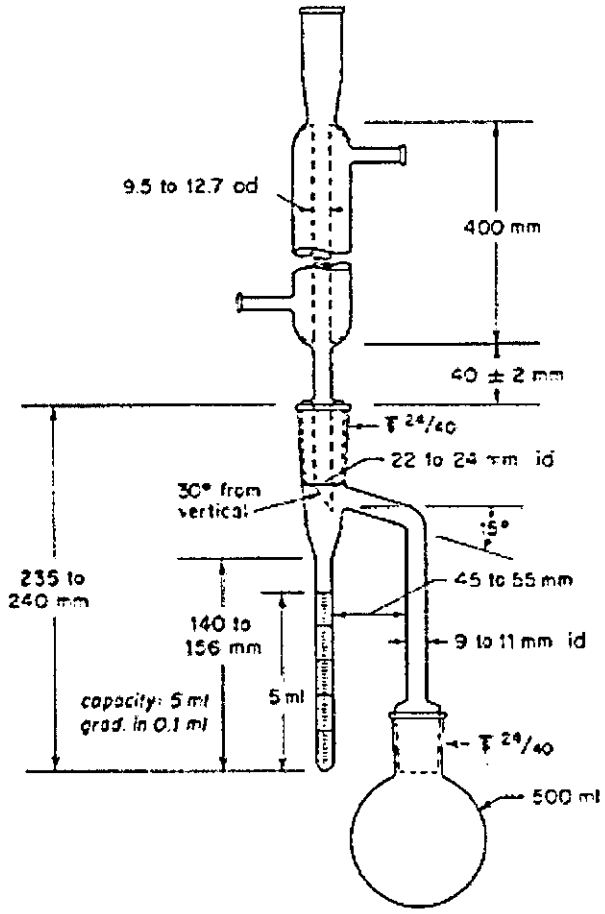

FIGURE 8 Moisture Distillation Apparatus.

1 mL of water, accurately measured, to 100 mL of toluene contained in the distillation flask. Conduct the distillation, and calculate the volume of water obtained as directed in the *Procedure*. Add another mL of water to the cooled apparatus, and repeat the distillation. Continue in this manner until five 1-mL portions of water have been added. The error at any indicated capacity should not exceed 0.05 mL. The source of heat is either an oil bath or an electric heater provided with a suitable means of temperature control. The distillation may be better controlled by insulating the tube leading from the flask to the receiver. It is also advantageous to protect the flask from drafts. Clean the entire apparatus with potassium dichromate–sulfuric acid cleaning solution, rinse thoroughly, and dry completely before using.

Procedure Place in the previously cleaned and dried flask a quantity of the substance, weighed accurately to the nearest 0.01 g, that is expected to yield from 1.5 to 4 mL of water. If the substance is of a pastelike consistency, weigh it in a boat of metal foil that will pass through the neck of the flask. If the substance is likely to cause bumping, take suitable precautions to prevent it. Transfer about 200 mL of ACS reagent-grade toluene into the flask, and swirl to mix it with the sample. Assemble the apparatus, fill the receiver with toluene by pouring it through the condenser until it begins to overflow into the flask, and insert a loose cotton plug in the top of the condenser. Heat the flask so that the distillation rate will be about 200

drops/min, and continue distilling until the volume of water in the trap remains constant for 5 min. Discontinue the heating, use a copper or nichrome wire spiral to dislodge any drops of water that may be adhering to the inside of the condenser tube or receiver, and wash down with about 5 mL of toluene. Disconnect the receiver, immerse it in water at 25° for at least 15 min or until the toluene layer is clear, and then read the volume of water. Conduct a blank determination using the same volume of toluene as used when distilling the sample mixture, and make any necessary correction (see *General Provisions*).

2.5.12. WATER: SEMI-MICRO DETERMINATION

The titration vessel, of about 60 ml capacity, is fitted with 2 platinum electrodes, a nitrogen inlet tube, a stopper which accommodates the burette tip, and a vent-tube protected by a desiccant. The substance to be examined is introduced through a side-arm which can be closed by a ground stopper. Stirring is effected magnetically or by means of a stream of dried nitrogen passed through the solution during the titration.

