2.2.3 LIMIT TEST FOR HEAVY METALS

Draft revision for *The International Pharmacopoeia*

(August 2018)

*DRAFT FOR COMMENTS*

Please send any comments you may have on the attached text to Dr Herbert Schmidt, Technical Officer, Medicines Quality Assurance, Technologies Standards and Norms (schmidt@who.int), with a copy to Ms Xenia Finnerty (finnertyk@who.int) by 30 September 2018.

Medicines Quality Assurance working documents will only be sent out electronically and will also be placed on the Medicines website for comment under “Current projects”. If you have not already receive our draft working documents, please send your email address to jonessi@who.int and we will add your name to our electronic mailing list.

© World Health Organization 2018

All rights reserved.

This draft is intended for a restricted audience only, i.e. the individuals and organizations having received this draft. The draft may not be reviewed, abstracted, quoted, reproduced, transmitted, distributed, translated or adapted, in part or in whole, in any form or by any means outside these individuals and organizations (including the organizations' concerned staff and member organizations) without the permission of the World Health Organization. The draft should not be displayed on any website.

Please send any request for permission to:

Dr Sabine Kopp, Group Lead, Medicines Quality Assurance, Technologies Standards and Norms, Regulation of Medicines and other Health Technologies, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland, fax: (41 22) 791 4856, email: kopps@who.int.

The designations employed and the presentation of the material in this draft do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this draft. However, the printed material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

This draft does not necessarily represent the decisions or the stated policy of the World Health Organization.
SCHEDULE FOR THE PROPOSED ADOPTION PROCESS OF DOCUMENT QAS/18.769:

Draft revision for The International Pharmacopoeia

2.2.3 LIMIT TEST FOR HEAVY METALS

<table>
<thead>
<tr>
<th>First draft prepared.</th>
<th>November 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discussion at informal consultation on new medicines,</td>
<td>2–4 May 2018</td>
</tr>
<tr>
<td>quality control and laboratory standards.</td>
<td></td>
</tr>
<tr>
<td>Preparation of a revised document based on feedback</td>
<td>June – July 2018</td>
</tr>
<tr>
<td>received at the informal consultation</td>
<td></td>
</tr>
<tr>
<td>Draft revision sent out for public consultation.</td>
<td>August – September 2018</td>
</tr>
<tr>
<td>Compilation of the feedback received</td>
<td>October 2018</td>
</tr>
<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations.</td>
<td>October 2018</td>
</tr>
<tr>
<td>Further follow-up action as required.</td>
<td></td>
</tr>
</tbody>
</table>

Note from the Secretariat. Feedback is being sought in relation to the revision of the method of analysis 2.2.3 Limit Test for Heavy Metals. It is proposed to change the provision as follows:

- to add a note to provide users of The International Pharmacopoeia with the option to apply ICH Q3D principles to control elemental impurities;
- to add a new procedure for the preparation of the test solution: procedure 5, a closed-vessel microwave digestion that shall be used as an alternative, in particular, for procedures 3 and 4 employing ignition techniques (in new and revised monographs, procedure 5 shall be preferred to procedures 3 or 4);
- to replace the reagent hydrogen sulfide R by thioacetamide R; and
- to align parts of text to the corresponding text included in the European Pharmacopoeia (2.4.8.), thereby keeping and further simplifying the structure of the existing text.
The aim of the limit test for heavy metals is to control metal contaminants potentially emanating from reagents, solvents, electrodes, reaction vessels, rubber seals, and so on. The test shall serve as a screening tool indicating the overall quality of the production process, with limits usually set at < 10 ppm or 20 ppm Pb.

The principle of the test is based on the precipitation of metal sulfides and assumes that all metals behave in a similar manner to a lead standard with which samples are compared. As investigations have shown, the test is effective for detecting Pb, Hg, Pd, Ag, V, Au and Cu which give dark brown or black precipitates. The colour of other precipitates varies from pale yellow to orange.

