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Cosmetics — Analytical methods — Measurement of traces of heavy metals in cosmetic finished products using ICP/MS technique

Cosmétiques — Méthodes d'analyse — Mesurage des éléments traces métalliques par ICP-MS dans les produits cosmétiques finis

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 217, *Cosmetics,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 392, *Cosmetics,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document specifies an analytical procedure for the determination of trace levels of heavy metals (e.g. chromium, cobalt, nickel, arsenic, cadmium, antimony and lead) in finished cosmetic products by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion of the sample. This type of analytical procedure is widely described in other areas such as environment [9][10][11], food[9][10][11] and pharmaceutical industry [12][13][14][15]. While it maximizes the detection of trace levels present in cosmetic products, it does not provide any methodology to directly evaluate systemic exposure of the consumers.

Cosmetics — Analytical methods — Measurement of traces of heavy metals in cosmetic finished products using ICP/MS technique

1 Scope

This document provides a method for quantification of trace levels of heavy metals in cosmetic products.

This document refers only to chromium, cobalt, nickel, arsenic, cadmium, antimony and lead. The methodology can apply to other elements, however, it is the responsibility of the analyst to demonstrate that it fits that purpose.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
validation range
range from the upper to the lower concentration of samples used for the method evaluation

3.2
validated range
range of concentrations between the upper and lower levels that the method performance has been demonstrated to be compliant with the method requirements

4 Principle

Trace levels of heavy metals in cosmetic products are quantified by ICP-MS measurement of the solutions following digestion of the cosmetic products. Digestion takes place with mineral acids in sealed vessels heated to 200 °C by microwaves, producing high pressures.

In the sample preparation procedure, cosmetic ingredients are digested by using a nitric acid/hydrochloric acid mixture allowing the trace levels of heavy metal to be solubilized for measurement. It is possible that some cosmetic inorganic ingredients, such as silica or titanium dioxide, are not completely digested under the conditions of this document and that heavy metal confined in such ingredients are not fully extracted. However, the level of heavy metal trapped in these inorganic materials is not considered to significantly contribute to the exposure level of consumers to these heavy metals. The use of ICP-MS ensures reliable measurement of trace levels of heavy metals due to its proven high sensitivity and selectivity.

In order to obtain comparable results, it is absolutely mandatory to follow all the conditions linked to the digestion of the samples.

5 Reagents

Care shall be taken to assure that the analyte background in the reagents and the water used is negligible and will not interfere analysis. Unless specified otherwise, solutions are understood to be aqueous solutions.

5.1 Water, conforming to Grade 1 of ISO 3696 (conductivity below 0,1 µS.cm^{-1} at 25 °C [16]).

5.2 Nitric acid, minimum mass fraction w = 60 %, density = 1,38 g/ml, suitable for ICP-MS analysis.

5.3 Hydrochloric acid, minimum w = 30 %, density = 1,15 g/ml, suitable for ICP-MS analysis.

5.4 Internal standard stock solutions

For storage and stability conditions of the internal standard stock solutions, follow the specifications of the suppliers. The internal standard solution should contain a certified, or traceable to a certified reference material (CRM) content.

5.4.1 Rhodium stock solution, 1 000 mg/l.

5.4.2 Lutetium stock solution, 1 000 mg/l.

5.5 Analytes stock solutions [chromium (Cr), cobalt (Co), nickel (Ni), arsenic (As), cadmium (Cd), antimony (Sb) and lead (Pb)] 1 000 mg/l for each element.

Commercially available single element or mixed stock solutions with a known certified, or traceable to a CRM content can be used. The used stock solutions shall not contain other elements that could interfere with the analytes to be quantified.

5.6 ICP-MS tune solution, containing, for example, Ce, Co, Li, Mg, Tl and Y (1 µg/l) according to instrument manufacturer's recommendations.

6 Apparatus and equipment

All apparatus and equipment that come into direct contact with sample or solutions shall be pre-cleaned with diluted nitric acid (see 7.2) and rinsed with ultrapure water (5.1) to ensure the lowest analyte background. To prevent contamination and adsorption, do not use lab materials made with borosilicate glass.

6.1 Digestion vessels

6.1.1 General case

Use commercially available, safety-tested pressure vessels and inserts made of acid-resistant, low-contamination materials. The assembled vessels shall be able to safely withstand temperatures up to at least 200 °C and pressures up to at least 40 bar.

It is recommended to keep digestion vessel used only for cosmetic analysis purposes to minimize cross contamination. In addition, the digestion vessels shall always be thoroughly washed after each use. After digesting highly loaded samples, it is recommended to perform a blank digestion with same conditions reported in this document to clean vessels before digesting subsequent samples.

6.1.2 Special care for quantification of antimony

Antimony has been reported to be prone to adsorb on the inner surface of walls of vessels and adsorption is empirically known to happen more frequently when using vessels with extensive period of usage.