The end-point is determined by amperometry. A suitable circuit consists of a potentiometer of about 2000 Ω connected across a 1.5 V battery to supply a variable potential. This potential is adjusted so that an initial low current passes through the platinum electrodes connected in series with a microammeter. On adding the reagent, the needle of the microammeter shows a deflection but returns immediately to its starting position. At the end of the reaction, a deflection is obtained which persists for not less than 30 s.

Use the *iodosulphurous reagent R* after determination of the water equivalent (*4.1.1*). The reagents and solutions used must be kept anhydrous and precautions must be taken throughout to prevent exposure to atmospheric moisture.

The *iodosulphurous reagent R* is protected from light, preferably stored in a bottle to which is fitted an automatic burette.

The composition of commercially available iodosulphurous reagents often differs from that of iodosulphurous reagent R by the replacement of pyridine with various other basic compounds. The use of these reagents must previously be validated, in order to verify, in each individual case, the stoichiometry and the absence of incompatibility between the substance under test and the reagent (1.1. General Notices).

Unless otherwise prescribed, use Method A.

Method A. Add about 20 ml of *anhydrous methanol R* or the solvent prescribed in the monograph to the titration vessel and titrate to the amperometric end-point with the *iodosulphurous reagent R*. Quickly transfer the prescribed amount of the substance to be examined to the titration vessel. Stir for 1 min and titrate again to the amperometric end-point using *iodosulphurous reagent R*.

Method B. Add about 10 ml of *anhydrous methanol R* or the solvent prescribed in the monograph to the titration vessel and titrate to the amperometric end-point with *iodosulphurous reagent R*. Quickly transfer the prescribed amount of the substance to be examined in a suitable state of division followed by an accurately measured volume of *iodosulphurous reagent R*, sufficient to give an excess of about 1 ml or the volume prescribed in the monograph. Allow the stoppered flask to stand protected from light for 1 min or the time prescribed in the monograph, stirring from time to time. Titrate the excess of *iodosulphurous reagent R* until the initial low current is again obtained, using *anhydrous methanol R* or the solvent prescribed in the monograph, to which has been added an accurately known amount of *water R* equivalent to about 2.5 g/l.

第７版　食品添加物公定書

乾燥減量試験法

乾燥減量試験法は、試料を規定された条件で乾燥するとき失われる水分及び揮発性物質の量を測定する方法である。

以下、本試験法を用いる場合において、「例えば、0.50%以下（105℃、３時間）」とあるのは、試料１～２ｇを精密に量り、105℃で３時間乾燥するとき、その減量が試料の採取量に対して0.50%以下であることを示し、また、「0.50%以下（0.5ｇ、1.3kPa 以下、24 時間）」とあるのは、試料約 0.5ｇを精密に量り、シリカゲルを乾燥剤としたデシケーターに入れ、1.3kPa 以下の減圧下で 24 時間乾燥するとき、その減量が試料の採取量に対して 0.50%以下であることを示す。

操作法

あらかじめひょう量瓶を別に規定する乾燥条件に準じて約 30 分間乾燥し、加熱した場合はデシケーター中で放冷した後、その重量を精密に量る。試料が大きな結晶又は塊の場合は、速やかに粉砕して径約２mm 以下の大きさとし、別に規定するもののほか、その１～２ｇを先のひょう量瓶に入れ、厚さ５mm 以下の層となるように広げた後、その重量を精密に量る。次にこれを乾燥器に入れ、栓をとってそばに置き、別に規定する条件で乾燥した後、栓をして乾燥器から取り出してその重量を精密に量る。加熱した場合は、別に規定するもののほか、デシケーター中で放冷した後、その重量を精密に量る。なお、試料が規定の乾燥温度より低い温度で融解する場合は、その融解温度より５～10℃低い温度で１～２時間乾燥した後、別に規定する乾燥条件で乾燥する。

10.　乾燥減量試験法

乾燥減量試験法は，試料を医薬品各条に規定する条件で乾燥し，その減量を測定する方法である．この方法は乾燥することによって失われる試料中の水分，結晶水の全部又は一部及び揮発性物質などの量を測定するために用いる．

