flask with a small filter funnel may be used), and filter. To the filtrate add 10 ml of sodium nitrite (10 g/l)TS; a yellow colour is produced. To 1.0 ml of this solution add a solution of 10 mg of 2-naphthol R in 5 ml of sodium carbonate (50 g/l)TS; a brownish red precipitate is formed. Add 4-5 ml of ethanol (~750 g/l)TS; the precipitate dissolves partially yielding a red solution.
2. Dissolve 0.05 g of the test substance in 5 ml of sodium hydroxide (~150 g/l)TS and add 3 drops of hydrogen peroxide (~330 g/l)TS; a pale yellow colour is produced.

PHENYTOIN SODIUM TABLETS

Description. Each tablet usually contains 25-100 mg of phenytoin sodium. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 0.08 g of phenytoin sodium.

2. Grind the tablets or cores, weigh out the above calculated equivalent amount to phenytoin sodium as powdered material and use it directly as the test substance. Divide the test substance into two equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of the test substance add 4 ml of chloroform R, 0.1 ml of cobalt(II) chloride (30 g/l)TS and shake; a blue-violet colour with a voluminous precipitate is produced (distinction from phenytoin).
2. To 1 part of the test substance add 2.0 ml of ammonia (~100 g/l)TS and heat until boiling begins. Add 1 drop of copper(II) sulfate (160 g/l)TS and shake; a blue-violet solution with a blue-green precipitate is produced. Allow to stand for 3 minutes, filter and wash with water; pink needles remain on the filter.

PROCAINAMIDE HYDROCHLORIDE INJECTION

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

Preparation of the sample

Pool the contents of the ampoules equivalent to 100 mg of procainamide hydrochloride, and use it directly as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To about 0.1 ml of the test solution add 1.0 ml of water, 5 drops of hydrochloric acid (~70 g/l)TS, 10 drops of sodium nitrite (10 g/l)TS, 1.0 ml of sodium hydroxide (~80 g/l)TS and 5 mg of 2-naphthol R; an orange-red coloured solution and a red precipitate are produced.
2. Dilute about 0.7 ml of the test solution to 1.0 ml of water, add 1.0 ml of potassium ferrocyanide (45 g/l)TS, 10 drops of hydrochloric acid (~70 g/l)TS and heat to boiling; a dark green precipitate is produced.
3. To about 0.1 ml of the test solution add 2.0 ml of water, and a few drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced which is insoluble in nitric acid (~130 g/l)TS, but soluble in an excess of ammonia (~100 g/l)TS.

PROPYLTHIOURACIL TABLETS

Description. Each tablet usually contains 50 mg of propylthiouracil.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 30 mg of propylthiouracil.

2. Grind the tablets, weigh out two portions of the above calculated equivalent amount to propylthiouracil as powdered material and use them directly as the test substance. Divide 1 portion of the test substance into three equal parts.

IDENTITY TESTS

Colour and other reactions

1. Shake 2 parts of the test substance with 2.0 ml of ammonia (~100 g/l)TS and filter. To the filtrate add 1.0 ml of silver nitrate (40 g/l)TS; a greyish white gel is produced.
2. Shake 1 part of the test substance with 2.0 ml of water and filter. To the filtrate add 3 drops of copper(II) sulfate (160 g/l)TS; a green solution is produced and a white to greyish precipitate is formed.
3. To 1 portion of the test substance add 10 ml of ethanol (~750 g/l)TS, filter and evaporate the filtrate to dryness on a water-bath. To the residue add 6-8 ml of bromine TS, shake a few minutes and warm until discoloured. Cool and filter. To the filtrate add 2.0 ml of barium chloride (50 g/l)TS; a white precipitate is produced which on the addition of 2.0 ml of sodium hydroxide (~150 g/l)TS does not turn violet (distinction from thiouracil).

