METHOD G

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 be conform to the manufacturer's instructions. The digestion cycle is suitable if a clear solution is obtained.

Test solution. 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 *sulphuric acid R*, 3.3 ml of *nitric acid R* and 2.0 ml of *strong hydrogen peroxide solution R* using a magnetic stirrer. Allow the substance to react with a reagent before adding the next one. Transfer the mixture to a dry high-pressure-resistant digestion vessel (fluoropolymer or quartz glass).

Reference solution (standard). Prepare as described for the test solution, using the prescribed volume of *lead standard solution (10 ppm Pb) R* instead of the substance to be examined.

Monitor solution. Prepare as prescribed for the test solution, adding to the substance to be examined the volume of *lead standard solution (10 ppm Pb) R* prescribed for the preparation of the reference solution.

Blank solution. Prepare as described for the test solution, omitting the substance to be examined.

Close the vessels and place in a laboratory microwave oven. Digest using a sequence of 2 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 *strong hydrogen peroxide solution R* 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 *strong hydrogen peroxide solution R* 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.

Using short-range pH indicator paper as external indicator, adjust the solutions to pH 3.0-4.0 with *concentrated ammonia R1* (*dilute ammonia R1* 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 *buffer solution pH 3.5 R* and 1.2 ml of *thioacetamide reagent R*. Mix immediately. Dilute to 50 ml with *water R*, mix and allow to stand for 2 min.

Filter the solutions through a membrane filter (pore size 3 µm; see Figure 2.4.8.-1, without the prefilter). 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.

Examine the spots on the filters. The brown colour from the spot of the test solution is not more intense than that from the reference solution.

The test is invalid if the reference solution spot does not show a brown colour compared to the blank spot, or if the spot from the monitor solution is not comparable with the spot from the reference solution.

Limit Tests for Heavy Metals

(Ph. Eur. method 2.4.8)

Test A

To 12 ml of the prescribed aqueous solution add 2 ml of *acetate buffer pH 3.5*, mix, add to 1.2 ml of *thioacetamide reagent*, mix immediately and allow to stand for 2 minutes. Any brown colour produced is not more intense than that obtained by treating in the same manner a mixture of 10 ml of either *lead standard solution (1 ppm Pb)* or *lead standard solution (2 ppm Pb)*, as prescribed, and 2 ml of the solution being examined. The standard solution exhibits a slightly brown colour when compared to a solution prepared by treating in the same manner a mixture of 10 ml of *water* and 2 ml of the solution being examined.

Test B

Dissolve the specified quantity of the substance being examined in an organic solvent containing a minimum percentage of water, such as *1,4-dioxan* or *acetone* containing 15% v/v of *water*, and carry out Test A but prepare the standard by diluting *lead standard solution (100 ppm Pb)* with the solvent used to prepare the test solution to contain 1 or 2 ppm of Pb, as specified. The standard solution exhibits a slightly brown colour when compared to a solution prepared by treating in the same manner a mixture of 10 ml of the solvent used to prepare the test solution and 2 ml of the solution being examined.

Test C

Place the prescribed quantity (usually not more than 2 g) of the substance being examined in a silica crucible with 4 ml of a 25% w/v solution of *magnesium sulphate* in 1M *sulphuric acid*. Mix using a fine glass rod and heat cautiously. If the mixture is liquid, evaporate gently to dryness on a water bath. Progressively heat to ignition, not allowing the temperature to exceed 800 , and continue heating until a white or at most greyish residue is produced. Allow to cool, moisten the residue with 0.2 ml of 1M *sulphuric acid*, evaporate, ignite again and allow to cool. The total period of ignition must not exceed 2 hours. Dissolve the residue using two 5-ml quantities of 2M *hydrochloric acid*. Add 0.1 ml of *phenolphthalein solution* and 13.5M *ammonia* dropwise until a pink colour is produced. Cool, add *glacial acetic acid* until the solution is decolorised and add a further 0.5 ml. Filter if necessary and dilute the solution to 20 ml with *water*.

To 12 ml of the resulting solution add 2 ml of *acetate buffer pH 3.5*, mix, add to 1.2 ml of *thioacetamide reagent*, mix immediately and allow to stand for 2 minutes. Any brown colour produced is not more intense than that obtained by treating in the same manner a mixture of 2 ml of the test solution obtained above and 10 ml of the 20 ml of solution obtained by repeating the procedure using the prescribed volume of *lead standard solution (10 ppm Pb)* in place of the substance being examined, adding 4 ml of a 25% w/v solution of *magnesium sulphate* in 1M *sulphuric acid* and beginning at the words 'Mix with a fine glass rod . . .'.

The standard solution exhibits a slightly brown colour when compared to a solution prepared by treating in the same manner a mixture of 10 ml of *water* and 2 ml of the solution being examined.