医薬品各条に，例えば 1.0 % 以下 (1 g, 105 °C, 4 時間) と規定するものは，本品約 1 g を精密に量り，105 °C で 4 時間乾燥するとき，その減量が本品 1 g につき 10 mg 以下であることを示し，また，0.5 % 以下 (1 g, 減圧, 酸化リン (V), 4 時間) と規定するものは，本品約 1 g を精密に量り，酸化リン (V) を乾燥剤としたデシケーターに入れ，4 時間減圧乾燥するとき，その減量が本品 1 g につき 5 mg 以下であることを示す．

操 作 法

はかり瓶をあらかじめ，医薬品各条に規定する方法に準じて 30 分間乾燥し，その質量を精密に量る．試料は医薬品各条に規定する量の ± 10 % の範囲内で採取し，はかり瓶に入れ，別に規定するもののほか，その層が 5 mm 以下になるように広げた後，その質量を精密に量り，これを乾燥器に入れ，医薬品各条に規定する条件で乾燥する．試料が大きいときは，手早く粉砕して径 2 mm 以下としたものを用いる．乾燥後，乾燥器から取り出し，質量を精密に量る．加熱して乾燥する場合は，加熱温度を医薬品各条に規定する温度の ± 2 °C の範囲とし，乾燥後，デシケーター (シリカゲル) で放冷する．

医薬品各条に規定する乾燥温度よりも低温で融解する試料は，融解温度より 5 ～ 10 °C 低い温度で，1 ～ 2 時間乾燥した後，医薬品各条に規定する条件で乾燥する．乾燥剤は医薬品各条に規定するものを用い，しばしば取り替える．

WATER CONTENT (LOSS ON DRYING)

Colours containing $-SO_3Na$ or $-COONa$ groups are usually hygroscopic and any water they retain from their manufacture (or subsequently adsorb from the atmosphere) is generally present in the colour in the form of a hydrate. When such colours are dried at 135° the loss in weight may generally be equated to the total water content, but this is not always the case. For example, Erythrosine and Ponceau 4R each retain one molecule of water of crystallization at 135° and it is normal practice to take this into account when totalling the amounts of main components present in a sample.

Procedure

Weigh 2.0 - 3.0 g of the sample in a tared weighing bottle fitted with a ground lid. A weighing bottle of squat form about 50 mm in diameter and 30 mm high is suitable. Heat at the prescribed temperature ± 5° until a constant weight is obtained. Express the loss in weight as a percentage of the weight of sample taken.

第７版　食品添加物公定書

重金属試験法

重金属試験法は、試料中に混在する重金属の許容される限度量を試験する方法である。この試験における重金属とは、酸性において硫化ナトリウム試液によって呈色する金属性物質をいい、その量は、鉛（Pb）の量として表す。

以下、本試験を用いる場合において、例えば、「Pbとして20μg/g以下（1.0g、第１法、比較液　鉛標準液 2.0ml）」とあるのは、本品 1.0gを量り試料とし、比較液には鉛標準液2.0mlを用い、第１法により操作し、試験を行うとき、重金属が、Pbとして20μg/g以下であることを示す。

操作法

（１）検液及び比較液の調製

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、ネスラー管に入れ、水約40mlを加えて溶かし、酢酸（１→20）２ml及び水を加えて50mlとし、検液とする。

別のネスラー管に別に規定する量の鉛標準液を量って入れ、酢酸（１→20）２ml及び水を加えて50mlとし、比較液とする。

第２法

別に規定する量の試料を量り、石英製又は磁製のるつぼに入れ、緩くふたをし、弱く加熱して炭化する。冷後、硝酸２ml及び硫酸５滴を加え、白煙が発生しなくなるまで加熱した後、450～550℃で灰化するまで強熱する。冷後、塩酸２mlを加え、水浴上で蒸発乾固し、残留物に塩酸３滴を加え、熱湯10mlを加えて２分間加温する。冷後、フェノールフタレイン試液１滴を加え、アンモニア試液を、液がわずかに赤くなるまで加えた後、水を用いて定量的にネスラー管に移す。更に酢酸（１→20）２ml及び水を加えて50mlとし、検液とする。別に、試料の場合と同質のるつぼに硝酸２ml、硫酸５滴及び塩酸２mlを入れ、加熱して蒸発乾固し、残留物に塩酸３滴を加え、以下検液の調製の場合と同様に操作して定量的に別のネスラー管に移す。更に別に規定する量の鉛標準液、酢酸（１→20）２ml及び水を加えて50mlとし、比較液とする。