Use only digestion vessels with minimal surface roughness to prevent antimony adhesion to the vessel surface[7],[16]. Thorough examination of the vessels shall be performed before their use to control for any scratch or damage or any deposit. If there is any doubt on the integrity of vessels inner surface, consider replacing with brand new digestion vessels.

This issue has been reported to more often happen when polytetrafluoroethylene digestion vessels are used because they can be easily damaged. Quartz containers are recommended because they are usually more resistant to attrition. Damage is also more easily visible because of their transparency. However, polytetrafluoroethylene vessels without any scratch or damage on its inner surface or any deposit are appropriate.

In case of doubt on the possible adsorption of antimony on vessels' walls (e.g. if significant deviations from the target value are observed), a control test is described in 8.4.2.

6.2 Microwave-assisted digestion instruments.

Microwave-heated systems shall be equipped with a temperature measurement unit, which simultaneously regulates the power control of the microwave. Calibrate the temperature sensor before use of at least once a year.

6.3 Membrane filter, 0,45 µm pore size.

The membrane filter used shall be suitable for inorganic traces analysis. It shall be inert with regard to the acid concentration of the measurement solution and shall not bring any contamination into the measurement solution or adsorption of the analytes. Several types of membrane material are commercially available [polytetrafluoroethylen (PTFE), polypropylene (PP), etc.] and their fit for purpose shall be verified by means of appropriate measurements [blanks, quality control (QC) samples, etc.].

6.4 ICP-MS

Mass spectrometer with inductively coupled argon plasma is composed of a sample introduction and an atomisation system, as well as an instrument control and evaluation unit. To prevent interferences with the masses of the heavy metals chromium, nickel, cobalt, arsenic and cadmium, use a mass spectrometer that is capable of compensating or minimizing such interferences (e.g. collision and/or reaction cell, resolution above 3 000, alternatively corrective formulae for higher concentrations).

7 Preparation of standards solutions

7.1 General

For all the solutions, the terminology "part" in the standard refers to either volume or weight. That means that standards and samples can be diluted by volume or weight. However, it should be consistent for both standards and samples.

7.2 Diluted nitric acid

Produced by mixing nitric acid (5.2) with pure water (5.1) at a ratio of approximately 1 + 9 parts respectively.

7.3 Diluting solution

The diluting solution shall have the same acid composition (total content and acid ratio) as the analytical solution (the diluted digest solution). This solution should contain:

— 2,5 parts of nitric acid (5.2);

— 0,5 part of hydrochloric acid (5.3);

— 97 parts of water (5.1).

7.4 Internal standard solutions

7.4.1 General

The internal standards (IS) selected should cover all the mass range of the considered analytes and have similar ionisation energy to the heavy metal for which it is used for correction purposes. It shall also be checked that the native concentration of the internal standards to be analysed is negligible and that they are not interfered by sample constituents.

Rhodium and lutetium have proven to be suitable as internal standards. Samples should be checked for negligible native concentrations and that the sample constituents do not interfere with it.

Rhodium is suitable for the determination of chromium, cobalt, nickel, arsenic, cadmium and antimony, whereas lutetium is suitable for the determination of lead. Alternatively, other elements may be used (for example indium or iridium). Scandium, however, is not suitable as IS due to calcium interferences. An IS with a mass (m/z) below 100 is not recommended because it may suffer from interferences from matrix components.

Internal standard solutions may be added in each sample and calibration solution at the same concentration or may be added through an online Y-fitting to a pump tube.

The concentration of the internal standard solutions shall be included in the range 1 mg/l to 2 mg/l. In 7.4.2 and 7.4.3, a concentration of 1 mg/l has been used for all the calculations.

7.4.2 Rhodium standard solution, 1 mg/l

Dilute the rhodium stock solution (5.4.1) 1 + 999 with diluting solution (see 7.3). This internal standard solution is stable at room temperature for 6 months.

Indium or iridium may also be used as internal standards.

7.4.3 Lutetium standard solution, 1 mg/l

Dilute the Lutetium stock solution (5.4.2) 1 + 999 with diluting solution (see 7.3). This internal standard solution is stable at room temperature for 6 months.

Indium or iridium may also be used as internal standards.

7.5 Standard solutions

7.5.1 General

The concentrations of these standard solutions are examples and should be adjusted to the specific conditions in the laboratories.

7.5.2 High concentration mixed standard solution, 10 mg/l

Dilute 100 times the analytes stock solution(s) (5.5) by adding:

— in the case of single analyte stock solutions, 1 part of each of these 7 solutions to 93 parts of the diluting solution (see 7.3);

— in the case of mixed stock solution, add 1 part of this solution to 99 parts of the diluting solution (see 7.3).

This high standard solution is stable at room temperature for 6 months.