It is proposed to complement this document by a review of all tests for heavy metals currently prescribed in The International Pharmacopoeia to evaluate the impact of the new provisions on existing test descriptions. In addition, a document could be developed that provides guidance for collaborating laboratories involved in the development of monographs for The International Pharmacopoeia on how to elaborate and validate this limit test.

Comments are sought in particular on the Note added to the text providing users with the option to assess and control elemental impurities according to ICH Q3D, for example, to accommodate decisions of responsible national or regional authorities.

Parts of the text are reproduced from the European Pharmacopoeia with permission and with appropriate editorial modifications.

Changes from the current monograph are indicated in the text by insert or delete.
Draft revision for The International Pharmacopoeia

2.2.3 LIMIT TEST FOR HEAVY METALS

Note. The Guideline for Elemental Impurities Q3D, published by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), presents a process to assess and control elemental impurities in finished pharmaceutical products using the principles of risk assessment. It is within a regulatory authority’s remit to decide whether or not they apply this guideline for the assessment of elemental impurities. If ICH Q3D is implemented, compliance of pharmaceutical substances with the limit test for heavy metals will no longer be required.

The limit test for heavy metals is provided to demonstrate that the content of metallic impurities that are precipitated as coloured sulfides by thioacetamide does not exceed the heavy metals limits given in the individual monographs in terms of micrograms of lead per gram of the test substance.

The test consists of three consecutive operations: preparation of the test solutions (procedures 1 to 5), development of the coloured precipitate by reaction with thioacetamide, and comparison of the colours thus obtained either by directly comparing the coloration of liquids in suitable comparison tubes (Method A) or by comparing the intensity of coloured residues obtained by filtering the liquid using an appropriate apparatus (Methods B or C). Method A is generally applicable only when the amount of heavy metals in the weight of the test substance used exceeds 5 μg; Methods B or C can also be used for amounts of 2 to 5 μg of heavy metals.

PREPARATION OF THE TEST SOLUTIONS

For the standard solution, unless otherwise specified, dilute lead PbTS containing 10 μg of lead per mL solvent to obtain a solution containing 1 μg of lead per mL or 2 μg of lead per mL, depending on the limit prescribed in the monograph. Use the solvent used to prepare the sample solution.
Procedure 1. For the sample solution, unless otherwise specified in the monograph, weigh the quantity of the substance to be examined and dissolve it in 25 mL of water R. For the reference solution, add 2 mL of the sample solution to 10 mL of the standard solution. For the blank solution, add 2 mL of the sample solution to 10 mL of water R.

Procedure 2. For the sample solution, unless otherwise specified in the monograph, weigh the quantity of the substance to be examined and dissolve it in about 25 mL of the organic solvent specified in the monograph, containing a minimum percentage of water R (for example, dioxan R containing 15% of water R or acetone R containing 15% of water R). For the reference solution, add 2 mL of the sample solution to 10 mL of the standard solution. For the blank solution, add 2 mL of the sample solution to 10 mL of the solvent used to prepare the sample solution.

Procedure 3. For the sample solution, place the prescribed quantity (not more than 2 g) of the substance to be examined in a silica crucible with 4 mL of a 250 g/L solution of magnesium sulfate R in sulfuric acid (~98 g/L) TS. Mix using a fine glass rod. Heat cautiously. If the mixture is liquid, evaporate gently to dryness on a water bath. Progressively heat to ignition and continue heating until an almost white or, at most, greyish residue is obtained. Carry out the ignition at a temperature not exceeding 800 °C. Allow to cool. Moisten the residue with a few drops of sulfuric acid (~98 g/L) TS. Evaporate, ignite again and allow to cool. The total period of ignition must not exceed two hours. Take up the residue in two quantities, each of 5 mL, of hydrochloric acid (~70 g/L) TS. Add 0.1 mL of diluted phenolphthalein/ethanol TS, then ammonia (~35 g/L) TS, until a pink colour is obtained. Cool, add anhydrous acetic acid R until the solution is decolorized and add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute with water R to 20 mL.

