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Cosmetics — Analytical approach for screening and quantification methods for heavy metals in cosmetics

Cosmétiques — Approche analytique des méthodes pour l'évaluation et la quantification des métaux lourds dans les cosmétiques

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Foreword

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The committee responsible for this document is ISO/TC 217, *Cosmetics*.

Introduction

Heavy metals occur naturally in the environment. Some heavy metals are utilized in many industries, and some in very small amount are essential minerals to life. On the other hand, heavy metals are often a concern due to their toxicity. Even for essential minerals, they can be a concern when excess amounts are ingested, or more generally, when the human exposure is too high, independently of the route of exposure.

Heavy metals are ubiquitous as they are found in nature (rocks, soil, water, amongst other sources). As such, these heavy metals can be found as impurities in raw materials, and, while not added intentionally to cosmetics, might be present as traces in finished products.[1][2]

The term "heavy metals" is widely used without a single definition. Commonly recognized heavy metals include, but are not limited to: lead, mercury, cadmium, arsenic, and antimony.

While it is acknowledged that heavy metal traces in cosmetic products are unavoidable due to the ubiquitous nature of these elements, companies have implemented numerous measures to monitor and control the amount that might be present.

This Technical Report presents the most common and typical analytical methods and tools for the detection of heavy metals in cosmetic raw materials and finished products.

Cosmetics — Analytical approach for screening and quantification methods for heavy metals in cosmetics

1 Scope

This Technical Report introduces most common and typical analytical approaches for screening and quantification of heavy metals of general interest at both raw material and finished product level. This Technical Report covers techniques from traditional colourimetric reaction, which can be executed without expensive instrument to the high-end one, like that of inductively coupled plasma-mass spectrometry (ICP-MS), which allows detection of elements at µg/kg level. Thus, this Technical Report covers the advantages and disadvantages of each analytical technique so that a suitable approach can be chosen.

The intent of this Technical Report is not to set or suggest acceptable concentration limits of heavy metals in both raw materials and finished products which have to be determined by each regulatory authority.

NOTE 1 The term “heavy metals” is widely used without single definition.

NOTE 2 Elements can be specified as heavy metals by one legislation, while not by others.

2 Principles

2.1 Planning

First, the approach is divided into screening and quantification of total heavy metals content. Heavy metals analysis requires not only technical knowledge and experience, but often requires expensive facilities and vigorous condition of sample preparation, especially when quantification of heavy metals content is investigated. The screening approach can contribute to identifying whether heavy metals levels should be determined using more quantitative methods.

An approach to analyse heavy metals in cosmetics products and raw materials consists of sample preparation method and detection method. Analytical testing conditions should be determined with appropriate combination of preparation method and detection method with acceptable validation data.

Sample preparation methods:

- leaching;
- digestion.

Detection tests and methods:

- colourimetric reaction;[3-8]
- x-ray fluorescence (XRF);
- atomic absorption spectrometry (AAS);
- inductively coupled plasma optical emission spectroscopy (ICP-OES), which is also known as inductively coupled plasma atomic emission spectroscopy (ICP-AES);
- inductively coupled plasma mass spectrometry (ICP-MS).

These approaches basically do not detect a difference between organic and inorganic compounds of an element. For example, they do not detect difference between metallic mercury and a phenylmercury compound. Also, they do not detect difference by valence state, such as, between chromium (III) and chromium (VI). If there is a specific interest in them, appropriate approaches should be taken, e.g. ICP-MS equipped with chromatography.

Typical approach for the screening and quantification on both raw materials and finished products are introduced in the Annex A, Annex B, and Annex C. Approaches other than introduced in the annexes can be effective.

2.2 Selection of a test substance

Screening and quantification of heavy metals can be performed on both raw materials and finished products.

As heavy metals are found in nature, certain raw materials, such as, inorganic materials can naturally contain heavy metals. Knowing the source and signature of raw materials is an effective approach to control the levels of heavy metals in finished products. Monitoring at raw material level can avoid the use of heavy-metal contaminated raw materials and is an effective way to control heavy metal concentration in finished products.

2.3 Preparation of samples

2.3.1 General

In many elemental analysis techniques, samples are converted into liquid. The preparation of the samples is related to the nature of the cosmetic matrix. The sample preparation techniques are basically classified into two: leaching method and digestion method.