7.5.3 Low concentration mixed standard solution, 0,1 mg/l

Dilute 100 times the high concentration standard solution (see 7.5.2) by adding 1 part of this solution to 99 parts of the diluting solution (see 7.3). This low standard solution is stable at room temperature for 3 months.

7.6 Calibration blank solution

The calibration blank solution corresponds to the matrix solution without any analyte of interest. Generally, it corresponds to the diluting solution with the suitable concentration of the appropriate internal standards if not added via a Y-fitting during the measurement.

7.7 Calibration solutions

Mixed calibration solutions are prepared by diluting the low concentration mixed standard solution (see 7.5.3) with the diluting solution (see 7.3) to levels in the linear range of the instrument and within the targeted concentration range. Include a suitable concentration of the appropriate internal standards, or add online the internal standards by means of pumping into the sample flow through a Y-fitting. At least 3 calibration solutions with various concentrations should be prepared. These calibration solutions shall be prepared daily.

Examples of preparation procedure of calibration solutions are detailed in Table 1 (with addition of the internal standards in all the calibration solutions) and Table 2 (with on line addition of the internal standards via a Y-fitting).

Table 1 — Example of calibration solutions of the ICP-MS — Addition of the internal standards in every calibration solution

Calibration solution	Part of low concentration mixed standard solution (7.5.3)	Part of rhodium standard solution (7.4.2)	Part of lutetium standard solution (7.4.3)	Part of the diluting solution (7.3)	Analyte concentration in the calibration solution (µg/l)
Calibration blank	0	2	2	496	0
Calibration solution 1	2,5	2	2	493,5	0,5
Calibration solution 2	5	2	2	491	1
Calibration solution 3	10	2	2	486	2
Calibration solution 4	25	2	2	471	5
Calibration solution 5	50	2	2	446	10

Table 2 — Example of calibration solutions of the ICP-MS — Online addition of the internal standards via a Y-fitting

Calibration solution	Part of low concentration mixed standard solution (7.5.3)	Part of the diluting solution (7.3)	Analyte concentration in the calibration solution (µg/l)
Calibration blank	0	500	0
Calibration solution 1	2,5	497,5	0,5
Calibration solution 2	5	495	1
Calibration solution 3	10	490	2
Calibration solution 4	25	475	5
Calibration solution 5	50	450	10

8 Procedure

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not address all the safety risks associated with its use. It is the responsibility of the analyst to take all appropriate measures for ensuring the safety and health of the personnel prior to application of the document.

8.1 Preparation of samples

Homogenize the samples by means of suitable devices. The sample preparation step shall ensure a homogeneous starting material for a weighed sample quantity. After homogenization, thoroughly clean the devices to rule out contamination of the subsequent sample. See Clause 6.

8.2 Pressure assisted digestion

WARNING 1 — Depending on the degree of reactivity of the sample, it can be required to weigh in lower quantities than specified in 8.2.2 in order to prevent extreme reactions or explosions. It shall be taken into account that digestion of samples with high carbon contents (e.g. carbohydrates, fats, oils, waxes) can cause explosions. Alcohols or solvents in combination with concentrated nitric acid can cause delayed severe reactions already at room temperature. Therefore, it is highly recommended to gently evaporate all volatile components before adding the acid (8.2.2).

WARNING 2 — Samples that are not covered by acid can cause local overheating of the digestion vessel and thus lead to local melting and subsequent bursting of the digestion vessel. Prior to digestion, ensure that the entire sample is fully covered by the acid mixture.

8.2.1 General

Temperature and pressure in the vessels shall be controlled to ensure a proper digestion (see 6.2). To avoid differences in temperature and pressure among vessels, one should only digest samples with similar composition in the same microwave-assisted digestion batch.

8.2.2 Preparation of sample by digestion — General case

Precisely weigh 200 mg of sample into a digestion vessel.

Add 1 ml of water (5.1) and thoroughly mix with a shaking device or manually until the sample is completely suspended in the water.

Add 5 ml nitric acid (5.2) to the mixture and mix again. The sample should be completely covered with the solution. Allow the mixture to rest in a closed digestion vessel to ensure that the preliminary reaction takes place. Depending on the reactive behaviour of the sample the duration of the preliminary reaction can require resting period of at least 30 min up to overnight.

Add then 1 ml of hydrochloric acid (5.3) and briefly mix. After addition of the hydrochloric acid, the pressure vessel shall be closed and sealed immediately to make sure that the formed chlorine gas is available for the reaction and does not evaporate.

8.2.3 Preparation of sample by digestion — Specific cases

— For cosmetic products with high water content, such as lotion, milky lotion, cleanser, or micellar water, a sample mass could reach 400 mg. In this case, no addition of water is required before addition of acids (see 8.2.2).

— For all the other specific cases, sample mass can be adapted but the ratio between sample mass and acid volumes (see 8.2.2) shall not be changed.