PYRANTEL EMBONATE TABLETS

Description. Each tablet contains pyrantel embonate usually equivalent to 250 mg of pyrantel.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.20 g of pyrantel.
2. Grind the tablets, weigh out the above calculated equivalent amount to pyrantel as powdered material, shake it with 20 ml of a mixture composed of chloroform R, methanol R and ammonia (~260 g/l)TS (10:10:1) and filter. Evaporate the filtrate to dryness on a water-bath, and recrystallize from a small volume of methanol R. Dry the separated crystals at 80 °C for 2 hours and use them as the test substance.

IDENTITY TESTS

Melting behaviour. About 251 °C.

Colour and other reactions

1. Dissolve 5 mg of the test substance in 1.0 ml of hydrochloric acid (~70 g/l)TS and add 1.0 ml of formaldehyde/sulfuric acid TS; a purple colour is produced.
2. To 5 mg of the test substance add about 1 ml of sodium hydroxide (~80 g/l)TS and 2.0 ml of potassium permanganate (10 g/l)TS; a green solution is obtained from which after boiling a brown precipitate separates.
3. Dissolve about 2 mg of the test substance in 2 ml of sulfuric acid (~1760 g/l)TS; a yellow colour is first produced which changes to orange and finally red.

QUININE SULFATE TABLETS

Description. Each tablet contains quinine sulfate usually equivalent to 300 mg of quinine.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent 0.06 g of quinine.
2. Grind the tablets, weigh out the above calculated equivalent amount to quinine as powdered material, shake it with 30 ml of water, filter, and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To 5 ml of the test solution add 2 drops of sulfuric acid (~100 g/l)TS; a blue fluorescence is produced.
2. To 5 ml of the test solution add, drop by drop, bromine TS until a light yellow colour persists, and then add 1.0 ml of ammonia (~100 g/l)TS; an emerald-green colour is produced.
3. To 5 ml of the test solution 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.

SALBUTAMOL SULFATE PESSARIES

Description. Each pessary contains salbutamol sulfate usually equivalent to 1.0-4.0 mg of salbutamol.

Preparation of the sample

1. Weigh 1 pessary and calculate the amount equivalent to 25 mg of salbutamol.
2. Grind the pessaries, weigh out the above calculated equivalent amount to salbutamol as powdered material, shake it with 10 ml of water and 50 ml of light petroleum R. Separate the aqueous layer, wash it once with 20 ml of chloroform R, and use the aqueous layer as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To 4 ml of the test solution add 0.10 ml of ferric chloride (25 g/l)TS; a reddish violet colour develops. Add 10 mg of sodium hydrogen carbonate R; a fleshy precipitate is produced with an evolution of gas. Add 1-2 drops of sulfuric acid (~1760 g/l)TS; the solution becomes colourless.
2. To 2.0 ml of the test solution add 0.5 ml of barium chloride (50 g/l)TS; a white precipitate is produced.
3. To 2.0 ml of the test solution add 2-3 drops of sulfuric acid (~100 g/l)TS and 2-3 drops of potassium permanganate (10 g/l)TS; the purple colour is discharged.

SALBUTAMOL SULFATE SYRUP

Description. The syrup contains salbutamol sulfate usually equivalent to 400 µg of salbutamol in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the well-homogenized contents of the containers equivalent to 1.2 mg and 16 mg of salbutamol and use it directly: 1.2 mg for test solution 1, dividing it in three equal volumes; 16 mg for test solution 2.
2. Transfer test solution 2 to separating funnel, add 5 ml of ammonia (~100 g/l)TS and sufficient sodium chloride R to saturate it. Extract three times with 30 ml volumes of chloroform R. Pass each chloroform extract through anhydrous sodium sulfate R, evaporate to reduce the volume to about 1 ml. Place 1 strip of filter-paper into it, and allow the solution to ascend for 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 and dry the remaining part of the strip in air at room temperature (test-paper).