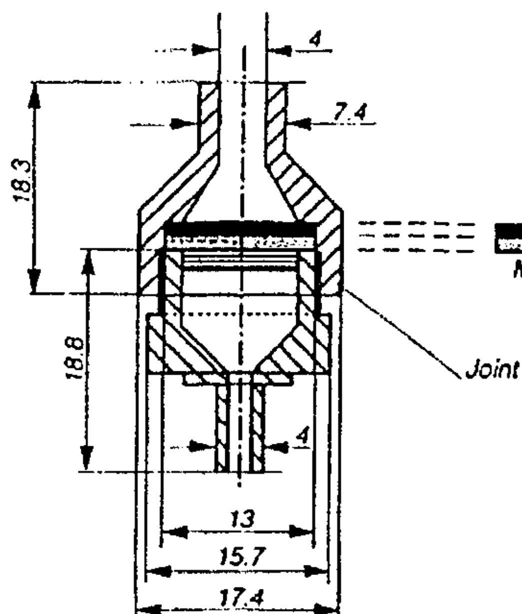
Test D

Mix the prescribed quantity of the substance being examined with 0.5 g of *magnesium oxide R1* in a silica crucible. Ignite to dull red heat until a homogeneous white or greyish white mass is produced. If after 30 minutes of ignition the mixture remains coloured, allow to cool, mix with a fine glass rod and repeat the ignition. If necessary, repeat the operation. Finally heat at 800° for about 1 hour, dissolve the residue using two 5-ml quantities of 5M *hydrochloric acid* and carry out the procedure described under Test C beginning at the words 'Add 0.1 ml of...'.

To prepare the standard add the prescribed volume of *lead standard solution (10 ppm Pb)* to 0.5 g of *magnesium oxide R1* contained in a silica crucible, dry the mixture in an oven at 100° to 105°, ignite as described above, dissolve the residue using two 5-ml quantities of 5M *hydrochloric acid* and carry out the procedure described under Test C beginning at the words 'add 0.1 ml of...', and use a mixture of 10 ml of the resulting solution and 2 ml of the test solution. The standard solution exhibits a slightly brown colour when compared to a solution prepared by treating in the same manner a mixture of 10 ml of *water* and 2 ml of the solution being examined.

Test E

Use a membrane filter holder, the dimensions of which are shown in Fig.7 3, fitted with a 50-ml syringe.
The membrane filter disc (C) should have a nominal pore diameter of 3 µm. It is protected by a prefilter (B). Dissolve the prescribed quantity of the substance being examined in 30 ml of *water* unless otherwise specified in the monograph. Filter the solution applying an even pressure. Dismantle the holder and check that the membrane filter remains uncontaminated; if necessary replace the membrane filter and repeat the filtration. To the filtrate, or the prescribed volume of the filtrate, add 2 ml of *acetate buffer pH 3.5* and add to 1.2 ml of *thioacetamide reagent*, mix and allow to stand for 10 minutes. Invert the order of the filters and filter the solution applying slow and even pressure. Remove the membrane filter and dry using filter paper. The intensity of any stain produced on the membrane filter is not more intense than that obtained by treating the prescribed volume of *lead standard solution (1 ppm Pb)* in the same manner.



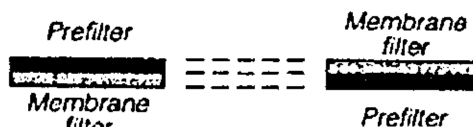


Fig. 7-3
Apparatus for Limit Test E for Heavy Metals

Dimensions in mm

Fig. 7-3a Pre-filtration of the solution

Fig. 7-3b Filtration of the solution after addition of the reagents

Test F

Test solution

Place the prescribed quantity or volume of the substance to be examined in a clean, dry, 100 ml Kjeldahl flask (a 300 ml flask may be used if the reaction foams excessively). Clamp the flask at an angle of 45°, and add a sufficient volume of a mixture of 8 ml of *sulphuric acid* and 10 ml of *nitric acid*. Warm gently until the reaction commences, allow the reaction to subside and add additional portions of the same acid mixture, heating after each addition, until a total of 18 ml of the acid mixture has been added. Increase the amount of heat and boil gently until the solution darkens. Cool, add 2 ml of *nitric acid* and heat again until the solution darkens. Continue the heating, followed by the addition of *nitric acid* until no further darkening occurs, then heat strongly until dense, white fumes are produced. Cool, cautiously add 5 ml of *water*, boil gently until dense, white fumes are produced and continue heating to reduce to 2 ml to 3 ml. Cool, cautiously add 5 ml of *water* and examine the colour of the solution. If the colour is yellow, cautiously add 1 ml of *strong hydrogen peroxide solution* and again evaporate until dense, white fumes are produced and reduce to a volume of 2 ml to 3 ml. If the solution is still yellow in colour, repeat the addition of 5 ml of *water* and 1 ml of *strong hydrogen peroxide solution* until the solution is colourless. Cool, dilute cautiously with *water* and rinse into a 50 ml colour comparison tube, ensuring that the total volume does not exceed 25 ml.

Adjust the solution to a pH between 3.0 and 4.0 using short range pH indicator paper as external indicator, with *concentrated ammonia R1* (*dilute ammonia R1* may be used, if

desired, as the specified range is approached), dilute with *water* to 40 ml and mix.

Add 2 ml of *buffer solution pH 3.5* and 1.2 ml of *thioacetamide reagent*. Mix immediately. Dilute to 50 ml with *water* and mix.

REFERENCE SOLUTION

Prepare at the same time and in the same manner using the prescribed volume of *lead standard solution (10 ppm Pb)*.

After 2 min, any brown colour in the test solution is not more intense than that in the reference solution.