ただし、試験に供する検液が澄明でない場合は、検液及び比較液を同一の条件でろ過する。

第３法　別に規定する量の試料を量り、石英製又は磁製のるつぼに入れ、初めは注意して弱く加熱し、次に強熱して灰化する。冷後、王水１mlを加え、水浴上で蒸発乾固し、残留物を塩酸３滴で潤し、熱湯10mlを加えて２分間加温する。次にフェノールフタレイン試液１滴を加え、アンモニア試液を液がわずかに赤くなるまで加えた後、酢酸溶液（１→20）

2ml を加え、必要がある場合はろ過し、水 10ml で洗い、ろ液及び洗液をネスラー管に入れ、水を加えて 50ml とし、検液とする。別に、試料の場合と同質のるつぼに王水 1 ml を入れ、水浴上で蒸発乾固し、以下検液の調製の場合と同様に操作し、ろ液及び洗液をネスラー管に入れ、別に規定する量の鉛標準液及び水を加えて 50ml とし、比較液とする。

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 を加えて混和し、エタノールに点火して燃焼させた後、徐々に加熱して炭化する。冷後、硫酸 1 ml を加え、注意して加熱した後、500～600℃で強熱して灰化する。この方法で炭化物が残る場合は、少量の硫酸で潤し、再び強熱して灰化する。冷後、残留物に塩酸 3 ml を加えて溶かし、水浴上で蒸発乾固し、この残留物を塩酸 3 滴で潤し、水 10ml を加え、加温して溶かす。次にフェノールフタレイン試液 1 滴を加え、アンモニア試液を、液がわずかに赤くなるまで加えた後、水を用いて定量的にネスラー管に移す。更に、酢酸（1→20）2ml 及び水を加えて 50ml とし、検液とする。別に、試料の場合と同質のるつぼに硝酸マグネシウムのエタノール溶液（1→10）10ml をとり、エタノールに点火して燃焼させる。冷後、硫酸 1 ml を加え、以下検液の調製の場合と同様に操作して定量的に別のネスラー管に移す。更に、別に規定する量の鉛標準液、酢酸（1→20）2ml 及び水を加えて 50ml とし、比較液とする。

ただし、試験に供する検液が澄明でない場合は、検液及び比較液を同一の条件でろ過する。

(2)　試験

別に規定するもののほか、検液及び比較液に硫化ナトリウム試液 2 滴ずつを加えて混和し、５分間放置した後、両ネスラー管を白色の背景を用い、上方及び側方から観察するとき、検液の呈する色は、比較液の呈する色より濃くない。

26. 重金属試験法

重金属試験法は，薬品中に混在する重金属の限度試験である．この重金属とは，酸性で硫化ナトリウム試液によって呈色する金属性混在物をいい，その量は鉛（Pb）の量として表す．

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

検液及び比較液の調製法

別に規定するもののほか，次の方法によって検液及び比較液を調製する．

（1） 第 1 法

医薬品各条に規定する量の試料をネスラー管にとり，水適量に溶かし 40 mL とする．これに希酢酸 2 mL 及び水を加えて 50 mL とし，検液とする．

比較液は医薬品各条に規定する量の鉛標準液をネスラー管にとり，希酢酸 2 mL 及び水を加えて 50 mL とする．

（2） 第 2 法

医薬品各条に規定する量の試料を石英製又は磁製のるつぼに量り，ゆるくふたをし，弱く加熱して炭化する．冷後，硝酸 2 mL 及び硫酸 5 滴を加え，白煙が生じなくなるまで注意して加熱した後，500 ～ 600 °C で強熱し，灰化する．冷後，塩酸 2 mL を加え，水浴上で蒸発乾固し，残留物を塩酸 3 滴で潤し，熱湯 10 mL を加えて 2 分間加温する．次にフェノールフタレイン試液 1 滴を加え，アンモニア試液を液が微赤色となるまで滴加し，希酢酸 2 mL を加え，必要ならばろ過し，水 10 mL で洗い，ろ液及び洗液をネスラー管に入れ，水を加えて 50 mL とし，検液とする．