IDENTITY TESTS

Colour and other reactions

1. To 1 volume of test solution 1 add 50 ml of water, 2.0 ml of ammonia (~100 g/l)TS, 1.0 ml of aminophenazone (30 g/l)TS, 4 ml of potassium ferricyanide (50 g/l)TS and 10 ml of chloroform R. Shake well and allow to separate; an orange-red colour is produced in the chloroform layer.
2. Place 1 drop of ferric chloride (25 g/l)TS onto the test-paper; a violet spot is produced.
3. To 2 volumes of test solution 1 add 1.0 ml of hydrochloric acid (~70 g/l)TS and 1.0 ml of barium chloride (50 g/l)TS; a white turbidity is produced.

SODIUM FLUORIDE TABLETS

Description. Each tablet usually contains 500 µg of sodium fluoride.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 15 mg of sodium fluoride.
2. Grind the tablets, weigh out the above calculated equivalent amount to sodium fluoride as powdered material and use it directly as the test substance. Divide the test substance into three equal parts.

IDENTITY TESTS

Colour and other reactions

1. Shake 1 part of the test substance with 10 ml of water and filter. Dilute separately 2 ml of ferric chloride (25 g/l)TS to 50 ml with water; dilute

1 ml of ammonium thiocyanate (75 g/l)TS to 10 ml with water. Mix 1.0 ml of both diluted solutions with 5 drops of hydrochloride acid (~70 g/l)TS; a red solution is produced. Add gradually the test solution to the reagent mixture; its colour is changed to yellow.

2. Shake 2 parts of the test substance with 20 ml of water and filter. Evaporate the filtrate on a water-bath to a volume of about 5 ml. Cool, add 1.0 ml of nitric acid (~130 g/l)TS and 1.0 ml of silver nitrate (40 g/l)TS; the solution remains unchanged (distinction from other halides).

SPIRONOLACTONE TABLETS

Description. Each tablet usually contains 25 mg of spironolactone.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 0.20 g of pironolactone.
2. Grind the tablets, weigh out the above calculated equivalent amount to spironolactone as powdered material, extract it twice with 10 ml portions of chloroform R, filter and evaporate the combined filtrate to dryness on a water-bath. Dissolve the residue in 3 ml of methanol R, filter, evaporate the filtrate to dryness and use the residue as the test substance.

IDENTITY TESTS

Melting point. The test substance melts at about 205 °C.

Colour and other reactions

1. Dilute 1 ml of sulfuric acid (~1760 g/l)TS with 1.0 ml of water, add 20 mg of the test substance and shake; an orange solution with an intense yellowish green fluorescence is produced. Gently heat the solution, it becomes deep red and the evolved hydrogen sulfide blackens lead acetate paper R held over the tube. Pour the solution into water; a greenish yellow opalescent solution is produced.
2. Dissolve about 1-2 mg of the test substance in 2.0 ml of blue tetrazolium/sodium hydroxide TS; a purple colour is produced.

STREPTOMYCIN SULFATE INJECTION

Description. The injection is a sterile solution of streptomycin sulfate usually containing the equivalent of 500 mg of streptomycin in 1.0 ml of a suitable vehicle.

Preparation of the sample

Pool the contents of the ampoules equivalent to 1.0 g of streptomycin and use it directly as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To about 0.2 ml of the test solution add 1.0 ml of sodium hydroxide (~80 g/l)TS and heat on a water-bath for 5 minutes. Cool, add 1.5 ml of hydrochloric acid (~70 g/l)TS and 3 drops of ferric chloride (25 g/l)TS; an intense violet colour is produced.
2. To about 0.1 ml of the test solution add 1.0 ml of pyridine R, 1.0 ml of sodium hydroxide (~80 g/l)TS and 3 drops of benzenesulfonyl chloride R, and shake well; a violet colour is produced.
3. Add 1 drop of the test solution to 2.0 ml of 4-dimethylaminobenzaldehyde TS and heat in a water-bath for 2 minutes; an orange-brown colour is produced.
4. Dilute about 0.2 ml of the test solution with 2.0 ml of water and add 1.0 ml of 1-naphthol TS and 2.5 ml of sodium hydrochlorite TS; a purple colour is produced.
5. To 2 drops of the test solution add 2.0 ml of water, shake and add 3 drops of barium chloride (50 g/l)TS; a white precipitate is produced.