HEAVY METALS TEST

This test is designed to limit the content of common metallic impurities colored by sulfide ion (Ag, As, Bi, Cd, Cu, Hg, Pb, Sb, Sn) by comparing the color with a standard containing lead ion (Pb) under the specified test conditions. It demonstrates that the test substance is not grossly contaminated by such heavy metals and, within the precision of the test, that it does not exceed the *Heavy Metals* limit given in the individual monograph as determined by concomitant visual comparison with a control solution. In the specified pH range, the optimum concentration of lead ion (Pb) for matching purposes by this method is 20 µg in 50 mL of solution.

The most common limitation of the *Heavy Metals Test* is that the color the sulfide ion produces in the *Sample Solution* depends on the metals present and may not match the color in the *Lead Solution* used for matching purposes. Lead sulfide is brown, as are Ag, Bi, Cu, Hg, and Sn sulfides. While it is possible that ions not mentioned here may also yield nonmatching colors, among the nine common metallic impurities listed above, the sulfides with different colors are those of As and Cd, which are yellow, and that of Sb, which is orange. If a yellow or orange color is observed, the following action is indicated: If the monograph does not include an arsenic requirement, As should be determined. Any As found should not exceed the requirement in the monograph, or 3 mg/kg if there is no requirement. If these criteria are met, Cd may be a contributor to the yellow color, so the Cd content should be determined. If an orange color is observed, the Sb content should be determined. These additional tests are in accord with the section on *Trace Impurities* in the *General Provisions* of this book, as follows: ". . . if other possible impurities may be present, additional tests may be required, and should be applied, as necessary, by the manufacturer, vendor, or user to demonstrate that the substance is suitable for its intended application in foods or in food processing."

Determine the amount of heavy metals by *Method I* unless otherwise specified in the individual monograph. Use *Method I* for substances that yield clear, colorless solutions before adding sulfide ion. Use *Method II* for those substances that do not yield clear, colorless solutions under the test conditions specified for *Method I* or for substances that by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion. Use *Method III*, a wet digestion method, only in those cases where neither *Method I* nor *Method II* can be used.

Special Reagents

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute with water to 1000.0 mL, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each mL of *Standard Lead Solution* contains the equivalent of 10 µg of lead ion (Pb).

Procedure (**Note**: In the following procedures, failure to accurately adjust the pH of the solution within the specified limits may result in a significant loss of test sensitivity.)

Method I

Solution A Pipet 2.0 mL of *Standard Lead Solution* (20 µg of Pb) into a 50-mL color-comparison tube, and add water to make 25 mL. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute with water to 40 mL, and mix.

Solution B Place 25 mL of the solution, prepared as directed in the individual monograph, into a 50-mL color-comparison tube that matches the one used for *Solution A*, adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute with water to 40 mL, and mix.

Solution C Place 25 mL of the solution prepared as directed in the individual monograph into a third color-comparison tube that matches those used for *Solutions A* and *B*, and add 2.0 mL of *Standard Lead Solution*. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid or 6 *N* ammonia, dilute with water to 40 mL, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*, and the intensity of the color of *Solution C* is equal to or greater than that of *Solution A*. If the color of *Solution C* is lighter than that of *Solution A*, the test substance is providing an interference with the test procedure, and *Method II* must be used for the substance under examination.

Method II

Solution A Prepare as directed under *Method I*.

Solution B Place the quantity, accurately weighed, of sample specified in the individual monograph in a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 mL of nitric acid and 5 drops of sulfuric acid, cautiously heat until white fumes evolve, then ignite, preferably in a muffle furnace, at 500° to 600° until all of the carbon is burned off. Cool, add 4 mL of

dilute hydrochloric acid (1 in 2), cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 min. Add 6 *N* ammonia dropwise until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 *N* acetic acid. Filter if necessary, rinse the crucible and the filter with 10 mL of water, transfer the solution and rinsings to a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

Method III

Solution A Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid into a 100-mL Kjeldahl flask, clamp the flask at an angle of 45°, and then add, in small increments, an additional volume of nitric acid equal to that added in the preparation of *Solution B*, below. Heat the solution to dense, white fumes, cool, and cautiously add 10 mL of water. Add a volume of hydrogen peroxide (30%) equal to that added in the preparation of *Solution B*, below, then boil gently to dense, white fumes, and cool. Cautiously add 5 mL of water, mix, and boil gently to dense, white fumes. Continue boiling until the volume is reduced to about 2 or 3 mL, then cool, and dilute cautiously with a few mL of water. Into this solution pipet 2.0 mL of *Standard Lead Solution*, and mix. Transfer into a 50-mL color-comparison tube, rinse the flask with water, adding the rinsings to the tube until the volume is 25 mL, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) with ammonium hydroxide initially, and then with 6 *N* ammonia as the desired range is neared, dilute with water to 40 mL, and mix.

Solution B Transfer the quantity, accurately weighed, of sample specified in the individual monograph into a 100-mL Kjeldahl flask (or into a 300-mL flask if the reaction foams excessively) clamp the flask at an angle of 45°, and then add a sufficient amount of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to moisten the sample thoroughly.

Note: For liquid samples, use 3 mL of the acid mixture.