For the reference solution, follow the procedure described for the sample solution, using the prescribed volume of dilute lead PbTS containing 10 µg of lead per mL instead of the substance to be examined. To 10 mL of the solution obtained, add 2 mL of the sample solution.
For the monitor solution, follow the procedure described for the sample solution, adding to the substance to be examined the volume of dilute lead PbTS prescribed for the preparation of the reference solution. To 10 mL of the solution obtained add 2 mL of the sample solution.

For the blank solution, add 2 mL of the sample solution to 10 mL of water R.

**Procedure 4.** For the sample solution, unless otherwise specified in the monograph, mix thoroughly in a silica crucible the prescribed quantity of the substance to be examined with 0.5 g of magnesium oxide R1. Ignite to a dull redness until a homogeneous white or greyish-white mass is obtained. If after 30 minutes of ignition the mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary, repeat the operation. Heat at 800 °C for about one hour. Take up the residue in two quantities, each of 5 mL, of a mixture of equal volumes of hydrochloric acid (~250 g/L) TS and water R. Add 0.1 mL of diluted phenolphthalein/ethanol TS and then ammonia (~35 g/L) TS until a pink colour is obtained. Cool, add anhydrous acetic acid R until the solution is decolorised, then add 0.5 mL in excess. Filter if necessary and wash the filter. Dilute with water R to 20 mL.

For the reference solution, follow the procedure described for the sample solution, using the prescribed volume of dilute lead PbTS containing 10 µg of lead per mL instead of the substance to be examined and drying in an oven at 100 °C to 105 °C. To 10 mL of the solution obtained, add 2 mL of the sample solution.

For the monitor solution, follow the procedure described for the sample solution, adding to the substance to be examined the volume of dilute lead PbTS prescribed for the preparation of the reference solution and drying in an oven at 100 °C to 105 °C. To 10 mL of the solution obtained add 2 mL of the sample solution.

For the blank solution, add 2 mL of the sample solution to 10 mL of water R.

**Procedure 5.** For the sample solution, unless otherwise specified in the monograph, place the prescribed amount of the substance to be examined (not more than 0.5 g) in a suitable, clean
beaker. Add successively 2.7 mL of cadmium-free and lead-free sulfuric acid (~1760 g/L) TS, 3.3 mL of cadmium-free and lead-free nitric acid (~1000 g/L) TS, and 2.0 mL of hydrogen peroxide (~330 g/L) TS using a magnetic stirrer. Allow the substance to react with the reagent before adding the next one. Transfer the mixture to a dry high-pressure digestion vessels (fluoropolymer or quartz glass).

For the reference solution, follow the procedure described for the sample solution, using the prescribed volume of dilute lead PbTS containing 10 µg of lead per mL instead of the substance to be examined.

For the monitor solution, follow the procedure described for the sample solution, adding to the substance to be examined the volume of dilute lead PbTS prescribed for the preparation of the reference solution.

For the blank solution, prepare the solution as described for the sample solution, omitting the substance to be examined.

**CAUTION.** When using high-pressure digestion vessels, the safety precautions and operating instructions given by the manufacturer must be followed. The digestion cycles have to be elaborated depending on the type of microwave oven to be used (for example, energy-controlled microwave ovens, temperature-controlled microwave ovens or high-pressure ovens). The cycle must conform to the manufacturer's instructions. The digestion cycle is suitable if a clear solution is obtained.

Close the vessels and place them in a laboratory microwave oven. Digest using a sequence of two separate suitable programmes. Design the programmes in several steps in order to control the reaction, monitoring pressure, temperature or energy depending on the type of microwave oven available. After the first programme, allow the digestion vessels to cool before opening.

Add to each vessel 2.0 mL of hydrogen peroxide (~330 g/L) TS and digest using the second programme. After the second programme, allow the digestion vessels to cool before opening. If
necessary to obtain a clear solution, repeat the addition of hydrogen peroxide (~330 g/L) TS and the second digestion programme.