TETRACYCLINE HYDROCHLORIDE OPHTHALMIC OINTMENT

Description. The ointment usually contains 10 mg of tetracycline hydrochloride per g of a suitable ointment base.

Preparation of the sample

Withdraw and weigh an amount equivalent to 30 mg of tetracycline hydrochloride and dissolve it in 25 ml of light petroleum R by warming carefully on a water-bath. Collect the residue by decanting, wash it with 3 portions of 25 ml of light petroleum R and use it as the test substance.

IDENTITY TESTS

Colour and other reactions

1. To about 1 mg of the test substance add 2 ml of sulfuric acid (~1760 g/l)TS; a red-violet colour is produced which on the addition of 5-6 ml of water changes to yellow.
2. In a porcelain dish warm 2.0 ml of zinc chloride (500 g/l)TS until a skin is formed on the surface of the solution or to a partial evaporation, add about 1 mg of the test substance and continue to warm for 1 minute; a yellow-orange colour is produced.
3. Dissolve 10 mg of the test substance in 1.0 ml of water and add a few drops of nitric acid (~130 g/l)TS and a few drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced which dissolves in 5-6 ml of ammonia (~100 g/l)TS.

TETRACYCLINE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 250 mg of tetracycline hydrochloride. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 0.10 g of tetracycline hydrochloride.
2. Grind the tablets or cores, weigh out the above calculated equivalent amount to tetracycline hydrochloride as powdered material, shake it with 10 ml of water, filter and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To about 2 ml of sulfuric acid (~1760 g/l)TS add 2 drops of the test solution; a purple violet colour is produced which remains unchanged for more than 2 minutes. Allow to stand for 5 minutes, then cautiously add 2.0 ml of water; a yellow colour is produced.
2. In a porcelain dish warm 2.0 ml of zinc chloride (500 g/l)TS until a skin is formed on the surface of the solution or to a partial evaporation, add 2 drops of the test solution and continue to warm for 1 minute; a yellow-orange colour is produced.
3. To 1.0 ml of the test solution add a few drops of nitric acid (~130 g/l)TS and a few drops of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced which dissolves in 5-6 ml of ammonia (~100 g/l)TS.

THIOPENTAL SODIUM POWDER FOR INJECTION

Description. Each vial contains a sterile powder usually equivalent to 0.5-1.0 g of thiopental sodium.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amounts equivalent to 0.05 g and 0.5 g of thiopental sodium.
2. Empty the vials, weigh out the above calculated equivalent amounts to thiopental sodium and use them directly: 0.05 g for test substance 1; 0.5 g for test substance 2 and divide it into two equal parts.

IDENTITY TESTS

Colour and other reactions

1. To 1 part of test substance 2 add 5 ml of water, acidify with hydrochloric acid (~70 g/l)TS, filter, wash the precipitate with water, recrystallize from ethanol (~150 g/l)TS and dry at 105 °C; melting point, about 160 °C.
2. Moisten a small amount of test substance 1 with a few drops of hydrochloric acid (~70 g/l)TS and introduce it into a nonluminous flame using a magnesia stick or a nichrome or platinum wire sealed to a glass rod; the flame acquires a bright yellow colour.
3. To the remaining test substance 1 add 2.0 ml of hot cobalt(II) acetate/methanol TS, heat the mixture, add about 40 mg of powdered sodium tetraborate R and heat again to boiling; a blue-violet colour is produced.