Warm gently until the reaction commences, allow the reaction to subside, and then add additional portions of the acid mixture, heating after each addition, until all of the 18 mL of acid mixture has been added. Increase the heat, and boil gently until the reaction mixture darkens. Remove the flask from the heat, add 2 mL of nitric acid, and heat to boiling again. Continue the intermittent heating and addition of 2-mL portions of nitric acid

until no further darkening occurs, then heat strongly to dense, white fumes, and cool. Cautiously add 5 mL of water, mix, boil gently to dense, white fumes, and continue heating until the volume is reduced to about 2 or 3 mL. Cool, cautiously add 5 mL of water, and examine. If the solution is yellow, cautiously add 1 mL of hydrogen peroxide (30%), and again evaporate to dense, white fumes and to a volume of about 2 or 3 mL. Cool, dilute cautiously with a few mL of water, and mix. Transfer

into a 50-mL color-comparison tube, rinse the flask with water, adding the rinsings to the tube until the volume is 25 mL, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) with ammonium hydroxide initially, and then with 6 *N* ammonia as the desired range is neared, dilute with water to 40 mL, and mix.

To each tube add 10 mL of freshly prepared hydrogen sulfide TS, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

HEAVY METALS LIMIT TEST

This is an empirical "catch-all" test for the following heavy metals in addition to lead: mercury, cadmium, antimony, arsenic (partial), silver, copper, and certain others. All these metals give a colour with hydrogen sulfide and the test is designed to demonstrate that the total amounts present, expressed in terms of lead, do not exceed the heavy metal limits laid down in the various monographs. Zinc and tin also form sulfides and although these are not coloured at the pH of the test (3-4) they may influence the test to some degree. The test is useful in indicating the presence of heavy metal

impurities in a raw material from a new source and in detecting accidental contamination of which the manufacturer might otherwise be unaware. It also provides confirmation of good manufacturing practice. However, if the method of manufacture or the grade of raw materials employed gives reason to believe that an impurity such as mercury or cadmium may be present, a specific test for the impurity in question should be employed.

Method I is used for substances that yield clear colourless solutions prior to addition of sulfide ion and should be used in the Procedure unless otherwise directed in the individual monograph. Method II is used for those substances that do not yield clear colourless solutions under the test conditions specified in Method I or for those which, by virtue of their complex nature, interfere with the precipitation of heavy metals by sulfide.

Reagents

Ammonia TS

Dilute 400 ml of reagent grade ammonium hydroxide to 1,000 ml with water.

Hydrochloric acid

Use reagent grade hydrochloric acid in preparing all solutions of hydrochloric acid employed in this test.

Lead nitrate stock solution

Dissolve 159.8 mg of lead nitrate, $Pb(NO_3)_2$, in 100 ml of water containing 1 ml of nitric acid, then dilute with water to 1,000 ml and mix. This solution should be prepared and stored in a glass container which is free from lead salts.

Standard lead solution

On the day of use, dilute 10.0 ml of Lead Nitrate Stock Solution, accurately measured, with water to 100.0 ml. Each ml of the solution so prepared contains the equivalent of 10 µg of lead ion (Pb).

Procedure

Note. In the following procedures for Methods I and II, failure to adjust accurately the pH of the solutions within the specified limits may result in a significant loss of test sensitivity.

METHOD I

Solution A

Pipet into a 50-ml Nessler tube 2 ml of Standard lead solution [20 µg of lead ion (Pb)] unless otherwise stated in the individual monograph. Adjust the pH to between 3.0 and 4.0 (short-range pH indicator paper) by the addition of dilute acetic acid TS or ammonia TS, dilute with water to 40 ml and mix.

Solution B

Place in a 50-ml Nessler tube that matches the one used for Solution A, 25 ml of the sample solution prepared as directed in the individual monograph, adjust the pH to between 3.0 and 4.0 (short-range pH indicator paper) by the addition of dilute acetic acid TS or ammonia TS, dilute to 40 ml with water, and mix.

Solution C

Place into a third 50-ml Nessler tube that matches the ones used for Solution A and B, 25 ml of the sample solution prepared as directed in the individual monograph and add the same volume of Standard lead solution as was added to Solution A. Adjust the pH to between 3.0 and 4.0 (short-range indicator paper) by the addition of dilute acetic acid TS or ammonia TS, dilute with water to 40 ml and mix.

Working in a fume hood, add to each tube 10 ml of freshly prepared hydrogen sulfide TS, mix, allow to stand for 5 min, and view downward over a white surface. The colour of Solution B is no darker than that of Solution A and the colour of Solution C is equal to or greater than that of Solution A. If the colour of Solution C is lighter than that of Solution A, the test substance is providing an interference and Method II must be used.

Method II

Proceed as directed under Method I preparing Solution B as follows:

Working in a fume hood, place the specified quantity of the sample, accurately weighed, in a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° to 600° until all the carbon is burned off. Cool, add 4 ml of dilute hydrochloric acid (1 in 2), cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness.

Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Add dropwise ammonia TS until the solution is just alkaline to litmus paper, dilute with water to 25 ml, and adjust the pH to between 3.0 and 4.0 (short-range pH indicator paper) by the addition of dilute acetic acid TS. Filter if necessary, wash the crucible and the filter with 10 ml of water, transfer the solution and rinsings to a 50-ml Nessler tube, dilute with water to 40 ml, and mix.

Prepare Solution C in the same manner as Solution B, adding to the sample in the crucible the same volume of Lead Standard Solution as was added to Solution A. Proceed as in Method I from "Working in a fume hood, add... ".