Cool, dilute cautiously with water R and rinse into a flask, ensuring that the total volume does not exceed 25 mL.

**Colour development and measurement**

**For Procedures 1 to 4**

*Method A.* Use matched flat-bottomed comparison tubes of transparent glass with a uniform internal diameter of 16 mm for the comparison of the colours. “Matched tubes” means tubes that are matched as closely as possible in internal diameter and in all other respects.

Transfer 12 ml of each of the test solutions prepared as described under *Preparations of the test solutions* to comparison tubes, add 2 mL of acetate buffer, pH 3.5, TS and mix. Add 1.2 mL of freshly prepared thioacetamide reagent TS, mix and allow to stand for two minutes.

Compare the colours of the solutions by viewing down the vertical axis of the tube in diffused light against a white or, if necessary, a black background, or by another suitable method. The test is not valid unless the colour of the reference solution is more intense than the colour of the blank solution. If the use of a monitor solution is prescribed, the colour of the monitor solution is at least as intense as the colour of the reference solution.

The sample complies with the requirements of the test when the colour of the test solution is not darker than the reference solution.

*Method B.* Transfer 12 ml of each of the test solutions prepared as described under *Preparations of the test solutions* to a beaker, add 2 mL of acetate buffer, pH 3.5, TS and mix. Add 1.2 mL of freshly prepared thioacetamide reagent TS, mix and allow to stand for two minutes.
Filter the solutions through a suitable membrane filter (nominal pore size 0.45 µm). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston.

Compare the intensity of the coloration of the residues obtained with the different test solutions on the membrane filters. The test is not valid unless the coloured residue obtained with the reference solution is more intense than the coloured residue obtained with the blank solution. If the use of a monitor solution is prescribed, the coloured residue obtained with the monitor solution is at least as intense as the coloured residue obtained with the reference solution.

The sample complies with the requirements of the test when the coloured residue obtained from the test solution is not more intense than the coloured residue from the lead standard.

*For Procedure 5:*

*Method C.* Using short-range pH indicator paper, adjust the test solutions to pH 3.0-4.0 with ammonia (~260 g/L) TS. (Ammonia (~100 g/L) TS may be used as the specified range is approached). To avoid heating of the solutions, use an ice bath and a magnetic stirrer. Dilute to 40 mL with water R and mix. Add 2 mL of acetate buffer, pH 3.5, TS and mix. Add to 1.2 mL of thioacetamide reagent R. Mix immediately. Dilute to 50 mL with water R, mix and allow to stand for two minutes. Filter the solutions through a suitable membrane filter (nominal pore size 0.45 µm). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston.

Compare the spots on the filters obtained with the different solutions. The test is not valid unless the coloured residue obtained with the reference solution is more intense than the coloured residue obtained with the blank solution. The coloured residue obtained with the monitor solution is at least as intense as the coloured residue obtained with the reference solution.

The sample complies with the requirements of the test when the coloured residue obtained with the sample solution is not more intense than the coloured residue obtained with the reference solution.
2.2.3 Limit test for heavy metals

The limit test for heavy metals is provided to demonstrate that the content of metallic impurities that are coloured by hydrogen sulfide does not exceed the heavy metals limit given in the individual monograph in terms of micrograms of lead per gram of the test substance.

The test consists of two consecutive operations: preparation of the test solution, and the colour development by reaction with hydrogen sulfide, followed by comparison of the colour obtained with that produced with standard lead solution.

The preparation of the test solution is carried out, as specified in the monograph, according to procedures 1 to 4 described below. A blank is prepared in a similar manner.

The reaction with hydrogen sulfide is carried out by mixing the test solution with freshly prepared hydrogen sulfide TS. The comparison of the colour thus obtained is carried out either by directly comparing the coloration of the liquid in suitable comparison tubes (Method A) or by comparing the intensity of coloration of spots obtained by filtering the liquid using an appropriate apparatus (Method B).

Method A is generally applicable only when the amount of heavy metals in the weight of test substance used exceeds 5 μg; for amounts of 2-5 μg of heavy metals Method B should be used.