比較液は硝酸 2 mL，硫酸 5 滴及び塩酸 2 mL を水浴上で蒸発し，更に砂浴上で蒸発乾固し，残留物を塩酸 3 滴で潤し，以下検液の調製法と同様に操作し，医薬品各条に規定する量の鉛標準液及び水を加えて 50 mL とする．

（3） 第 3 法

医薬品各条に規定する量の試料を石英製又は磁製のるつぼに量り，初めは注意して弱く加熱し，次に強熱して灰化する．冷後，王水 1 mL を加え，水浴上で蒸発乾固し，残留物を塩酸 3 滴で潤し，熱湯 10 mL を加えて 2 分間加温する．次にフェノールフタレイン試液 1 滴を加え，アンモニア試液を液が微赤色となるまで滴加し，希酢酸 2 mL を加え，必要ならばろ過し，水 10 mL で洗い，ろ液及び洗液をネスラー管に入れ，水を加えて 50 mL とし，検液とする．

比較液は王水 1 mL を水浴上で蒸発乾固し，以下検液の調製法と同様に操作し，医薬品各条に規定する量の鉛標準液及び水を加えて 50 mL とする．

（4） 第 4 法

医薬品各条に規定する量の試料を白金製又は磁製のるつぼに量り，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 を加えて混和し，エタノールに点火して燃焼させた後，徐々に加熱して炭化する．冷後，硫酸 1 mL を加え，注意して加熱した後，500 ～ 600 °C で強熱し，灰化する．もしこの方法で，なお炭化物が残るときは，少量の硫酸で潤し，再び強熱して灰化する．冷後，残留物に塩酸 3 mL を加えて溶かし，水浴上で蒸発乾固し，残留物を塩酸 3 滴で潤し，水 10 mL を加え，加温して溶かす．次にフェノールフタレイン試液を 1 滴加えた後，アンモニア試液を液が微赤色となるまで滴加し，希酢酸 2 mL を加え，必要ならばろ過し，水 10 mL で洗い，ろ液及び洗液をネスラー管に入れ，水を加えて 50 mL とし，検液とする．

比較液は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 をとり，エタノールに点火して燃焼させる．冷後，硫酸 1 mL を加え，注意して加熱した後，500 ～ 600 °C で強熱する．冷後，塩酸 3 mL を加え，以下検液の調製法と同様に操作し，医薬品各条に規定する量の鉛標準液及び水を加えて 50 mL とする．

操作法

検液及び比較液に硫化ナトリウム試液 1 滴ずつを加えて混和し，5 分間放置した後，両管を白色の背景を用い，上方又は側方から観察して液の色を比較する．

検液の呈する色は，比較液の呈する色より濃くない．

⟨231⟩ HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in terms of the percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* ⟨851⟩) with a control prepared from a *Standard Lead Solution*. [NOTE—Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I*, unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations 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, or for fixed and volatile oils. *Method III*, a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be utilized.

Special Reagents

Lead Nitrate Stock Solution—Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble 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. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Method I

pH 3.5 Acetate Buffer—Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation—Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, dilute with water to 40 mL, and mix.

Test Preparation—Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0/(1000L),$$

in which L is the *Heavy metals* limit, in percentage. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, dilute with water to 40 mL, and mix.

Monitor Preparation—Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation*, and add 2.0 mL of *Standard Lead Solution*. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, dilute with water to 40 mL, and mix.

Procedure—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of *pH 3.5 Acetate Buffer*, then add 1.2 mL of

thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the intensity of the color of the *Monitor Preparation* is equal to or greater than that of the *Standard Preparation*. [NOTE—If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation*, use *Method II* instead of *Method I* for the substance being tested.]

Method II

pH 3.5 Acetate Buffer—Prepare as directed under *Method I*.

Standard Preparation—Prepare as directed under *Method I*.