第７版　食品添加物公定書

ヒ素試験法

ヒ素試験法は、試料中に混在するヒ素の許容される限度量を試験する方法である。その量は、三酸化ヒ素（As_2O_3）の量として表す。

以下、本試験法を用いる場合において、例えば、「As_2O_3として4.0μg/g以下（0.25ｇ、第１法、装置A）」とあるのは、本品0.25ｇを量り、試料とし、第１法により検液を調製し、装置Aを用いる方法により試験を行うとき、ヒ素が、As_2O_3として4.0μg/g以下であることを示す。

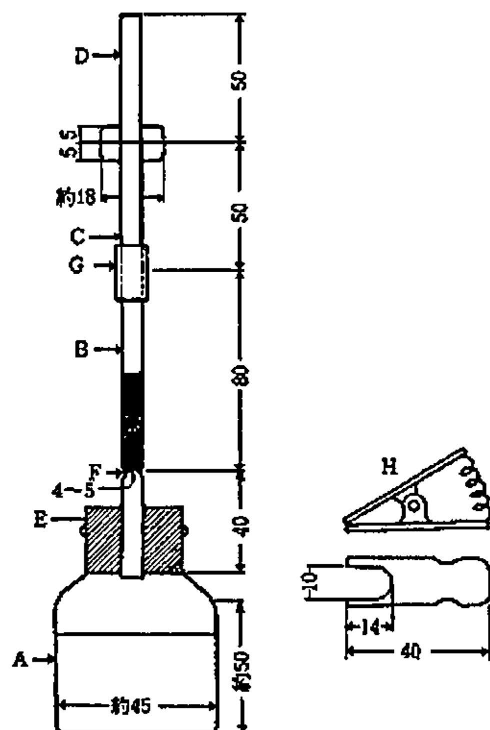
装置A

概略は、図１による。

ガラス管Bにはガラス繊維をF部から約30mmの高さまで詰め、酢酸鉛試液及び水の等容量の混液で均等に潤し、管の下端から静かに吸引してガラス繊維及び器壁から過量の液を除いておく。

使用の直前、ガラス管C及びDの接続部に臭化第二水銀紙を挟み、クリップHで両管を固定する。

A：発生瓶（容量約60mlで、40mlの標線があるもの）
B：内径約6.5mmのガラス管
C及びD：接続部が内径6.5mm、外径約18mmで、すり合わせとなっているガラス管で、接続部の内縁と外縁が同心円をなしているもの
E：ゴム栓
F：ガラス管Bに付けたへこみで、ガラス繊維を支える。
G：ゴム管
H：クリップ



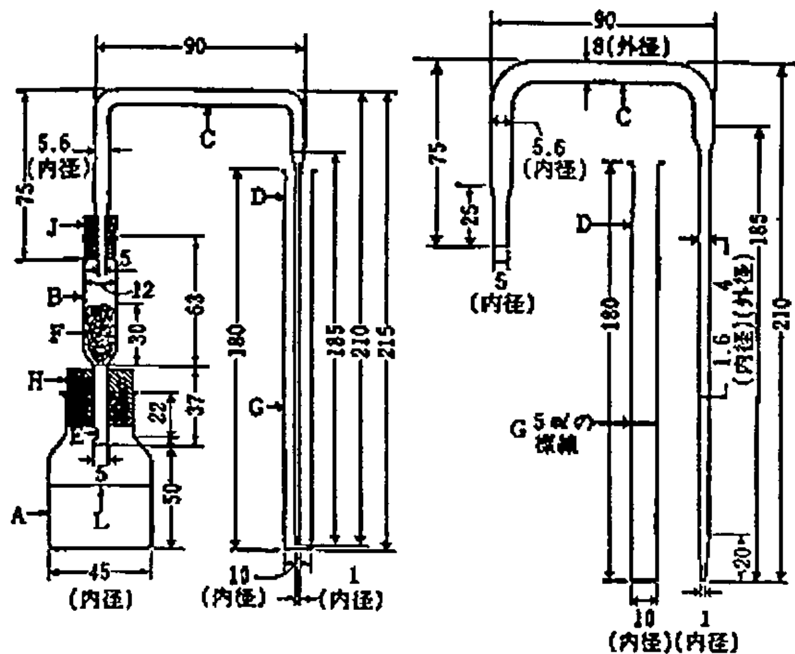
（単位 mm）
図１

出典：食品衛生関係法規集

装置Ｂ

概略は、図２による。

排気管Ｂに約 30mm の高さにガラス繊維Ｆを詰め、酢酸鉛試液及び水の等容量混液で均等に潤した後、下端から弱く吸引して、過量の液を除く。これをゴム栓Ｈの中心に垂直に差し込み、Ｂの下部の小孔Ｅは下にわずかに突きでるようにして発生瓶Ａに付ける。Ｂの上端にはガラス管Ｃを垂直に固定したゴム栓Ｊを付ける。Ｃの排気管側の下端はゴム栓Ｊの下端と同一平面とする。



(単位mm)

