REVISION OF CHAPTER 2.1:
GENERAL IDENTIFICATION TESTS

Draft proposal for revision in The International Pharmacopoeia
(July 2020)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to Dr Herbert Schmidt, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidt@who.int) by 18 September 2020.

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SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/20.854:

**REVISION OF CHAPTER 2.1:**

**GENERAL IDENTIFICATION TESTS**

<table>
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<td>Need to revise Chapter 2.1, General identification tests identified at the consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.</td>
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[Note from the Secretariat. *It is proposed to revise chapter 2.1: General identification tests.*

Changes from the current text are indicated in the text by [insert] or [delete].]
REVISION OF CHAPTER 2.1:

GENERAL IDENTIFICATION TESTS

2.1 General identification tests

Acetylated substances

Place a quantity of the test substance as specified in the monograph in a test tube (of maximum 18 mm diameter) and treat it with 3 drops of phosphoric acid (~1440 g/L) TS. Close the tube with a stopper through which passes a smaller test tube filled with water and, on the outside of which, hangs a drop of lanthanum nitrate (30 g/L) TS. Heat the apparatus in a boiling water bath for 5 minutes. Transfer the drop of lanthanum nitrate to a white porcelain spot plate and mix with a drop of iodine (0.02 mol/L) VS. Place at the edge of the mixture a drop of ammonia (~100 g/L) TS. A blue colour slowly appears at the interface of the two liquids and persists for a short time.

Amines, primary aromatic

Dissolve a quantity of the test substance as specified in the monograph in 2 mL of hydrochloric acid (~70 g/L) TS with the aid of heat, if necessary. Cool in ice, treat it with 4 mL of sodium nitrite (10 g/L) TS and pour the mixture into 2 mL of 2-naphthol TS1 containing 1 g of sodium acetate R. A heavy precipitate, coloured as specified in the monograph, is produced.

Ammonia and volatile aliphatic amines

Dissolve a quantity of the substance as specified in the monograph, place the solution in a test-tube and add 1 g of magnesium oxide R; warm, if specified in the monograph. Alkaline vapours evolve gradually and turn manganese/silver paper R black, the reagent paper being placed in the upper part of the test-tube.

Ammonium

Carry out the test in an apparatus consisting of stoppered test tubes A and B connected by a bent glass tube to permit a stream of air to pass consecutively through test tubes A and B.
Place the solution as specified in the monograph and 0.2 g of magnesium oxide R into test-tube A, and 1 mL of hydrochloric acid (0.1 mol/l) VS containing 1 drop of methyl red/ethanol TS in test-tube B. Bubble air through the apparatus. Evolved ammonia turns the colour of the solution in test-tube B to yellow. On the addition of 1 mL of sodium cobaltinitrite (100 g/l) TS to this solution, a yellowish-brown precipitate is formed.

**Bismuth**

A. Prepare the solution in hydrochloric acid (~250 g/l) TS as specified in the monograph and dilute 10 times with water. A white precipitate is formed, which turns dark brown on the addition of sodium sulfide TS.

B. Treat the solution in nitric acid (~1000 g/l) TS as specified in the monograph with potassium iodide (80 g/l) TS. A black precipitate is formed, which is soluble in an excess of the reagent to give a yellowish-brown or orange solution. Dilute this solution with several volumes of water and heat; an orange or copper-coloured precipitate is obtained. The black precipitate that is first formed on the addition of potassium iodide (80 g/l) TS also becomes orange or copper-coloured when heated with water.

**Bromides**

A. Prepare a solution as specified in the monograph, acidify with nitric acid (~130 g/L) TS and add silver nitrate (40 g/L) TS. A yellowish curdy precipitate is produced, which is partially soluble in ammonia (~260 g/L) TS, but almost insoluble in ammonia (~100 g/L) TS and in nitric acid (~1000 g/L) TS.

B. **NOTE: For testing bromides or hydrobromides of insoluble or sparingly soluble bases.** Prepare the solution as specified in the monograph, add ammonia (~100 g/L) TS, filter, acidify the filtrate with nitric acid (~130 g/L) TS and proceed with test A.

C. Prepare the solution as specified in the monograph, acidify with sulfuric acid (~100 g/L) TS, and mix with chlorine TS. A brown solution results; after shaking with dichloromethane chloroform R, it becomes colourless whereas the dichloromethane chloroform layer turns reddish.
**Calcium**

A. Prepare the solution as specified in the monograph and add to it ammonium oxalate (25 g/L) TS. A white precipitate is formed, which is soluble in hydrochloric acid (~250 g/L) TS but is practically insoluble in acetic acid (~300 g/L) TS.

B. Treat 1 drop of a solution as specified in the monograph with 4 drops of glyoxal bis(2-hydroxyanil) TS, and 1 drop of sodium hydroxide (~80 g/L) TS. A reddish brown precipitate is formed which dissolves in dichloromethane chloroform R to give a red solution.