In case of high volatile content products, it is highly recommended for safety reason to completely remove volatile portions through a careful heat-up monitoring the loss of sample weight (e.g. in a water bath at 60 °C) after weighing them into the digestion vessel but prior to adding the acid (see 8.2.2).

Due to sample heterogeneity concern, a weight below 100 mg is not recommended.

8.2.4 Microwave digestion procedure

WARNING 1 — Pay special attention when performing digestion under elevated temperature as it might increase internal pressure resulting in higher safety risks in operation. During all steps of the digestion process, the manufacturer's safety information shall be accurately followed.

Process the samples using a 3-step heating program:

a) ramp the heat up from room temperature to 200 °C in approximately 30 min;

b) hold the temperature at 200 °C for 30 min;

c) cool down to 50 °C, before removing the vessels from the microwave.

It is mandatory to maintain a temperature of 200 °C for 30 min to obtain comparable results, since complete digestion is not possible for all types of samples.

WARNING 2 — Depending on reactivity of the sample, a lower heat-up rate may be used in order to prevent extreme reactions or explosions.

For example, a 7-step heating program with a slower heating ramp has been efficiently used:

a) ramp the heat up from room temperature to 160 °C in 25 min;

b) hold the temperature at 160 °C for 15 min;

c) ramp the heat up from 160 °C to 180 °C in 10 min;

d) hold the temperature at 180 °C for 10 min;

e) ramp the heat up from 180 °C to 200 °C in 35 min;

f) hold the temperature at 200 °C for 30 min;

g) cool down to 50 °C, before removing the vessels from the microwave.

NOTE This alternative digestion program was not included in the validation studies. It is up to the user to show equivalency when used.

8.2.5 Preparation of measurement solutions

Cool the vessels to room temperature before opening following any manufacturer's provisions.

Quantitatively transfer the digestion solution to a vessel and dilute to 20 ml with water (5.1). Further dilute the solution 1 + 9 with water (5.1). If internal standards are not added on line via a Y-fitting to the sample during measurement, add the volume of internal standard solutions (see 7.4.2 and 7.4.3) during the dilution step to get consistent IS concentration with the calibration solutions.

NOTE A different intermediate dilution volume can be used when the digest solution is quantitatively transferred from the digestion vessel as long as the final dilution remains unchanged. For example, transfer the digestion solution and dilute to 50 ml with water (5.1) then further dilute this solution 1 + 3 with water (5.1).

Further dilution of the samples can be performed if required using the diluting solution (see 7.3) to bring analyte concentration to within the linear calibration range. Ensure that internal standard concentration in the diluted measurement solution matches that in the calibration solutions.

Remove any residue by decanting or filtering the final solution by a membrane filter (6.3).

8.3 Inductively coupled plasma mass spectrometry

8.3.1 ICP-MS operating conditions

Adjust the instrument according to the manufacturer's instructions and ignite the plasma. After appropriate heat-up and stabilisation of the device (approximately 20 min to 30 min), optimise the settings.

The instrument settings shall be selected such that both high sensitivity and low level of interferences (e.g. oxide ratio, doubly charged ions) are reached.

For this purpose, measure an ICP-MS tune solution (5.6). The formation rate of oxides and doubly charged ions should be, for example, <3 %, depending on the recommendations of the instrument manufacturer.

If using collision or reaction cells to reduce polyatomic interferences, optimize the flow of the cell gas(es).

Follow the recommendations of the ICP-MS manufacturer for adjusting the resolution windows for a low, medium, and high mass. Check that the measured mass is at the centre of the resolution window.

Measure at least one internal standard for each mass where resolution is checked.

Commercially available mass spectrometers frequently use various detectors or detector operating modes (e.g. pulse and analog mode) to cover a larger linear range of concentration. In such cases, the instrument shall ensure that the sensitivity transition among detectors and/or operating modes is continuous and consistent.

8.3.2 Quantification of the analytes by ICP-MS

Once optimization of the instrument is finished, start measurements. Table 3 gives recommended isotope masses for the elements to be analysed.