2.3.2 Leaching method

Leaching method is a preparation method in order to determine an amount of heavy metals extracted from a sample under acidic conditions. The principle of the leaching method is modelling the conditions of a gastrointestinal fluid or sweat to liberate heavy metals that might be present in products. This allows estimating an amount of heavy metals to which users can be exposed.

2.3.3 Digestion method

Digestion method is a preparation method in order to determine the total amount of heavy metals present in a sample. When full digestion method is used, it reliably reveals the worst case scenario of exposure. Also, full digestion of the matrix reduces interferences in the detection, especially in ICP-MS.

Samples are sometimes simply heated to ashes (dry ashing) in order to remove organic matter. Dry ashing can be carried out with magnesium nitrate as ashing aids.[9][10] Other ashing aids might be applicable such as magnesium sulfate with sulphuric acid.[8] Since cosmetic matrix is complex, insoluble matter often remains after ashing and further digestion is often conducted.

Samples are digested by heating, usually with a single acid, sometimes with multiple acids (wet digestion), rarely with alkali (fusion), in open or closed vessels and are fully or almost entirely dissolved. It often requires vigorous conditions and cautions concerning possible volatilisation for some metals (such as cadmium, arsenic, or mercury) to obtain acceptable recovery.[8][11]

Recent trends are for closed vessel digestion with microwave assistance which can reduce losses of volatile elements and also improve efficiency in routine analysis. Choice of acids is the important factor to fully digest samples. For cosmetic products, the usage of hydrofluoric acid (HF) can be considered highly effective in digestion of silica compounds. The treatment with hydrofluoric acid needs a post-treatment with boric acid in order to mask remaining HF. Nitric acid, hydrochloric acid, sulphuric acid, and other acids are also selected to digest samples. Each acid, including HF, has their own advantage and therefore often used by combination to effect full digestion. There are many publications for heavy metals analysis, including assessments of sample digestion methods. There is a digestion method recently published with inter-laboratory results for lead, cadmium, and mercury on different finished products containing inorganic materials. This method describes a digestion process using nitric acid with hydrochloric acid in a closed vessel under high pressure heat to around 200 °C. The method specifies the detailed conditions in order to get reproducible results.[12] The study by the authority reports that analytical results obtained by nitric acid and those by nitric acid with HF, in comparison. Nitric acid digestion gave lower results than nitric acid with HF on some cosmetic products.[13] Nevertheless, if possible, it is recommended to avoid the use of hydrofluoric acid for safety and hygiene reasons, within the digestion.

2.4 Detection tests and methods

2.4.1 Colourimetric reaction

This technique has been described as detection test, mostly for raw materials, for heavy metals which form yellow to dark brown-coloured insoluble sulphide under pH 3,0 to 3,5 condition. Elements which can be detected by this technique are for instance, lead, bismuth, copper, cadmium, antimony, tin, and mercury.[8] The insoluble sulphide produced in the reaction shows dark colour in diluted solutions due to its colloidal dispersion. As the source of sulphide ion, either sodium sulphide or thioacetamide is normally used. The density of colour is increased in proportion to the concentration of heavy metals. The quantity of heavy metals is expressed in terms of concentration of lead, in comparison with a lead reference solution. The advantage of the technique is that it can be performed without expensive instruments. The colourimetric test is only applicable for sample solutions which are uncoloured and free from insoluble matter. Recovery should be determined in an accurate and suitable way, especially if dry-ashing is used to obtain such solutions. This technique cannot detect selenium and chromium. Also, zinc produces white precipitate which can cause interference. For this reason, it is important to confirm the reliability of the test by appropriate validation.

When difference in the hue of the developed colour is observed between samples solution and standard solution, other techniques should be explored.

NOTE Applications of colourimetric tests are found in several compendia for cosmetics and pharmaceuticals[3-7] such as Japanese Standards of Quasi-drug Ingredients (JSQI)[3] and European Pharmacopoeia.[4] Also, the Japanese Standards of Cosmetics Ingredients[5] and Japanese Cosmetics Ingredients Codex[6] can still be referred for actual applications, especially for English description, although they are not active compendia anymore as they have basically been consolidated to JSQI.