**Chlorides**

A. Prepare a solution as specified in the monograph, acidify with nitric acid (~130 g/L) TS and add silver nitrate (40 g/L) TS. A white curdy precipitate is produced, which is soluble in ammonia (~100 g/L) TS but is practically insoluble in nitric acid (~1000 g/L) TS.

B. **NOTE: For testing chlorides or hydrochlorides of insoluble or sparingly soluble bases.** Prepare the solution as specified in the monograph, add ammonia (~100 g/L) TS, filter and acidify the filtrate with nitric acid (~130 g/L) TS and proceed with test A.

C. Mix the quantity of the test substance as specified in the monograph with an equal quantity of manganese dioxide R, moisten with sulfuric acid (~1760 g/L) TS and heat gently. The evolved chlorine is recognizable by its greenish colour and produces a blue coloration of moistened starch/iodide paper R. Carry out the reaction preferably under a hood.

**Citrates**

A. Treat at ambient temperature a neutral solution as specified in the monograph with calcium chloride (55 g/L) TS. No precipitate is formed but, on boiling, a white solid is produced which is soluble in acetic acid (~300 g/L) TS.
B. Boil a solution with mercuric sulfate TS as specified in the monograph and filter if necessary. After the addition of a few drops of potassium permanganate (10 g/l) TS to the filtrate, the colour is discharged and a white precipitate is produced.

B. To 10 mg of the test substance or the solution described in the monograph, add 4 mL of pyridine R and 2 mL of acetic anhydride R, and shake; a yellow colour is immediately produced. Heat on a water bath for 2 minutes; a light pink to red colour is produced.

[Note from the Secretariat. Identity test C in the monograph on Diethycarbamazine dihydrogen citrate tablets describes a colour reaction using mercuric sulfate. It is proposed to replace the text by the following: Filter the aqueous layer obtained from identity test B. The filtrate yields reaction B described under 2.1 General identification tests as characteristic of citrates.]

Ferric salts:

Dissolve a quantity of the test substance to be examined equivalent to not less than 1 mg of iron (Fe3+) in 1 mL of water R or use 1 mL of the prescribed solution. Add 1 mL of potassium ferrocyanide (~53 g/L) TS. A blue precipitate is formed that does not dissolve on addition of 5 mL of hydrochloric acid (~70 g/L) TS.

Ferrous salts

A. Prepare a solution as specified in the monograph and add potassium ferricyanide (10 g/L) TS. A dark-blue precipitate is formed which is practically insoluble in hydrochloric acid (~70 g/L) TS.

B. Prepare a solution as specified in the monograph, acidify with sulfuric acid (~100 g/L) TS and treat with o-phenanthroline (1 g/L) TS. An intense red colour is produced which is discharged by the addition of ceric sulfate (35 g/L) TS.

Iodides

A. Prepare a solution as specified in the monograph, acidify with nitric acid (~130 g/L) TS and add silver nitrate (40 g/L) TS. A yellow curdy precipitate is formed which is practically insoluble in ammonia (~100 g/L) TS and in nitric acid (~1000 g/L) TS.
B. NOTE: For testing iodides of insoluble or sparingly soluble bases. Prepare a solution as specified in the monograph, add ammonia (~100 g/L) TS, filter and acidify the filtrate with nitric acid (~130 g/L) TS and proceed with test A.

C. Prepare a solution as specified in the monograph, acidify with sulfuric acid (~100 g/L) TS and add potassium nitrite (100 g/L) TS. A brown solution results; after shaking with dichloromethane chloroform R, it becomes colourless whereas the dichloromethane chloroform layer turns violet.

Nitrates

A. Prepare a solution as specified in the monograph and treat it with ferrous sulfate (15 g/L) TS. No brown colour appears unless sulfuric acid (~1760 g/L) TS is cautiously added to form a lower layer. A brown colour is then produced at the interface of the two liquids.

B. Add 2 mg of the finely ground test substance to a mixture of 0.1 mL of nitrobenzene R and 0.2 mL of sulfuric acid (~1760 g/L) TS. Allow to stand at room temperature for 5 minutes, cool in ice, and add slowly while mixing 5 mL of water and 3 mL of sodium hydroxide (~400 g/L) TS. Add 5 mL of acetone R, shake and allow to separate. An intense violet colour is produced in the upper phase.

Orthophosphates

A. Add, drop by drop, a quantity of nitric acid (~130 g/L) TS to 5 mL of ammonium molybdate (95 g/L) TS until any precipitate that may appear dissolves. Divide this solution into 2 portions, add to one portion a test solution acidified with nitric acid (~130 g/L) TS as specified in the monograph and boil both portions. A yellow precipitate is formed with the test solution while the other shows no more than a slight opalescence.