図２

Ａ：発生瓶（肩までの容量約70ml）

Ｂ：排気管

Ｃ：ガラス管（内径5.6mm、吸収管に入れる部分は先端を内径１mmに引き伸ばす。）

Ｄ：吸収管（内径10mm）

Ｅ：小孔

Ｆ：ガラス繊維（約0.2g）

Ｇ：５mlの標線

Ｈ及びＪ：ゴム栓

Ｌ：40mlの標線

出典：食品衛生関係法規集

装置C

概略は、図3による。

A：定量ポンプ

B_1、B_2：ミクシングジョイント

C：反応管

D：圧力計

E：流量計

F：気液セパレータ

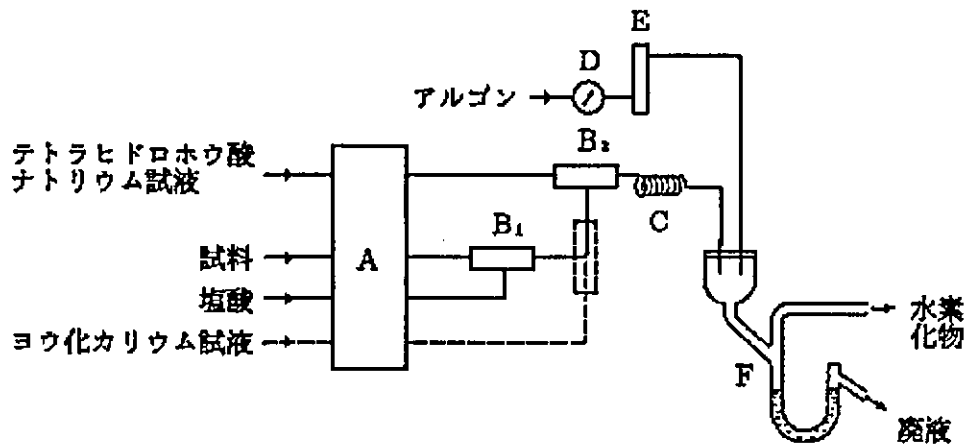


図3

出典：食品衛生関係法規集

操作法

(1) 検液の調製

別に規定するもののほか、次の方法による。

第1法 別に規定する量の試料を量り、水5ml を加え、必要があれば加温して溶かし、検液とする。

第2法 別に規定する量の試料を量り、水5ml 及び硫酸1ml を加える。ただし、無機酸の場合には硫酸を加えない。これに亜硫酸 10ml を加え、小ビーカーに入れ、水浴上で加熱して亜硫酸がなくなり約2ml となるまで蒸発し、水を加えて5ml とし、検液とする。

第3法 別に規定する量の試料を量り、白金製、石英製又は磁製のるつぼに入れ、硝酸マグネシウムのエタノール溶液(1→50) 10ml を加え、エタノールに点火して燃焼させた後、徐々に加熱して 450～550℃で灰化する。なお炭化物が残るときは、少量の硝酸マグネシウムのエタノール溶液（1→50）で潤し、再び強熱して 450～550℃で灰化する。冷後、残留物に塩酸3ml を加え、水浴上で加温して溶かし、検液とする。

第4法 別に規定する量の試料を量り、白金製、石英製又は磁製のるつぼに入れ、硝酸マ

グネシウムのエタノール溶液（1→10）10ml を加え、エタノールに点火して燃焼させた後、徐々に加熱した後、450～550℃で灰化する。なお炭化物が残るときは、少量の硝酸マグネシウムのエタノール溶液（1→50）で潤し、再び強熱して、450～550℃で灰化する。冷後、残留物に塩酸3ml を加え、水浴上で加温して溶かし、検液とする。

（2） 試験

別に規定するもののほか、次の方法による。

①装置Aを用いる方法

検液を発生瓶に入れ、ブロモフェノールブルー試液1滴を加え、アンモニア水、アンモニア試液又は塩酸（1→4）で中和し、塩酸（1→2） 5ml 及びヨウ化カリウム試液5ml を加え、2～3分間放置した後、酸性塩化第一スズ試液5ml を加えて 10 分間放置する。次に水を加えて 40ml とし、無ヒ素亜鉛2ｇを加え、直ちにガラス管B、C及びDを付けたゴム栓Eを施し、25℃の水中に発生瓶の肩まで浸し、1時間放置した後、直ちに臭化第二水銀紙の色を観察するとき、この色は、次の標準色より濃くない。

標準色の調製は、検液の試験と同時に行い、ヒ素標準液 1.0ml を量り、発生瓶に入れ、塩酸（1→2） 5ml 及びヨウ化カリウム試液5ml を加え、以下検液の場合と同様に操作して得た臭化第二水銀紙の呈色を標準色とする。

②装置Bを用いる方法

検液を発生瓶に入れ、装置Aを用いる方法と同様に操作し、酸性塩化第一スズ試液5ml を加えて室温で 10 分間放置したのち、次に水を加えて 40ml とし、無ヒ素亜鉛2ｇを加え、直ちにB及びCを連結したゴム栓Hを発生瓶に付ける。Cの細管部の端はあらかじめヒ化水素吸収液5ml を入れた吸収管Dの底に達するように入れておく。次に発生瓶は25℃の水中に肩まで浸し、1時間放置する。吸収管をはずし、必要があればピリジンを加えて5ml とし、吸収液の色を観察するとき、この色は、次の標準色より濃くない。

標準色の調製は、検液の試験と同時に行い、ヒ素標準液 2.0ml を量り、発生瓶に入れ、塩酸（1→2） 5ml 及びヨウ化カリウム試液5ml を加えて2～3分間放置した後、酸性塩化第一スズ試液5ml を加え、室温で 10 分間放置する。以下検液の場合と同様に操作して得た吸収液の呈色を標準色とする。