Table 3 — Recommended isotopes masses

Element	Mass Amu	Isotope frequency %	Potential interferences[a]
Cr	52	83,8	$^{35}Cl^{16}O^{1}H^{+}$, $^{40}Ar^{12}C+$, $^{36}Ar^{16}O^{+}$
	53	9,5	$^{37}Cl^{16}O^{+}$, $^{38}Ar^{15}N^{+}$, $^{38}Ar^{14}N^{1}H^{+}$
Co	59	100	$^{43}Ca^{16}O^{+}$, $^{42}Ca^{16}O^{1}H^{+}$, $^{24}Mg^{35}Cl^{+}$, $^{36}Ar^{23}Na^{+}$
Ni	60	26,2	$^{44}Ca^{16}O^{+}$, $^{23}Na^{37}Cl^{+}$, $^{48}Ti^{12}C^{+}$
	62	3,6	$^{46}Ti^{16}O^{+}$, $^{23}Na^{39}K^{+}$
As	75	100	$^{40}Ar^{35}Cl^{+}$, $^{59}Co^{16}O^{+}$
Rh (IS)	103	100	$^{40}Ar^{63}Cu^{+}$
Cd	111	12,8	$^{94}Zr^{16}O^{1}H^{+}$, $^{95}Mo^{16}O^{+}$
	114[b]	28,7	$^{114}Sn^{+}$, $^{98}Mo^{16}O^{+}$, $^{96}Zr^{18}O^{+}$
In (IS)	115	95,7	$^{99}Ru^{16}O^{+}$
Sb	121	57,4	$^{105}Pd^{16}O^{+}$
	123	47,6	$^{94}Zr^{16}O2$, $^{107}Ag^{16}O^{+}$
Lu (IS)	175	97,4	$^{159}Tb^{16}O^{+}$
Ir (IS)	193	62,7	$^{177}Hf^{16}O^{+}$
Pb	206	24,1	$^{190}Pt^{16}O^{+}$
	207	22,1	$^{191}Pt^{16}O^{+}$
	208	52,4	$^{192}Pt^{16}O^{+}$

a Inter-element interference from isobars, doubly charged ions and polyatomic ions.

b ^{114}Sn may interfere with ^{114}Cd. When SnO is an ingredient in the cosmetic finished product, do not use the ^{114}Cd isotope.

Measure the calibration blank solution (see 7.6) and calibration solutions (see 7.7). Create a calibration curve for each isotope of the analytes. Each curve is the concentration of the analyte as a function of the instrument response (in counts/second). The instrument response corresponds to the analyte isotope counting rate normalised by the selected internal standard isotope counting rate.

Pump the sample solution for measurement. For each analyte isotope, convert the measured instrument response into concentration units from the calibration curve.

Standard addition procedure can be also of interest in case of doubt in the results or for a double check.

Blank subtraction is not recommended. In case of contaminations that have an influence on the contents in the digestion solutions, the whole series should be generally discarded. Before starting a new digestion series, the source of contamination shall be identified, and its cause eliminated.

8.4 Quality control of the analysis

8.4.1 General

Quality control of the analysis shall be ensured in every step of the standard by means of:

Blank solutions:

— digestion blank: a random digestion vessel containing all reagents [water/nitric acid/hydrochloric acid (1/5/1 parts respectively)], but no sample

— analysis blank: same composition as the diluting solution [water/nitric acid/hydrochloric acid (97/2,5/0,5 parts respectively)]

— calibration blank: same composition as the diluting solution [water/nitric acid/hydrochloric acid (97/2,5/0,5 parts respectively)].

Calibration blank and analysis blank have the same composition but are two independent solutions.

Quality controls:

— quality control sample: a sample with a known analyte content value, which will run through all procedure steps, beginning with the digestion. To ensure a relevant assessment of the analytical procedure including the digestion step, quality control sample should contain matrix components. Physicochemical nature of the QC sample shall be as close as possible to that of actual sample. This QC sample can be a well characterised material or a certified reference material. For non-liquid cosmetic sample analysis, it is strongly advised to use a non-liquid (e.g. solid or pasty) QC sample;

— analysis quality control: a mid-calibration standard or a reference material solution.

Recovery and relative standard deviation (RSD) mentioned in the 8.4.2 and 8.4.3 are acceptance criteria obtained by a single laboratory and enabling to assess the quality of the measurement. This intralaboratory variability shall be lower than the total error of the standard (±40 %) that has been determined by the means of interlaboratory ring test and detailed in Annex A.

8.4.2 During digestion

8.4.2.1 General case

Each digestion batch should include the digestion blank(s) and quality control sample(s) that are subject to the same preparation steps and digestion as the cosmetic product samples.

Digestion blanks are used to assess contamination from other sources than the cosmetic sample (i.e. containers, acids, water, filters, etc.).

Quality control sample is used to evaluate the whole analytical procedure for effectiveness by comparing the determined value with the reference value. Acceptance criteria of quality control sample determined value are defined by the operator[3], according to their experience and can be notably designed by the operating laboratories thanks to control charts[17]. Recovery acceptance criterion commonly used for a QC in a single laboratory is ±20 %. For information, in the case of spiked samples, the recovery acceptability has been set by USP to 70 % to 150 %[1].