Test Preparation—Use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0/(1000L),$$

in which L is the *Heavy metals* limit, in percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 mL of 6 N hydrochloric acid, cover, digest on a steam bath for 15 minutes, 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 minutes. Add 6 N ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

Procedure—To each of the tubes containing the *Standard Preparation* and the *Test Preparation*, add 2 mL of *pH 3.5 Acetate Buffer*, then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*.

Method III

pH 3.5 Acetate Buffer—Prepare as directed under *Method I*.

Standard Preparation—Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation*. Heat the solution to the production of dense, white fumes, cool, cautiously add 10 mL of water and, if hydrogen peroxide was used in treating the *Test Preparation*, add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested, and boil gently to the production of dense, white fumes. Again cool, cautiously add 5 mL of

water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test **Preparation**—

If the substance is a solid—Transfer the quantity of the test substance specified in the individual monograph to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and add a sufficient quantity of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to moisten the substance thoroughly. 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 addition of nitric acid until no further darkening occurs, then heat strongly to the production of dense, white fumes. Cool, cautiously add 5 mL of water, boil gently to the production of dense, white fumes, and continue heating until the volume is reduced to a few mL. Cool, cautiously add 5 mL of water, and examine the color of the solution. If the color is yellow, cautiously add 1 mL of 30 percent hydrogen peroxide, and again evaporate to the production of dense, white fumes and a volume of 2 to 3 mL. If the solution is still yellow in color, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid—Transfer the quantity of the test substance specified in the individual monograph to a clean, dry, 100-mL Kjeldahl flask. [NOTE—A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed under *If the substance is a solid*, beginning with "add additional portions of the same acid mixture."

Procedure—Treat the *Test Preparation* and the *Standard Preparation* as follows: Adjust the solution to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer*, then add 1.2 mL of thioacetamide-glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the *Test Preparation* is not darker than that of the *Standard Preparation*.

E P

2.4.8. HEAVY METALS

The methods described below require the use of *thioacetamide reagent R*. As an alternative, *sodium sulphide solution RI* (0.1 ml) is usually suitable. Since tests prescribed in monographs have been developed using *thioacetamide reagent R*, if *sodium sulphide solution RI* is used instead, it is necessary to include also for methods A and B a monitor solution, prepared from the quantity of the substance to be examined prescribed for the test, to which has been added the volume of lead standard solution prescribed for preparation of the reference solution. The test is invalid if the monitor solution is not comparable with the reference solution.

METHOD A

Test solution. 12 ml of the prescribed aqueous solution of the substance to be examined.

Reference solution (standard). A mixture of 10 ml of *lead standard solution (1 ppm Pb) R* or *lead standard solution (2 ppm Pb) R*, as prescribed, and 2 ml of the prescribed aqueous solution of the substance to be examined.

Blank solution. A mixture of 10 ml of *water R* and 2 ml of the prescribed aqueous solution of the substance to be examined.

To each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix. Add 1.2 ml of *thioacetamide reagent R*. Mix immediately. Examine the solutions after 2 min. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution. The substance to be examined complies with the test if any brown colour in the test solution is not more intense than that in the reference solution.

If the result is difficult to judge, 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.

METHOD B

Test solution. 12 ml of the prescribed solution of the substance to be examined prepared using an organic solvent containing a minimum percentage of water (for example, dioxan containing 15 per cent of water or acetone containing 15 per cent of water).

Reference solution (standard). A mixture of 10 ml of lead standard solution (1 or 2 ppm Pb), as prescribed, and 2 ml of the prescribed solution of the substance to be examined in an organic solvent. Prepare the lead standard solution (1 or 2 ppm Pb) by dilution of *lead standard solution (100 ppm Pb) R* with the solvent used for the substance to be examined.

Blank solution. A mixture of 10 ml of the solvent used for the substance to be examined and 2 ml of the prescribed solution of the substance to be examined in an organic solvent.

To each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix. Add 1.2 ml of *thioacetamide reagent R*. Mix immediately. Examine the solutions after 2 min. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution. The substance to be examined complies with the test if any brown colour in the test solution is not more intense than that in the reference solution.

If the result is difficult to judge, 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.