2.4.2 X-ray fluorescence

2.4.2.1 General

When a sample is irradiated with X-rays which have energy above a certain level, core electrons in atoms are excited, ejected, and then core holes are created. Subsequently, peripheral electrons fall into the created core holes and the excess energy which corresponds to the energy level difference are released as electromagnetic waves in the X-ray region called “X-ray fluorescence”[14]. Since the energy level difference is unique to each element, the emitted X-ray fluorescence is also termed a characteristic X-ray. Identification of the element is possible using these X-ray spectra and the elemental concentration in the sample can be estimated from the X-ray intensity. The advantage of this technique is that it is non-destructive analysis. Various sample forms such as solid, liquid, or powder are applicable for the measurement. The measurements are performed easily and quickly without complicated sample preparation. Complexity would be realized in quantitative or semi-quantitative analysis because this technique is matrix-dependent, and therefore, correction or appropriate validation would be required. For certain elements, sufficient sensitivity can not be obtained, particularly with portable equipment.

2.4.2.2 Types of equipment

Equipment can roughly be classified into two according to detection principles, one is the energy-dispersive type and the other one is wavelength-dispersive type. Each type has particular features, therefore, their advantages and disadvantages should be considered to select suitable one.

2.4.2.2.1 Energy-dispersive type

Its feature is a semiconductor detector. Since the detector itself has energy resolution, the configuration of the equipment can be simplified in comparison to the wavelength type. For this reason, size of the equipment is smaller than the wavelength-dispersive type. Disadvantages are lower sensitivity, and generally resolution is low as well compared to wavelength-dispersive type. The elements to be detected are generally from sodium to uranium, and sensitivity tends to be lower in lighter elements.

2.4.2.2.2 Wavelength-dispersive type

The advantages are high detection sensitivity and high energy resolution. The disadvantage is large equipment size. The elements to be detected are generally from beryllium to uranium. The X-ray fluorescence generated from the sample goes through a solar slit to be a parallel luminous flux. Then it strikes an analysing crystal to be diffracted so that specific wavelength is picked up by the detector.

Several types of analysing crystals are available. A crystal with appropriate intervals between crystalline surfaces has to be selected according to the wavelength range to be analysed. As for an X-ray detector, a proportional counter tube type is generally used for light elements (beryllium to scandium), and a scintillation counter is generally used in the detection of X-ray fluorescence having a short wavelength of around 0,2 nm to 0,3 nm or less (titanium to uranium).

2.4.3 Atomic absorption spectrometry (AAS)[15]

2.4.3.1 General

Electrons of atoms in the atomizer can be promoted to an excited state in nanoseconds by absorbing a defined quantity of energy radiation of a given wavelength. This amount of energy is specific to a particular electron transition for each element. In general, each wavelength corresponds to only one element. The signal without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration using Beer-Lambert law.

The technique requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on Beer-Lambert law.

The AAS is composed of radiation source, atomization chamber, monochromator, detector, and readout device. In order to analyse a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and graphite tube atomizers.

AAS is a very common technique with a good sensitivity and a good specificity. Interference can occur for some elements in the presence of nitric acid with high amounts of iron, aluminium, and silicium. The main disadvantages are its mono-elemental capability requirement for complete dissolution of the samples (except the special application of graphite-furnace AAS with solid sample introduction) and the relatively high cost.

2.4.3.2 Flame AAS

In flame AAS, the sample solution is introduced into a flame of acetylene and an oxidation gas, such as air or nitric oxide, and the elements can be atomized. Flame AAS shows high sensitivity on the detection of alkaline metals and alkaline earth metals.

2.4.3.3 Hydride generation AAS (HG-AAS)

This technique is effective for the elements which are reduced to volatile hydrides by sodium tetrahydroborate ($NaBH_4$). Therefore, the applicable elements are limited, such as arsenic, bismuth, antimony, and selenium. Volatile hydrides are separated from matrices by introduction into an atomisation chamber. As a result, HG-AAS shows high sensitivity for these elements.