If the value is not in the pre-defined range, the following actions shall be investigated:

— recalibrate the instrument and reanalyse the sample solutions measured since the last acceptable calibration to assess analytical part of the process;

— spike the sample with a known amount of analyte to allow a control of the analytical part of the procedure (quantification by ICP-MS) and check for any loss of analyte (e.g. precipitation, sorption, evaporation, etc.) during the process;

— control of the quality of the vessels and parameters of the microwave digestion;

— then investigate on the digestion part of the process by performing a new digestion of the samples;

— finally, a control of the quality of the QC sample can be performed by using another QC sample (another batch of the same QC sample or another type of QC sample).

If significant deviations from the target value are observed for antimony, it is highly recommended to test for surface adsorption as described in 8.2.3.

Prepare duplicate digests of the cosmetic product, ideally, in two different batches, to evaluate the sample homogeneity and precision. If the relative standard deviation (RSD) between the 2 duplicates is above 20 %, repeat the digestion and analysis.

8.4.2.2 Suggested confirmatory test to check adsorption of antimony on vessels

If antimony recovery or relative standard deviation between digestion replicates does not meet QC lab requirements, an adsorption test can be performed. The highest concentration of HCl is empirically known to help stabilizing antimony in solution and avoiding adsorption of antimony on the surface of digestion vessel.

For that purpose, it is recommended to check adsorption on vessel walls by digesting one portion of an antimony-containing standard or sample using 1 ml hydrochloric acid (5.3) as described in 8.2.2, and another portion using 2 ml hydrochloric acid. If significant deviations are observed, assume the digestion vessels are damaged and should no longer be used for determination of antimony. In that case, replace the digestion vessels with brand new ones.

This document does not allow antimony values to be reported using the higher quantity of hydrochloric acid.

Pay special attention when performing digestion with extra hydrochloric acid as it can increase internal pressure resulting in higher safety risks in operation.

8.4.3 During analysis

During the analysis, further QC samples shall be added to ensure that quantification is reliable.

Internal standard shall be added in all the solutions (blanks, calibration standards, samples, QC samples) at the same concentration in order to compensate for a potential plasma drift.

Analysis blanks may be regularly analysed (i.e. every 10 samples) to verify that no carry-over exists. They can also be added after the highest calibration standard or after a sample suspected to get high analyte content. The element contents in the blank solutions (digestion, calibration and analysis blanks) shall be low enough. If that is not the case, identify the causes for the element contents in the blank solutions and if required, repeat the digestion step.

By analysing samples in duplicates, one after the other, ICP-MS rinsing procedure and cross contamination effect can be evaluated too. If such kind of contamination is detected, rinsing procedure of the instrument shall be modified until elimination of the contamination (increase of the rinsing time or change the rinsing solutions).

A quality control sample shall be regularly run (i.e. every 10 samples) to check instrument consistency response. Recovery acceptance criterion commonly used for a QC in a single laboratory is ±20 %. If recovery of the analyte is not within this range, recalibrate the instrument and reanalyse sample solutions measured since the last acceptable calibration check.

8.4.4 Example of ICP-MS sequence

An example of an ICP-MS sequence taking into account all the suggested QC samples is reported in the Table 4.

Table 4 — Example of ICP-MS sequence

1	Analysis blank	Analyte background level
2	Analysis blank	Analyte background level
3	Calibration blank	Calibration curve
4	Calibration solution 1 – 0,5 µg/l	
5	Calibration solution 2 – 1 µg/l	
6	Calibration solution 3 – 2 µg/l	
7	Calibration solution 4 – 5 µg/l	
8	Calibration solution 5 – 10 µg/l	

Table 4 *(continued)*

9	Analysis blank	Analyte background level
10	Quality control of the analysis 5 µg/l	Analysis control sample
11	Analysis blank	Analyte background level
12	Digestion blank replicate 1	Digestion blank
13	Digestion blank replicate 2	
14	Analysis blank	Analyte background level
15	QC sample replicate 1	Procedure control sample
16	QC sample replicate 2	
17	Analysis blank	Analyte background level
18	Cosmetic sample 1 replicate 1	Cosmetic sample 1
19	Cosmetic sample 1 replicate 2	
20	Analysis blank	Analyte background level
21	Cosmetic sample 2 replicate 1	Cosmetic sample 2
22	Cosmetic sample 2 replicate 2	
23	Analysis blank	Analyte background level
24	Cosmetic sample 3 replicate 1	Cosmetic sample 3
25	Cosmetic sample 3 replicate 2	
26	Analysis blank	Analyte background level
27	Quality control of the analysis 5 µg/l	Analysis control sample
28	Analysis blank	Analyte background level

9 Calculation

Calculate the analyte concentration of the element in the cosmetic sample, C_C, in mg/kg, as shown in Formula (1):

$$C_C = \frac{(a \cdot V \cdot F)}{(1\ 000 \cdot m)} \quad (1)$$

where

a is the analyte concentration in the sample measurement solution, in µg /l;

V is the volume of the filled-up diluted digestion solution, in ml;

F is the dilution factor of the sample measurement solution;

m is the weight of the cosmetic sample, in g.