METHOD C

Test 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 sulphate R* in *dilute sulphuric acid R*. 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 *dilute sulphuric acid R*. Evaporate, ignite again and allow to cool. The total period of ignition must not exceed 2 h. Take up the residue in 2 quantities, each of 5 ml, of *dilute hydrochloric acid R*. Add 0.1 ml of *phenolphthalein solution R*, then *concentrated ammonia R* until a pink colour is obtained. Cool, add *glacial acetic acid R* until the solution is decolorised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water R*.

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. To 10 ml of the solution obtained add 2 ml of the test solution.

Monitor solution. Prepare as described for the test solution, adding to the substance to be examined the volume of *lead standard solution (10 ppm Pb) R* prescribed for preparation of the reference solution. To 10 ml of the solution obtained add 2 ml of the test solution.

Blank solution. A mixture of 10 ml of *water R* and 2 ml of the test solution.

To 12 ml of each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix. Add 1.2 ml of *thioacetamide reagent R*. Mix immediately. Examine the solutions after 2 min. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution or if the monitor solution is not comparable with the reference solution. The substance to be examined complies with the test if any brown colour in the test solution is not more intense than that in the reference solution.

If the result is difficult to judge, 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.

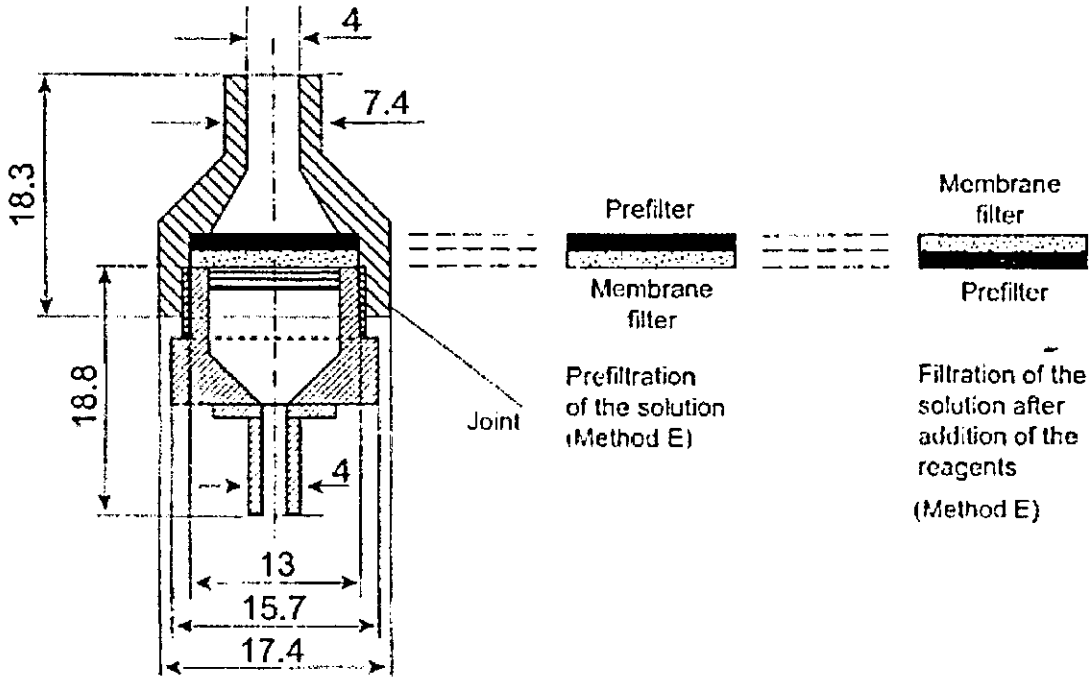

Figure 2.4.8.-1. – *Apparatus for the test for heavy metals*
Dimensions in millimetres

METHOD D

Test solution. In a silica crucible, mix thoroughly the prescribed quantity of the substance to be examined with 0.5 g of *magnesium oxide R1*. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. If after 30 min 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 1 h. Take up the residue in 2 quantities, each of 5 ml, of a mixture of equal volumes of *hydrochloric acid R1* and *water R*. Add 0.1 ml of *phenolphthalein solution R* and then *concentrated ammonia R* until a pink colour is obtained. Cool, add *glacial acetic acid R* until the solution is decolorised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water R*.