2.4.3.4 Graphite furnace AAS (GF-AAS)

In GF-AAS, an either liquid or solid sample is introduced into the graphite tube where it dries through electrical heating, and the residues are ashed. In order to achieve repeatability, accuracy, and higher sensitivity, use of platform for graphite tube is recommended. Matrix modifier is used when target elements are highly volatile in order to minimize the loss of the elements during heating. In a subsequent heating step at very high temperatures, elements present in the residue are atomized. During this phase, the attenuation of the lamp radiation by the atomization in the narrow volume of the graphite tube can be measured. GF-AAS generally shows higher sensitivity than flame-AAS on many elements, while background correction is required due to the use of high temperature. A background correction often applied is Zeeman background correction or deuterium background correction.

2.4.3.5 Cold vapour AAS (CV-AAS)

The analysis of mercury sometimes requires specially designed sample preparation techniques due to its physico-chemical behaviour and requires either a dedicated preparation of the sample or a dedicated technique. Since mercury does not require high temperature to be atomized, the technique called cold vapour is often used for the analysis by taking advantage of its property. Mercury is atomized either by reduction using reducing agents such as stannous chloride or by heating. Heating method is sometimes followed by amalgamation with gold to selectively introduce mercury to a cell to obtain higher sensitivity. Instruments specialized for mercury analysis are commercially available by taking advantage of its property.[10][16][17]

2.4.4 Inductively coupled plasma (ICP)

2.4.4.1 General

ICP has excellent ability to excite or ionize elements because of its very high temperature plasma.[18-20] When the torch of the ICP is turned on, an intense electromagnetic field is created. Argon gas flowing through the torch is ignited, and then, the plasma is created. The flow of argon to maintain the plasma is high (~20 l/min), and the temperature of the plasma is approximately 7 000 K or higher. In most cases, sample solution is introduced into a nebulizer by a peristaltic pump to create a mist. The mist is introduced directly into the argon plasma, immediately collides with the electrons and charged ions of the plasma, and elements of sample become ions. Molecules are destroyed into their respective atoms which lose electrons, and provoke light emission at the characteristic wavelengths of the elements involved. Detectors are either MS or OES, MS detectors detect ionized atoms on the basis of m/z, OES detectors utilizes the light emitted by excited atoms.[21]

2.4.4.2 ICP-OES

If the ICP is equipped with an optical spectrometer (ICP-OES), the intensity of this emission corresponds to the concentration of the element within the sample. The ICP-OES has good sensitivity, but some spectral interference should be considered (many emission lines as in the case of iron). Some interference equation can decrease this phenomenon.

2.4.4.3 ICP-MS

If the ICP is equipped with a mass detector (ICP-MS), the abundance of ions (isotopes) corresponds to the concentration of the sample. For ICP-MS, the plasma has higher temperature to increase ion production. Some mass interferences, such as polyatomic or isotopic can occur. Interference equation can be used, or some modern equipment install collision cell or high resolution analysers to decrease or eliminate this problem.

The great advantages of the ICP-OES and ICP-MS are the multi-element capability and the linear dynamic range. Cost and the fact that samples typically should be in solution are the main disadvantages.

Annex A
(informative)

Colourimetric reaction[3][8]

A.1 General

Elements which can be detected by this technique are, lead, bismuth, copper, cadmium, antimony, tin, and mercury.

The outline of test procedure is as follows.

1) A test solution (TS) is prepared by dissolving a sample in water containing diluted acetic acid to adjust pH to 3,0 to 3,5. If necessary, use sulphuric acid, nitric acid, or other acids to incinerate and remove organic interferences in the sample. Heavy metals in the test solution react with added sodium sulphide to form coloured-insoluble sulphides.

2) A lead reference solution is processed by the same procedure for a sample.

3) The test solution and the lead reference solution are transferred to Nessler tubes separately, and the colours of the solutions are compared by observing the tubes from up and side against a white background.

The use of sodium sulphide TS as colour development reagent is found in Japanese Standards of Quasi-drug Ingredients.[3] The sodium sulphide TS can be replaced by thioacetamide as adopted by European Pharmacopoeia 7.0,[4][8] USP35-NF 30[7] and other pharmacopeia.

A.2 Solutions and standard solutions

A.2.1 General

Only essential solutions are introduced in this Technical Report. All reagents should be analytical grade.