The dilution factor, F, shall take into consideration all the dilution steps from filled up digestion solution to measurement solution.

10 Method performance

Method performance has been determined by the mean of the accuracy profile methodology (as described in ISO/TS 22176) that enables to evaluate the global precision and the validated range for each element considered. Taking into account all the results obtained during the method validation interlaboratory tests, on cosmetic matrix containing a certified reference material as the only source of

heavy metals, the acceptance limit has been set to ±40 % for all the elements. All the accuracy profiles are presented in Annex A. As a consequence, the validated ranges are as follows:

— chromium: from 8,6 mg/kg to 51 mg/kg;

— cobalt: from 0,4 mg/kg to 22,3 mg/kg;

— nickel: from 1,8 mg/kg to 28,3 mg/kg;

— arsenic: from 0,9 mg/kg to 25,1 mg/kg;

— cadmium: from 0,5 mg/kg to 23 mg/kg;

— antimony: from 0,5 mg/kg to 10 mg/kg;

— lead: from 1,3 mg/kg to 27,1 mg/kg.

Another interlaboratory test has been performed in 2015 for the elements nickel, arsenic, cadmium, antimony and lead and was evaluated according to ISO 5725-2[1]. In total seven samples (e.g. lipstick, body lotion, toothpaste and eyeshadow) spiked with varying content of the element specified above were analysed.

The interlaboratory test has been performed in the following working ranges:

— nickel in a range from 1,30 mg/kg to 30,45 mg/kg;

— arsenic in a range from 0,47 mg/kg to 8,55 mg/kg;

— cadmium in a range from 0,11 mg/kg to 4,12 mg/kg;

— antimony in a range from 0,45 mg/kg to 13,77 mg/kg;

— lead in a range from 0,56 mg/kg to 17,36 mg/kg.

The statistical characteristics of the second interlaboratory test are provided in Annex B.

11 Test report

The test report should contain the data according to ISO/IEC 17025[12] and at least the following information:

a) all information necessary for the identification of the sample (kind of sample, origin of sample, designation);

b) a reference to this document, i.e. ISO 21392:2021;

c) the date and type of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

f) the test results and the units in which they have been expressed;

g) any particular points observed in the course of the test;

h) any operations not specified in the method or regarded as optional, which can have affected the results.

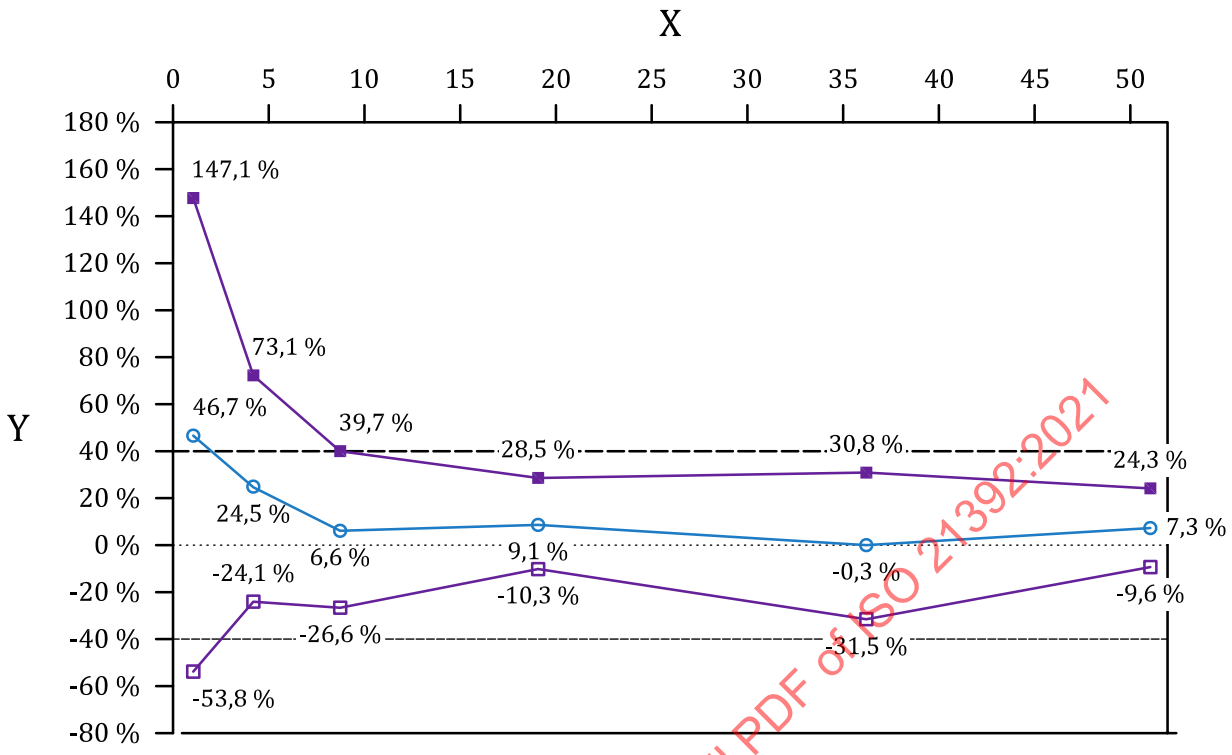
Annex A
(informative)