4. Fuse 1 part of test substance 2 with 1 g of sodium hydroxide R in a test-tube until the glass glows red; the melt turns red-brown and vapours are evolved. Insert a piece of moistened pH-indicator paper R into the vapours; its coloration is changed to an alkaline range. Cool the melt, add 2.0 ml of water, mix well and filter. Acidify the filtrate with sulfuric acid (~100 g/l)TS and heat gently; the vapours evolved turn a strip of lead nitrate paper R to brown and then to black.

VERAPAMIL HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 40-80 mg of verapamil hydrochloride. The tablets may be sugar-coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amounts equivalent to 0.10 g and 20 mg of verapamil hydrochloride.
2. Grind the tablets or cores, weigh out the above calculated equivalent amounts to verapamil hydrochloride as powdered material and use them directly: 0.10 g for test substance 1; 20 mg for test substance 2.
3. Shake test substance 1 with 10 ml of water, filter and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. To 2.0 ml of the test solution add 0.20 ml of mercuric chloride (65 g/l)TS; a white precipitate is produced.
2. To 2.0 ml of the test solution add about 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 dissolves gradually to form a pale yellow solution.
3. Shake 0.20 g of citric acid R with 10 ml of acetic anhydride R and to 1.0 ml of the supernatant solution add test substance 2, and heat on a water-bath; a purple colour is produced.

VINCRIStINE SULFATE POWDER FOR INJECTION

Description. Each vial contains a sterile powder usually equivalent to 1-5 mg of vincristine sulfate.

Preparation of the sample

1. Weigh the contents of 1 vial and calculate the amount equivalent to 2.0 mg of vincristine sulfate.
2. Empty the vials, weigh out the above calculated equivalent amount to vincristine sulfate, shake it with 3 ml of a mixture of 9 volumes of chloroform R and 1 volume of methanol R, and filter. Evaporate the filtrate to dryness at 40 °C in a water-bath and use the residue as the test substance. Divide the test substance into two equal parts.

IDENTITY TESTS

Colour and other reactions

1. Dissolve 10 mg of ceric ammonium sulfate R in about 1 ml of phosphoric acid (~1440 g/l)TS and to 2 drops of this solution add 1 part of the test substance; a blue-violet colour is observed which changes slowly to brown.
2. To the remaining part of the test substance add about 0.2 ml of vanillin/hydrochloric acid TS and allow to stand for 1 minute; an orange colour is observed.

REAGENTS

The preparation of the required reagents is described in "Basic tests for pharmaceutical substances". A list of additional reagents is as follows:

Calcium chloride (100 g/l)TS.

Cobalt(II) acetate/methanol TS.

Procedure. Dissolve 20 mg of cobalt(II) acetate R in 10 ml of methanol R.

Cobalt(II) acetate R.

Sodium tetraborate R.

ANNEX 1

Tests that require final validation or improvement

In the process of verification several tests were modified according to suggestions from collaborators. Additional work is needed with regard to certain products as indicated below:

Cimetidine tablets

The colour of the precipitate in test 2 needs validation.

Dexamethasone sodium phosphate injection

The results of test 1 need validation.

Diazepam injection

Difficulties were encountered with test 2; it needs validation or modification.

Ergometrine hydrogen maleate injection

Test 3 needs validation.

Ergometrine hydrogen maleate tablets

Test 3 needs validation.

Erythromycin estolate capsules

Tests 1, 2 and 3 need validation.

Erythromycin stearate tablets

Tests 1, 2 and 3 need validation.

Fluorouracil injection

Test 3 needs validation.

Glyceryl trinitrate tablets

The whole test needs validation.

Haloperidol solution

A further test is desired, which could be based on a reaction with ammonium molybdate or the melting point.

Nikethamide injection

Tests 2 and 4 need validation.

Phenylbutazone tablets

The whole test needs validation.

Pyrantel embonate tablets

The whole test needs validation.

Salbutamol sulfate syrup

In test 1 difficulties occurred with the extraction of the orange colour into the chloroform layer; the test needs validation or modification.

Sodium fluoride tablets

The whole test needs validation.

Spirolactone tablets

The whole test needs validation.