A.2.2 Standard lead solution (10 mg/l)

A.3 Apparatus

A.3.1 Nessler tube, colourless, glass-stoppered cylinders with 1,0 mm to 1,5 mm thickness, made of hard glass. The difference of the height of the graduation line of 50 ml from the bottom among cylinders does not exceed 2 mm.

A.4 Preparation of sample and control solution

A.4.1 Calculation of sample amount for testing

Use a quantity, in g, of the sample to be tested as calculated by the formula. Amount of standard lead solution is often 2,0 ml, but can be changed in order to achieve the required limit and optimize the test conditions to improve method reliability.

$$w = 10 \times V/L \qquad \text{(A.1)}$$

where

w is the weight of the sample, expressed in g;

V is the amount of standard lead solution, expressed in ml;

L is the heavy metals limit interested, expressed in µg/g.

A.4.2 Selection of sample preparation method

Method 1 can be applied to raw materials that dissolve in water and do not produce precipitation when diluted acetic acid is added to adjust the pH of the solution 3,0 to 3,5. When these requirements were not met, or recovery was not enough, explore the applicability of Method 2 to Method 4. If none of these techniques were applicable, other techniques should be employed.

Method 2 can be commonly applied to organic materials. However, it is not applicable to the raw materials containing reducing metal, such as copper and insoluble metal oxides arising from ashing, because hydrochloric acid which is employed in this method cannot fully dissolve these materials.

Method 3 is often effective for raw materials that cannot be fully ashed by Method 2. It is designed to dissolve these residues by aqua regia. Platinum crucible cannot be used in this method.

Method 4 can be applied when enough recovery was not obtained by Method 2. Burning with ethanol to ash in the presence of magnesium nitrate suppress the evaporation of metals during ignition.

Method 2 to 4 involve open vessel incineration or digestion. Mercury and arsenic can evaporate during the process. Separate approach might be necessary when these elements are involved.

A.4.3 Method 1

Place the appropriate calculated amount of the sample in a Nessler tube, and dissolve in sufficient water to make 40 ml. Add 2 ml of diluted acetic acid and water to make 50 ml, and use this solution as the test solution. Place the appropriate amount of standard lead solution in another Nessler tube, add 2 ml of diluted acetic acid and water to make 50 ml, and use this solution as the control solution.

A.4.4 Method 2

Place the appropriate calculated amount of the sample in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle heating. After cooling, add 2 ml of nitric acid and 5 drops of sulphuric acid, heat carefully until no more white fumes evolve, and ignite at between 450 °C to 600 °C to incinerate. After cooling, add 2 ml of hydrochloric acid, evaporate to fully dry on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 ml of hot water, and warm for 2 min. Add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red colour develops. If excess ammonia TS is added, the solution might become red, rather than pale red; and the solution might not adjust to approximately pH 3,5 by the addition of 2 ml of diluted acetic acid, and accurate judgment becomes difficult. If excess amount of ammonia TS have been added, diluted hydrochloric acid can be added to neutralize until the solution becomes pale red colour again.

Add 2 ml of diluted acetic acid, filter if necessary, wash with 10 ml of water, transfer the filtrate and the washing to a Nessler tube, add water to make 50 ml, and use this solution as the test solution.

Prepare the control solution as follows: take 2 ml of nitric acid, 5 drops of sulphuric acid, and 2 ml of hydrochloric acid, evaporate them on a water bath, then evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Proceed in the same manner as the preparation of the test solution, and add the appropriate calculated amount of standard lead solution and water to make 50 ml.

A.4.5 Method 3

Place the appropriate calculated amount of the sample in a quartz or porcelain crucible. Start with gentle, careful heating, then ignite to incinerate. After cooling, add 1 ml of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 ml of hot water, and warm for 2 min. Add 1 drop of phenolphthalein TS, then add ammonia TS dropwise until the solution shows a pale red colour. Add 2 ml of diluted acetic acid, filter if necessary, wash with 10 ml of water, transfer the filtrate and the washings to a Nessler tube, add water to make 50 ml, and use this solution as the test solution.

Prepare the control solution as follows: evaporate to dryness 1 ml of aqua regia on a water bath, proceed in the same manner as the preparation of the test solution, and then add the appropriate calculated amount of standard lead solution and water to make 50 ml.