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 and drying in an oven at 100-105 °C. To 10 ml of the solution obtained add 2 ml of the test solution.

Monitor solution. Prepare as described for the test solution, adding to the substance to be examined the volume of *lead standard solution (10 ppm Pb) R* prescribed for preparation of the reference solution and drying in an oven at 100-105 °C. To 10 ml of the solution obtained add 2 ml of the test solution.

Blank solution. A mixture of 10 ml of *water R* and 2 ml of the test solution.

To 12 ml of each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix. Add 1.2 ml of *thioacetamide reagent R*. Mix immediately. Examine the solutions after 2 min. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution or if the monitor solution is not comparable with the reference solution. The substance to be examined complies with the test if any brown colour in the test solution is not more intense than that in the reference solution.

If the result is difficult to judge, 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.

METHOD E

Test solution. Dissolve the prescribed quantity of the substance to be examined in 30 ml of *water R* or the prescribed volume.

Reference solution (standard). Unless otherwise prescribed, dilute the prescribed volume of *lead standard solution (1 ppm Pb) R* to the same volume as the test solution.

Prepare the filtration apparatus by adapting the barrel of a 50 ml syringe without its piston to a support containing, on the plate, a membrane filter (pore size 3 µm) and above it a prefilter (Figure 2.4.8.-1).

Transfer the test solution into the syringe barrel, put the piston in place and then apply an even pressure on it until the whole of the liquid has been filtered. In opening the support and removing the prefilter, check that the membrane filter remains uncontaminated with impurities. If this is not the case replace it with another membrane filter and repeat the operation under the same conditions.

To the prefiltrate or to the prescribed volume of the prefiltrate add 2 ml of *buffer solution pH 3.5 R*. Add to 1.2 ml of *thioacetamide reagent R*. Mix and allow to stand for 10 min and again filter as described above, but inverting the order of the filters, the liquid passing first through the membrane filter before passing through the prefilter (Figure 2.4.8.-1). The filtration must be carried out slowly and uniformly by applying moderate and constant pressure to the piston of the syringe. After complete filtration, open the support, remove the membrane filter, and dry using filter paper.

In parallel, treat the reference solution in the same manner as the test solution.

The colour of the spot obtained with the test solution is not more intense than that obtained with the reference solution.

METHOD F

Test solution. Place the prescribed quantity or volume of the substance to be examined in a clean, dry, 100 ml long-necked combustion flask (a 300 ml flask may be used if the reaction foams excessively). Clamp the flask at an angle of 45°. If the substance to be examined is a solid, add a sufficient volume of a mixture of 8 ml of *sulphuric acid R* and 10 ml of *nitric acid R* to moisten the substance thoroughly; if the substance to be examined is a liquid, add a few millilitres of a mixture of 8 ml of *sulphuric acid R* and 10 ml of *nitric acid R*. 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 R* and heat again until the solution darkens. Continue the heating, followed by the addition of *nitric acid R* until no further darkening occurs, then heat strongly until dense, white fumes are produced. Cool, cautiously add 5 ml of *water R*, boil gently until dense, white fumes are produced and continue heating to reduce to 2-3 ml. Cool, cautiously add 5 ml of *water R* and examine the colour of the solution. If the colour is yellow, cautiously add 1 ml of *strong hydrogen peroxide solution R* and again evaporate until dense, white fumes are produced and reduce to a volume of 2-3 ml. If the solution is still yellow in colour, repeat the addition of 5 ml of *water R* and 1 ml of *strong hydrogen peroxide solution R* until the solution is colourless. Cool, dilute cautiously with *water R* and rinse into a 50 ml colour comparison tube, ensuring that the total volume does not exceed 25 ml. Adjust the solution to pH 3.0-4.0, 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 R* to 40 ml 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* and mix.

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

Monitor solution. Prepare as described 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.

Examine the solutions vertically against a white background. After 2 min, any brown colour in the test solution is not more intense than that in the reference solution.

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

If the result is difficult to judge, 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.