The standard lead solution used in the test: dilute lead PbTS contains 10 μg of lead in 1 mL. When 0.1 mL of this solution is employed to prepare the standard for comparison with a solution of 1 g of the substance being tested, the standard solution thus prepared contains 1 μg of Pb and represents the equivalent of 1 μg of lead per g of the substance tested.

Apparatus
For determination of heavy metals by Method A carry out the test in matched flat-bottomed comparison tubes of transparent glass of about 70 mL capacity and about 23 mm internal diameter bearing a 40-mL and a 50-mL mark. Nessler cylinders complying with the above dimensions are suitable. The expression "matched tubes" means tubes that are matched as closely as possible in internal diameter and in all other respects. For mixing the solution use a stirring rod preferably having a loop at the lower end.

For determination of heavy metals by Method B use a 50-mL syringe made of suitable material (usually plastic) with a detachable plunger and a male Luer conical joint of 9 mm internal diameter at the lower end (Millipore syringe XX 11 050 05 is suitable) to which an adaptor for filtration is attached.

The adaptor is made of suitable material (a filtration adapter Millipore SX00 013 00 in polypropylene is suitable) and has a female joint for connecting it with the syringe. It is devised so as to be separable into two parts to permit the exchange of filters, the lower part containing a support for membrane filters 13 mm in diameter. A suitable prefilter (Millipore prefilter AP 2001 300 is suitable) and a membrane filter made of mixed cellulose esters, 13 mm in diameter, with a pore opening of 3 μm (a Millipore filter SSWP 013 00 is suitable) are used for the filtration.

Recommended procedure

Preparation of test solution

Procedure 1. Weigh the quantity of substance specified in the monograph, dissolve it in 25 mL of water, adjust the pH of the solution to 3–4 with acetic acid (~60 g/l) PbTS, or with ammonia (~100 g/l) PbTS, as necessary, then dilute to 40 mL with water and mix.

Procedure 2. Weigh the quantity of substance specified in the monograph, dissolve it in about 30 mL of solvent specified (ethanol (~750 g/l) TS, methanol R, acetone R, or dioxan R may be used), add 0.5 mL of acetic acid (~300 g/l) TS, and dilute to 40 mL with the solvent.
Procedure 3. Place the quantity of the substance specified in the monograph in a suitable crucible, preferably made of silica, and carefully ignite at a low temperature until the contents are thoroughly charred. The crucible may be loosely covered with a lid during the charring. Add to the contents of the crucible 2 mL of nitric acid (~1000 g/l) TS and 5 drops of sulfuric acid (~1760 g/l TS), and cautiously heat until white fumes are evolved, and then ignite, preferably in a muffle furnace, at 500°C until all the carbon is burned off. Cool, add 2 mL of hydrochloric acid (~250 g/l) TS, and slowly evaporate in a water bath to dryness. Moisten the residue with 1 drop of hydrochloric acid (~250 g/l) TS, add 10 mL of hot water, and digest for 2 minutes. Add, drop by drop, ammonia (~100 g/l) PbTS, until the pH of the solution is between 8 and 8.5, then add, drop by drop, acetic acid (~60 g/l) PbTS, to adjust the pH to between 3 and 4. Filter if necessary, wash the crucible and the filter with about 10 mL of water, dilute with water to 40 mL, and mix.

Procedure 4. Place the quantity of substance specified in the monograph in a suitable crucible, preferably made of silica, mix it well with about 0.5 g of magnesium oxide R and incinerate until a homogeneous white mass is obtained. If after 15 minutes of incineration the residue is still coloured, let the crucible cool, mix the contents well with a glass rod and resume heating. Next, dissolve the residue in hydrochloric acid (~70 g/l) TS, add, drop by drop, a solution of ammonia (~100 g/l) PbTS, until the pH of the solution is between 8 and 8.5, then add, also drop by drop, acetic acid (~60 g/l) PbTS, to adjust the pH to 3-4, filter, dilute with water to 40 mL, and mix.