A.4.6 Method 4

Place the appropriate calculated amount of the sample in a platinum or porcelain crucible, add 10 ml of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), mix well. Ignite the ethanol to burn, and carbonize by gentle heating. After cooling, add 1 ml of sulphuric acid, heat carefully, and ignite at 500 °C to 600 °C to incinerate. If any carbonized substance remains in this method, moisten the residue with a small amount of sulphuric acid, ignite again to incinerate. After cooling, add 3 ml of hydrochloric acid to the residue, evaporate to dryness on a water bath. Moisten the residue with 3 drops of hydrochloric acid, add 10 ml of hot water, and warm to dissolve. Add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red colour develops. Add 2 ml of diluted acetic acid, filter if necessary, wash with 10 ml of water, and transfer the filtrate and the washings to a Nessler tube. Add water to make 50 ml, and use this solution as the test solution.

Prepare the control solution as follows: take 10 ml of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and ignite the ethanol to burn. After cooling, add 1 ml of sulphuric acid, heat carefully, and ignite at 500 °C to 600 °C. After cooling, add 3 ml of hydrochloric acid and proceed in the same manner as the preparation of the test solution, add an appropriate amount of standard lead solution and water to make up to 50 ml.

A.5 Determination

To the test solution and the control solution, add 1 drop each of sodium sulphide TS, mix well, and allow to stand for 5 min. It is important to add the sodium sulphide TS to the test and control solution simultaneously because the colour development is time-dependent. Observe the solutions horizontally or vertically against a white background to compare their colours. The test solution should not be more coloured than the control solution.

When comparing the colour of solutions, analysts should pay careful attention on the light source to avoid false judgement. Observation should be carried out under sufficient intensity of the light. Also, test solution and standard solution should be evenly illuminated. The best position of Nessler tube and white background such as paper or board in visual observation is shown in Figure 1. Stopper of Nessler tube has to be removed before observation. If colour difference was not clear, change the position of test solution and standard solution and observe again. It helps to avoid false judgement in visual observation.

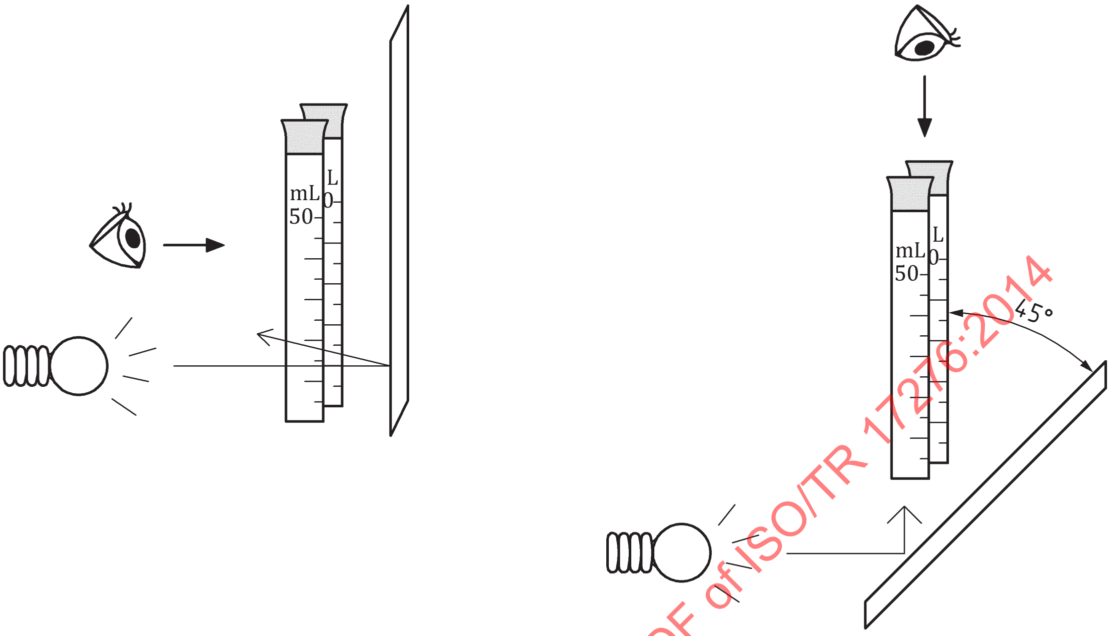

Figure 1 — The position of apparatuses in visual observation

Annex B
(informative)

X-ray fluorescence

B.1 General

Elements which can be detected by this technique are described in 2.4.3.1 and 2.4.3.2.