A N N E X 2

Revised Basic Tests Requiring Validation

AMITRIPTYLINE HYDROCHLORIDE TABLETS

Description. Each tablet usually contains 25 mg of amitriptyline hydrochloride. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amounts equivalent to 5 mg and 0.10 g of amitriptyline hydrochloride.
2. Grind the tablets or cores, weigh out the above calculated equivalent amounts to amitriptyline hydrochloride as powdered material and use them directly: 5 mg for test substance 1; two portions of 0.10 g for test substance 2.
3. For the test solution, shake 1 portion of test substance 2 with 5 ml of water, filter and use the filtrate.

IDENTITY TESTS

Colour and other reactions

1. To test substance 1 add about 3 ml of sulfuric acid (~1760 g/l)TS; a red colour is produced. Add a few drops of potassium dichromate (100 g/l)TS; the colour turns to dark brown.
2. Shake 1 portion of test substance 2 with 10 ml of sulfuric acid (~100 g/l)TS and add 2.0 ml of a saturated solution of potassium permanganate R; the violet colour of the solution disappears quickly. Heat the mixture on a water-bath until the formed brown precipitate is almost dissolved. Allow to cool. To the supernatant liquid add 5 ml of ammonia (~250 g/l)TS and shake for 2 minutes. Add 3 ml of chloroform R and shake again; a violet-red colour is produced in the chloroform layer.
3. To the test solution add 0.10 ml of nitric acid (~130 g/l)TS; a white precipitate which may appear dissolves on stirring. Check the solution with pH-indicator paper R to assure that it is acidic and add 2.0 ml of silver nitrate (40 g/l)TS; a white, curdy precipitate is produced.

CHLORAMBUCIL TABLETS

Description. Each tablet usually contains 2.0 mg of chlorambucil. The tablets may be coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 0.05 g of chlorambucil.
2. Grind the tablets or cores, weigh out the above calculated equivalent amount to chlorambucil as powdered material, shake it with 20 ml of chloroform R, filter, and evaporate the filtrate to dryness on a water-bath. Use the residue as the test substance.

IDENTITY TESTS

Colour and other reactions

1. Dissolve 10 mg of the test substance in a mixture of 1.0 ml of acetone R and 1.0 ml of water. Add 1 drop of sulfuric acid (~1760 g/l)TS and a few drops of silver nitrate (40 g/l)TS; no opalescence is immediately observed. Warm the solution on a water-bath for 2-3 minutes; an opalescence is obtained.
2. To 30 mg of the test substance add 3.0 ml of hydrochloric acid (~70 g/l)TS, mix and allow to stand for 30 minutes, shaking occasionally. Filter, wash the residue with 5 ml of water (keep the filtrate for tests 3 and 4) and dry the residue at 105 °C for 3 hours; melting point, about 146 °C.
3. To 5 ml of the filtrate from test 2 add 0.5 ml of potassio-mercuric iodide TS; a light beige coloured precipitate is produced.
4. To the remaining filtrate from test 2 add 3 drops of potassium permanganate (10 g/l)TS; the colour is discharged.

HALOPERIDOL INJECTION

Description. The injection is a sterile solution usually containing 5.0 mg of haloperidol in 1.0 ml of a suitable vehicle.

Preparation of the sample

1. Pool the contents of the ampoules equivalent to 20 mg of haloperidol and use it directly as test solution 1. Divide test solution 1 into two equal volumes.
2. Transfer 1 volume of test solution 1 to a platinum crucible, add 20 mg of anhydrous sodium carbonate R and evaporate to dryness on a water-bath. Heat until a white residue is obtained, dissolve it in 2.0 ml of water warming gently on a water-bath, cool, neutralize with hydrochloric acid (~70 g/l)TS and use it as test solution 2.
3. To 1 volume of test solution 1 add 10 ml of water and 0.5 ml of sodium hydroxide (~80 g/l)TS, extract with 10 ml of chloroform R, filter and evaporate the filtrate to dryness. Use the residue as the test substance.