Colour development and measurement

Method-A

To 40 mL of the liquid contained in the comparison tube add 10 mL of freshly prepared hydrogen sulfide TS, mix and allow to stand for 5 minutes.

In another comparison tube place a volume of solution of dilute lead PbTS, containing the lead equivalent of the heavy metals limit specified in the monograph, dilute with water, adjust the pH with ammonia (~100 g/l) PbTS and acetic acid (~60 g/l) PbTS to 3-4, dilute with water or the
solvent used to 40 mL, mix, add 10 mL of freshly-prepared hydrogen sulfide TS, mix and allow to stand for 5 minutes.

Compare the colours by viewing down the vertical axis of the tube in diffused light against a white background, or by another suitable method. The colour of the test solution is not darker than that of the lead standard.

Method B

Take the filtration syringe, arrange the prefilter and the membrane filter as indicated in the diagram for prefiltration, remove the plunger from the syringe, place the test solution inside the syringe, replace the plunger, and filter the test solution slowly by exerting a regular pressure on the plunger. Collect the filtrate in a beaker or a test tube. Open the adapter and check whether the membrane filter is free from impurities. If not, replace it and repeat the operation in the same manner. Then rearrange the prefilter and membrane filter as indicated in Fig. 4. Adjust the pH of the filtrate with ammonia (~100 g/l) PbTS and acetic acid (~60 g/l) PbTS to 3-4, add 10 mL of freshly prepared hydrogen sulfide TS, all the reagents previously filtered through a membrane filter, mix, allow to stand for 5 minutes, take out the plunger, place the solution inside the syringe, and filter it through the membrane filter by exerting slowly a regular and moderate pressure on the plunger. Open the adaptor and take out the membrane filter.

FIG. 4. LIMIT TEST FOR HEAVY METALS: METHOD B
Take a volume of solution of dilute lead PbTS containing the lead equivalent to the heavy metals limit specified in the monograph, dilute with water, adjust the pH with ammonia (~100 g/l) PbTS and acetic acid (~60 g/l) PbTS to 3–4, dilute with water to 40 mL, mix, and proceed as described above.

Compare the intensity of the coloration of spots obtained on the membrane filters. The colour obtained from the test solution is not more intense than that from the lead standard.

**REAGENTS TO BE ADDED OR REVISED**

**Acetate buffer, pH 3.5, TS**

*Procedure.* Dissolve 25.0 g of ammonium acetate R in 25 mL of water R and add 38.0 mL of hydrochloric acid (~250 g/l) TS. Adjust the pH, if necessary, with hydrochloric acid (~70 g/L) TS or ammonia (~100 g/L) TS. Dilute with water R to 100.0 mL.
Magnesium oxide R1
Complies with the requirements prescribed for magnesium oxide R with the following modifications:
Arsenic: maximum 2 ppm.
Heavy metals (2.2.3): maximum 10 ppm.
Iron: maximum 50 ppm.

Phenolphthalein/ethanol TS, diluted
Procedure. Dissolve 0.1 g of phenolphthalein R in sufficient ethanol (~750 g/L) TS to produce 100 mL.
Sensitivity test. To 0.1 mL of the phenolphthalein solution add 100 mL of carbon dioxide-free water R. The solution is colourless. Not more than 0.2 mL of 0.02 M sodium hydroxide is required to change the colour to pink.
Colour change: pH 8.2 (colourless) to pH 10.0 (red).

Thioacetamide R
C_2H_5NS = 75.13 (62-55-5).
General reagent grade of commerce.
White crystals or crystalline powder; melting point, about 113 °C.

Thioacetamide reagent TS
Add 1 mL of a mixture of 15 mL of 1m sodium hydroxide, 5 mL of water and 20 mL of glycerol (85%) to 0.2 mL of thioacetamide solution, heat in a water bath for 20 seconds, cool and use immediately.

Thioacetamide solution TS
A 4% w/v solution of thioacetamide R.

***