③装置Cを用いる方法

検液4ml に塩酸1ml 及びヨウ化カリウム溶液（1→10） 1ml を加え、水浴上 70℃で4分間加温した後、水を加えて 20ml とする。装置にアルゴンを流しながら、この溶液及び適当な濃度の塩酸（1～6mol／L）、テトラヒドロホウ酸ナトリウム試液を、定量ポンプAを用いてそれぞれ1～10ml／L分の適当な流量で連続的に装置内に導入して順々に混合させ、水素化ヒ素を発生させる。なお、ヨウ化カリウム溶液（1→10）を定量ポンプで連続的に装置内に導入する方式にあっては、検液を直接又は水で適当な濃度に希釈後、この溶液及び適当な濃度の塩酸（1～6mol／L）、ヨウ化カリウム溶液（1→10）、テトラヒドロホウ酸ナトリウム試液を、上と同様な操作で装置に導入して順々に混合させ、水素化ヒ素を発

生させる。発生した水素化ヒ素と廃液を気液セパレータＦで分離した後、水素化ヒ素を含む気体を加熱吸収セルを取り付けた原子吸光度測定装置に導入し、波長 193.7nm の指示値を読むとき、その値は、比較液のものより大きくない。

ただし、比較液の調製は、検液の試験と同時に行い、別に規定する量のヒ素標準液を用いて、検液の場合と同様に操作して行う。

操作上の注意

（１） 試験に用いる器具・試薬及び試液は、ヒ素を含まないか、又はほとんど含まないものを用い、必要があれば空試験を行う。

（２） 装置Ａを用いる場合は発生ガスが漏れないように、臭化第二水銀紙を挟むすり合わせ部は、緊密につなぐ。

（３） 装置Ａを用いる場合は臭化第二水銀紙の呈色は、光、熱、湿気などによって退色するので、比色は、速やかに行う。デシケーター中に光を遮っておけば、しばらく保存することができる。

（４） 装置Ｃを用いる場合は、装置により試料、塩酸、テトラヒドロホウ酸ナトリウム試液、ヨウ化カリウム溶液の流量や、塩酸及びヨウ化カリウム溶液の濃度は異なり、更にテトラヒドロホウ酸ナトリウム試液とは異なる濃度のテトラヒドロホウ酸ナトリウム溶液を使用する場合もある。

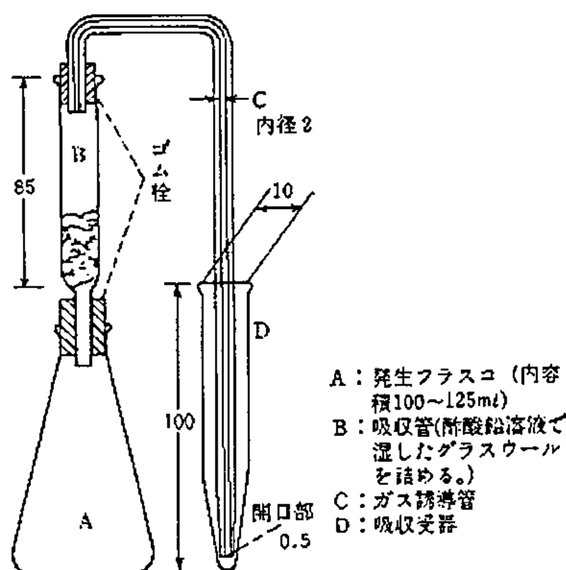
グットツァイト法

試験溶液３ml を採り、第２　添加物の部Ｂ　一般試験法の項のヒ素試験法中の装置Ａを用いる方法により試験を行うとき、その呈色は標準色より濃くてはならない。ただし、この場合の標準色は、空試験溶液３ml にヒ素標準液 1.2ml を加えた溶液について試験溶液の場合と同様に操作して作る。

ジエチルジチオカルバミン酸銀法

① 装置

概略は、次の図による（単位 mm)。



出典：食品衛生関係法規集

② 試薬・試液

次に示すもの以外は、第２　添加物の部Ｃ　試薬・試液等の項に示すものを用いる。

ジエチルジチオカルバミン酸銀ピリジン溶液：ジエチルジチオカルバミン酸銀１ｇをピリジン 200ml に溶かし、遮光して冷所に保存する。

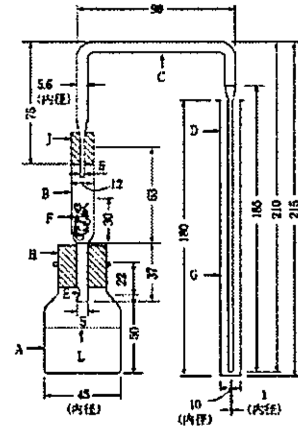
砂状亜鉛：20～30 メッシュの無ヒ素亜鉛を１％硫酸銅溶液に黒化するまで浸し、洗浄した後、乾燥する。