IDENTITY TEST

Melting point. The test substance melts at about 150 °C.

Colour and other reactions

In a test-tube mix 1 drop of ferric chloride (25 g/l)TS with 1 drop of ammonium thiocyanate (75 g/l)TS, dilute with 10 ml of water and acidify with 1 drop of hydrochloric acid (~70 g/l)TS. To 1.0 ml of this solution, add drop by drop, the test solution; the red colour is discharged.

HALOPERIDOL TABLETS

Description. Each tablet usually contains 2-5 mg of haloperidol.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 20 mg of haloperidol.
2. Grind the tablets, weigh out the above equivalent amount to haloperidol as powdered material and use directly as test substance 1. Divide test substance 1 into two equal parts.
3. Shake 1 part of test substance 1 with 10 ml of chloroform R for 5 minutes, filter into a platinum crucible, add 20 mg of anhydrous sodium carbonate R and evaporate to dryness on a water-bath. Heat until a white residue is obtained, dissolve it in 2.0 ml of water warming gently on a water-bath, cool, neutralize with hydrochloric acid (~70 g/l)TS and use it as the test solution.
4. To 1 part of test substance 1 add 10 ml of water and 1.0 ml of sodium hydroxide (~80 g/l)TS, extract with 10 ml of chloroform R, filter and evaporate the filtrate to dryness. Use the residue as test substance 2.

IDENTITY TEST

Melting point. Test substance 2 melts at about 150 °C.

Colour and other reactions

In a test-tube mix 1 drop of ferric chloride (25 g/l)TS with 1 drop of ammonium thiocyanate (75 g/l)TS, dilute with 10 ml of water and acidify with 1 drop of hydrochloric acid (~70 g/l)TS. To 1.0 ml of this solution add, drop by drop, the test solution; the red colour is discharged.

SODIUM VALPROATE TABLETS

Description. Each tablet usually contains 200-500 mg of sodium valproate. The tablets may be sugar-coated.

Preparation of the sample

1. In the event that tablets are coated, carefully remove the coating by scraping. Weigh 1 tablet or core and calculate the amount equivalent to 0.40 g of sodium valproate.
2. Grind the tablets or cores, weigh out the above calculated equivalent amount to sodium valproate as powdered material, add 5 ml of water, stir well, filter, and use the filtrate as the test solution.

IDENTITY TESTS

Colour and other reactions

1. Dip a magnesia stick or a nichrome or platinum wire sealed to a glass rod first into hydrochloric acid (~420 g/l)TS, then into the test solution and introduce it into a nonluminous flame; a bright yellow colour is observed.
2. Add about 0.5 ml of cobalt(II) chloride (30 g/l)TS to 1.0 ml of the test solution; a violet precipitate is produced which is soluble in carbon tetrachloride R.
3. To 1.0 ml of the test solution add a few drops of potassium iodobismuthate/acetic acid TS; a violet precipitate is produced.

TRIMETHOPRIM TABLETS

Description. Each tablet usually contains 100-200 mg of trimethoprim.

Preparation of the sample

1. Weigh 1 tablet and calculate the amount equivalent to 25 mg of trimethoprim.
2. Grind the tablets, weigh out the above calculated equivalent amount to trimethoprim as powdered material, shake it with 5 ml of chloroform R, filter, evaporate the filtrate to dryness and use the residue as the test substance.

IDENTITY TESTS

Colour and other reactions

1. To 10 mg of the test substance add 5 ml of sulfuric acid (~1760 g/l)TS, 1 drop of ferric chloride (25 g/l)TS and warm the solution in a water-bath for 3 minutes. Cool, add to the yellow solution 1 drop of nitric acid (~130 g/l)TS; the colour of the solution turns red.
2. Shake 5 mg of the test substance with 0.2 ml of formaldehyde/sulfuric acid TS; and intense orange-red colour is produced.

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