B. Prepare a neutral solution as specified in the monograph and add silver nitrate (40 g/L) TS. A yellow precipitate is produced which does not darken upon heating the solution to boiling. The precipitate is soluble in ammonia (~100 g/L) TS and in nitric acid (~130 g/L) TS.
Potassium

Prepare an alkaline solution as specified in the monograph and treat it with sodium tetraphenylborate (30 g/L) TS. A white precipitate is produced.

Salicylates

Treat a neutral solution as specified in the monograph with ferric chloride (25 g/L) TS. An intense reddish violet colour appears which remains on the addition of a small amount of acetic acid (~300 g/L) TS but disappears on the addition of hydrochloric acid (~70 g/L) TS, with separation of a white crystalline precipitate.

Sodium

A. Moisten a quantity of the substance with hydrochloric acid (~250 g/L) TS. An intense yellow colour is produced when the solution is introduced into a nonluminous flame.

NOTE: Perform test B or C if, for technical reasons, test A cannot be carried out.

B. Acidify a solution as specified in the monograph with acetic acid (~60 g/l) TS, filter, if necessary, and treat it with uranyl/zinc acetate TS. A yellow crystalline precipitate is produced.

B. Dissolve 0.1 g of the test substance in 2 mL of water R or use 2 mL of the solution described in the monograph. Add 2 mL of potassium carbonate (150 g/L) TS and heat to boiling. No precipitate is formed. Add 4 mL of potassium pyroantimonate (13 g/L) TS and heat to boiling. Allow to cool in iced water and, if necessary, rub the inside of the test tube with a glass rod. A dense white precipitate is produced.

C. Dissolve a quantity of the substance to be examined equivalent to about 2 mg of sodium (Na+) in 0.5 mL of water R or use 0.5 mL of the prescribed solution. Add 1.5 mL of methoxyphenylacetic R and cool in ice-water for 30 min. A voluminous, white, crystalline precipitate is produced. Place in water at 20 °C and stir for 5 min. The precipitate does not disappear. Add 1 mL of ammonia (~100 g/L) TS. The precipitate dissolves completely. Add 1 mL of ammonium carbonate solution R. No precipitate is produced.
Sulfates

A. Prepare a solution as specified in the monograph and add barium chloride (50 g/L) TS. A white precipitate is formed which is practically insoluble in hydrochloric acid (~250 g/L) TS.

B. To a solution as specified in the monograph, add lead acetate (80 g/L) TS. A white precipitate is formed which is soluble in ammonium acetate (80 g/L) TS and in sodium hydroxide (~80 g/L) TS but practically insoluble in hot water.

Tartrates

A. Acidify a solution as specified in the monograph with acetic acid (~300 g/L) TS and add 1 drop of ferrous sulfate (15 g/L) TS, a few drops of hydrogen peroxide (~60 g/L) TS and enough sodium hydroxide (~80 g/L) TS to make the solution alkaline. A purple or violet colour is produced.

B. Mix a few mL of sulfuric acid (~1760 g/L) TS with a few drops of resorcinol (20 g/L) TS and a few drops of potassium bromide (100 g/L) TS and add 2 or 3 drops of a solution as specified in the monograph. Warm the liquid in a water-bath for 5 to 10 minutes. An intense blue colour is produced. Cool the liquid and pour it into water. The solution becomes red.

Reagents to be added:

Ammonium carbonate (158 g/L) TS

Ammonium carbonate dissolved in water R to contain 158 g of ammonium carbonate R in 1000 mL.

Methoxyphenylacetic R

Procedure. Dissolve 2.7 g of methoxyphenylacetic acid R in 6 mL of tetramethylammonium hydroxide (~100 g/L) TS and add 20 mL of dehydrated ethanol R.

Storage. Store in a polyethylene container.
Methoxyphenylacetic acid R

*C*<sub>9</sub>H<sub>10</sub>O<sub>3</sub>

**Description.** White, crystalline powder or white or almost white crystals.

**Solubility.** Sparingly soluble in water, freely soluble in ethanol (~750 g/L) TS.

**Melting point.** About 70° C.

**Potassium carbonate (150 g/L) TS**

**Procedure.** Dissolve 15 g of anhydrous potassium carbonate R in 100 mL of water R.

**Potassium pyroantimonate (13 g/L) TS**

Dissolve 1.95 g of potassium pyroantimonate R in 95 mL of hot water R. Cool quickly and add a solution containing 2.5 g of potassium hydroxide R in 50 mL of water R and 1 mL of dilute sodium hydroxide (~85 g/L) TS. Allow to stand for 24 hours, filter and dilute to 150 mL with water R.

**Potassium pyroantimonate R**

*K*<sub>Sb(OH)</sub><sub>6</sub>

**Description.** White or almost white, crystals or crystalline powder.

**Solubility.** Sparingly soluble in water R.

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