塩化第一スズ溶液：塩化第一スズ４ｇを無ヒ素塩酸 125ml に溶かし、水を加えて 250ml とし、共栓瓶に入れ、密栓して保存する。

③　試験操作

試験溶液 10ml を発生フラスコに採り、水を加えて 25ml とし、塩酸（１→２）５ml、ヨウ化カリウム溶液２ml 及び塩化第一スズ溶液５ml を加え、室温で 15 分間放置する。次いで、この発生フラスコに砂状亜鉛３ｇを加え、直ちに吸収管及びガス誘導管を連結し、あらかじめジエチルジチオカルバミン酸銀ピリジン溶液３ml を入れた吸収受器を接続して 20～25°で１時間放置する。次に、装置をはずし、ガス誘導管内の液を吸収受器内の吸収液に合わせてよく混和した後、この吸収液を１cm の吸収セルに採り、30 分以内にジエチルジチオカルバミン酸銀ピリジン溶液を対照液として波長 525nm 付近で吸光度を測定するとき、試験溶液の吸光度は、空試験溶液 10ml にヒ素標準液４ml を加えた後、水を加えて 25ml とした溶液について、試験溶液の場合と同様に操作して得られる吸光度を超えてはならない。

51.　ヒ素試験法



ヒ素試験法は，薬品中に混在するヒ素の限度試験である．その限度は三酸化二ヒ素（As_2O_3）の量として表す．

医薬品各条には，ヒ素（As_2O_3 として）の限度を ppm で（　）内に付記する．

装　置

図 51-1 に示す装置 B を用いる．

排気管 B に約 30 mm の高さにガラス繊維 F を詰め，酢酸鉛（Ⅱ）試液及び水の等容量混液で均等に潤した後，下端から弱く吸引して，過量の液を除く．これをゴム栓 H の中心に垂直にさし込み，B の下部の小孔 E は下にわずかに突き出るようにして発生瓶 A に付ける．B の上端にはガラス管 C を垂直に固定したゴム栓 J を付ける．C の排気管側の下端はゴム栓 J の下端と同一平面とする．

検液の調製法

別に規定するもののほか，次の方法による．

（1）　第 1 法

医薬品各条に規定する量の試料を量り，水 5 mL を加え，必要ならば加温して溶かし，検液とする．

（2）　第 2 法

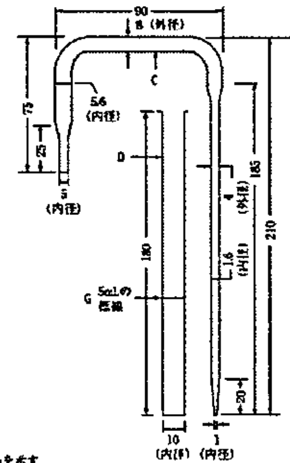
医薬品各条に規定する量の試料を量り，水 5 mL 及び硫酸 1 mL を加える．ただし，無機酸の場合には硫酸を加えない．これに亜硫酸水 10 mL を加え，小ビーカーに入れ，水浴上で加熱して亜硫酸がなくなり約 2 mL となるまで蒸発し，水を加えて 5 mL とし，検液とする．

（3）　第 3 法

医薬品各条に規定する量の試料を量り，白金製，石英製又は磁製のるつぼにとる．これに硝酸マグネシウム六水和物のエタノール（95）溶液（1 → 50）10 mL を加え，エタノールに点火して燃焼させた後，徐々に加熱して灰化する．もしこの方法で，なお炭化物が残るときは，少量の硝酸で潤し，再び強熱して灰化する．冷後，残留物に塩酸 3 mL を加え，水浴上で加温して溶かし，検液とする．

（4）　第 4 法

医薬品各条に規定する量の試料を量り，白金製，石英製又は磁製のるつぼにとる．これに硝酸マグネシウム六水和物のエタノール（95）溶液（1 → 10）10 mL を加え，エタノールに点火して燃焼させた後，徐々に加熱した後，強熱して灰化する．もしこの方法で，なお炭化物が残るときは，少量の硝酸で潤し，徐々に加熱した後，強熱して灰化する．冷後，残留物に塩酸 3 mL を加え，水浴上で加温して溶かし，検液とする．



A：発生瓶（肩までの内容約 70 mL）
B：排気管
C：ガラス管（内径 5.6 mm，吸収管に入れる部分は先端を内径 1 mm に引き伸ばす．）
D：吸収管（10 mm）
E：小孔
F：ガラスウール（約 0.2 g）
G：5 mL の標線
H及びJ：ゴム栓
L：40 mL の標線

図 51－1　ヒ素試験装置 B

(5) 第5法

医薬品各条に規定する量の試料を量り，N,N-ジメチルホルムアミド 10 mL を加え，必要ならば加温して溶かし，検液とする.

試 液

ヒ化水素吸収液：N,N-ジエチルジチオカルバミド銀 0.50 g をピリジンに溶かし 100 mL とする. この液は遮光した共栓瓶に入れ，冷所に保存する.

ヒ素標準原液：三酸化二ヒ素標準試薬を微細の粉末とし，105 °C で 4 時間乾燥し，その 0.100 g を正確に量り，水酸化ナトリウム溶液 (1 → 5) 5 mL に溶かす. この液に希硫酸を加えて中性とし，更に希硫酸 10 mL を追加し，新たに煮沸して冷却した水を加えて正確に 1000 mL とする.

ヒ素標準液：ヒ素標準原液 10 mL を正確に量り，希硫酸 10 mL を加え，新たに煮沸して冷却した水を加えて正確に 1000 mL とする. この液 1 mL は三酸化二ヒ素 (As_2O_3) 1 μg を含む. この液は用時調製し，共栓瓶に保存する.

操 作 法

別に規定するもののほか，装置 B を用いて試験を行う.