B.2 Apparatus

B.1.1 X-ray fluorescence spectrometer, with suitable detector.

B.1.2 Apparatus for sample preparation as necessary, e.g. pellet die, sample cells, and thin film.

B.3 Preparation of sample

To analyse a powder sample, a binder can be added as necessary to form a pellet by pressurization. A liquid sample is measured by sealing it into a suitable container with a thin film. If the sample is liquid and the concentration of the element to be measured is low, practical concentration can be increased by using various types of concentration methods.

The analytical depth of the sample by X-ray can often become several micro meters (10^{-6} m) depending on the composition of the sample and the element to be measured. Careful attention has to be paid to the thickness and surface condition of the sample.

B.4 Detection

Cool the detector with liquid nitrogen well in advance of the analysis if the detector requires to be cooled. Set up X-ray tube voltage, tube current, measurement time, and others, necessary in accordance with instructions from the instrument supplier. Specify the elements of interest and run the analysis.

Fundamental parameter method is available for some instruments. The method estimates the amount of elements in the samples without the establishment of calibration curve, but estimates by theoretical calculation using amount of every elements contained in the sample to correct matrix effect. Also, some equipment offers conversion factors using the same theoretical equation as the fundamental parameter method. These factors are only for a simplified quantification.

Quantification is normally done using calibration curve which is established by the intensity of fluorescent X-rays from the target element. Intensity of X-ray fluorescence is affected either by constituent elements, especially by major ones, or particle size. This is called matrix effect. Therefore, in order to obtain linear calibration curve, standard samples should have similar chemical composition and surface state to interested samples.

Aside from the above, the so-called internal standard method can be employed in which an internal standard element with a constant concentration is added to the sample. The concentration is obtained from the ratio of intensities of the internal standard element and the target element.

Annex C
(informative)

Quantification of elements in samples

C.1 Quantification using AAS[9][22]

C.1.1 General

Sample can be digested using microwave, and the solutions are used to determine the amount of arsenic, cadmium, lead, using GF-AAS by external calibration method. Since application of AAS is not limited to these elements, other elements can be analysed by this technique with adequate preparations.

C.1.2 Reagents and solutions

C.1.2.1 General

All reagents should be analytical grade. Use elemental analysis grade if available.

C.1.2.2 Modifier for arsenic, palladium solution, 1 000 µg/ml in 0,5 % volume fraction of nitric acid.

C.1.2.3 Modifier for lead and cadmium, mix 1:1 of 2,0 g/l magnesium nitrate in 0,5 % volume fraction of nitric acid and 2,0 g/l ammonium phosphate monobasic in 0,5 % volume fraction of nitric acid.

C.1.2.4 Standard solution, solutions of arsenic, cadmium, and lead, 1 000 µg/ml for each element.

C.1.2.5 Calibration standard solutions, dilute standard solutions (C.1.2.4) with 0,5 % volume fraction of nitric acid to prepare different concentration of standard calibration solutions. Upper and lower concentration of the target value has to be covered.

C.1.3 Apparatus

C.1.3.1 Filter paper, particle retention 11 µm or equivalent.

C.1.3.2 Microwave digestion system, with pressure and temperature control.

C.1.3.3 Quartz or tetrafluoromethane (TFM) vessel, 50 ml capacity.

C.1.4 Procedure

C.1.4.1 Preparation of samples

Accurately weigh 0,15 g to 0,20 g of sample, in duplicate, into a high pressure resistance 50 ml quartz or TFM vessel. If the sample contains volatile components, remove volatile components before microwave digestion. Add 3 ml concentrated nitric acid (at least 65 % in volume fraction) and a 1 ml of 30 % volume fraction of hydrogen peroxide using a graduated pipette. If sample contains talcum or pigment, add 1 ml of concentrated hydrochloric acid. Ensure the sample is completely wet. Close the vessel lid. Leave to stand for at least 15 min depending on the matrix to ensure complete reaction. Digest the sample in microwave digestion system. Set the condition of microwave digestion and run the programme. In order to obtain acceptable recovery, conditions of microwave digestion have to be optimized according to the samples. Consult the supplier of the microwave as necessary.

- maximum temperature: 200 °C;
- maximum pressure: 7,5 MPa;
- digestion time: 40 min to 50 min, depends on the sample.

If the precision of obtained results or recovery, or both of them was not acceptable, it is recommended to review either sample amount or amount of acids for digestion, or microwave program to make a modification.

After sample digestion, cool to room temperature, add 20 ml deionised water to the digested solution, rinse the inner wall and lid thoroughly. Filter through filter paper into 50 ml volumetric flask and dilute to volume with deionised water.

Prepare a method blank in a same manner as sample preparation but without adding the sample.

C.1.4.2 Spiked sample preparation for determination of percent recovery

Accurately weigh, to the nearest 0,1 mg, in duplicate, 0,15 g to 0,20 g of sample into a 50 ml quartz or TFM vessel. Pipette 300 µl each of 5 µg/ml lead standard solution, 5 µg/ml arsenic standard solution, and 0,5 µg/ml cadmium standard solution into the vessel. Digest to obtain sample solution in a same manner as described in C.1.4.1.

C.1.4.3 Detection

Table 1 shows the example of condition of GF-AAS. Set-up analytical program appropriately. To run a sequence, follow the instruction manual provided by the supplier of the instrument.

Table 1 — Condition of GF-AAS

Element	Wavelength nm	Pyrolysis °C	Atomized temperature °C	Modifier volume µl	Injection volume µl
Arsenic	193,7	1 250	2 100	5	20
Cadmium	228,8	650	1 650	5	20
Lead	283,3	550	1 550	5	20

C.1.5 Calculation

The linear relationship of increasing concentration of the element interested in the calibration standards versus their corresponding peak area is expressed in Formula (C.1):

$$y = ax + b \quad \text{(C.1)}$$

where

a is the slope of the regression line;

b is the y-intercept.

Use slope and intercept determined from the calibration standards and instrument response from test solution to calculate concentration in test solution, µg/g. The amount of the element of interest in the sample is calculated from the following formulae:

$$\rho = (A - b)/a \quad \text{(C.2)}$$

$$w = \rho \times V/m \tag{C.3}$$

where

ρ is the concentration of the element interested in the sample solution, expressed in µg/ml;

A is the instrument response of the sample solution, intensity;

b is the y-intercept;

a is the slope of the calibration curve;

m is the weight of the sample under investigation, expressed in grams (g);

V is 50 ml;

w is the amount of the element interested in the sample, expressed in µg/g.

C.2 Quantification using ICP

C.2.1 General

This method describes the quantification of lead, cadmium, arsenic, nickel, cobalt, chromium in various matrices. Since application of ICP is not limited to these elements, other elements can be analysed by this technique with adequate preparations. Using ICP, different detection modes can be used such as OES or MS. In the following, ICP-MS is given as an example.

C.2.2 Reagents and solutions

Use elemental reagents suitable grade for ICP-MS analysis.

C.2.3 Standard solutions

C.2.3.1 Multi-element standard solution contains lead, cadmium, arsenic, nickel, cobalt, and chromium, 10 µg/ml or element standards for lead, cadmium, arsenic, nickel, cobalt, and chromium, 10 µg/ml.

C.2.3.2 Indium ICP-MS, 1 000 µg/ml (internal standard 1, optional).

C.2.3.3 Rhodium ICP-MS, 1 000 µg/ml (internal standard 2, optional).

C.2.4 Apparatus

C.2.4.1 Microwave digestion system, suitable for ICP sample preparation.

C.2.4.2 Inductively coupled plasma-mass spectrometer, with collision cell (He) and reaction (H_2).

C.2.4.3 Analytical balance, accurate to 0,1 mg.

C.2.4.4 Polypropylene flask, 50 ml capacity.

C.2.4.5 Syringe filters, 25 mm, 0,45 µm with hydrophilic polyethersulfone or equivalent.

C.2.4.6 Disposable syringes, measures up to 5 ml.