Performance of the method determined by the accuracy profile methodology

To assess performance of the method described in this document, a set of 6 samples have been analysed by several laboratories in triplicates. Those samples consist in tailor made lipsticks with known contents of heavy metals that were only originating from a solid certified reference material (CRM).

A lipstick base without any source of heavy metals has first been prepared and controlled by using this document. Then precise amount of solid certified reference material has been dispersed in the lipstick base along with free from heavy metals colorants. Lipstick sample bulks were then carefully homogenized in order to ensure the proper distribution of the CRM. 6 lipstick bulks with different amounts of dispersed CRM (corresponding to 6 different levels per element of interest) have been created and their heavy metals content controlled. Every of the 6 lipstick bulks has then been aliquoted in several containers, every aliquot controlled prior to sending to 8 participating labs for analysis.

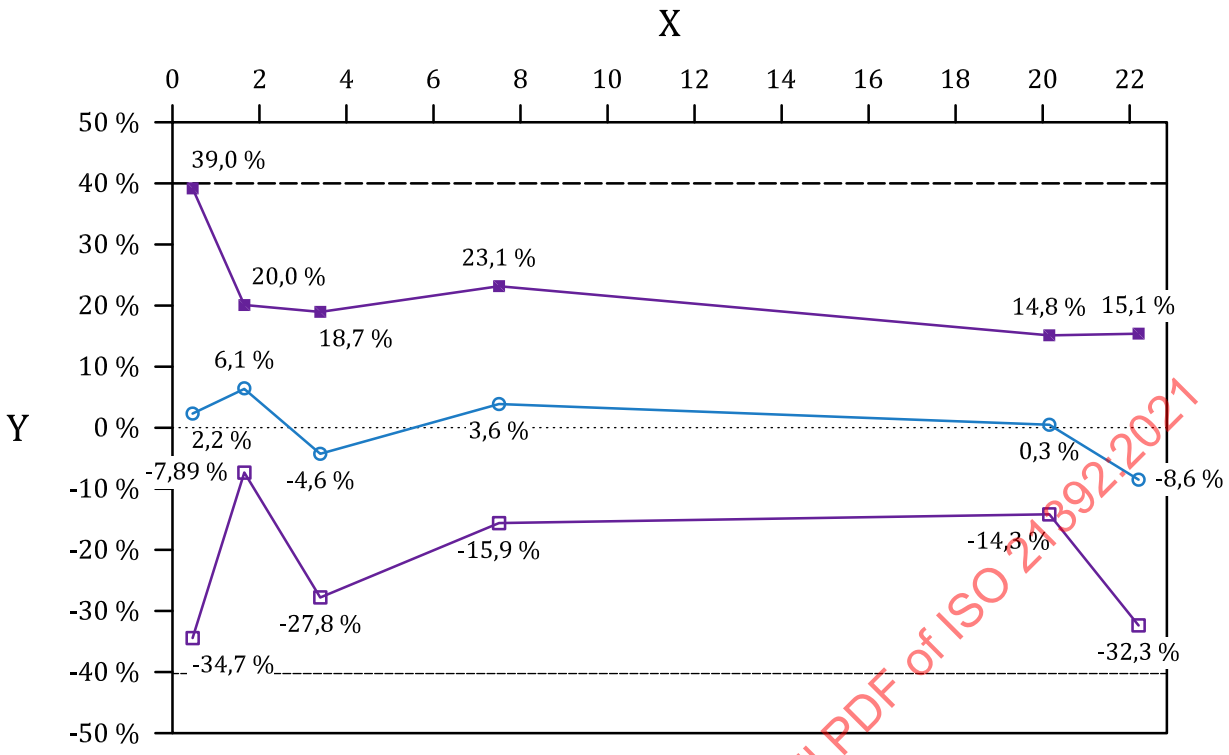
Results obtained for each of the 7 elements (Cr, Co, Ni, As, Cd, Sb, Pb) are presented in Figures A.1 to A.7 and allow the evaluation of the method performance by the mean of the accuracy profile methodology (as described in ISO TS 22176).



Key

X	chromium concentration (mg/kg)
Y	recovery (%)
——■——	relative high tolerance limit
——○——	mean recovery
- - - -	lower acceptance limit
——□——	relative low tolerance limit
— — —	upper acceptance limit
.....	zero level

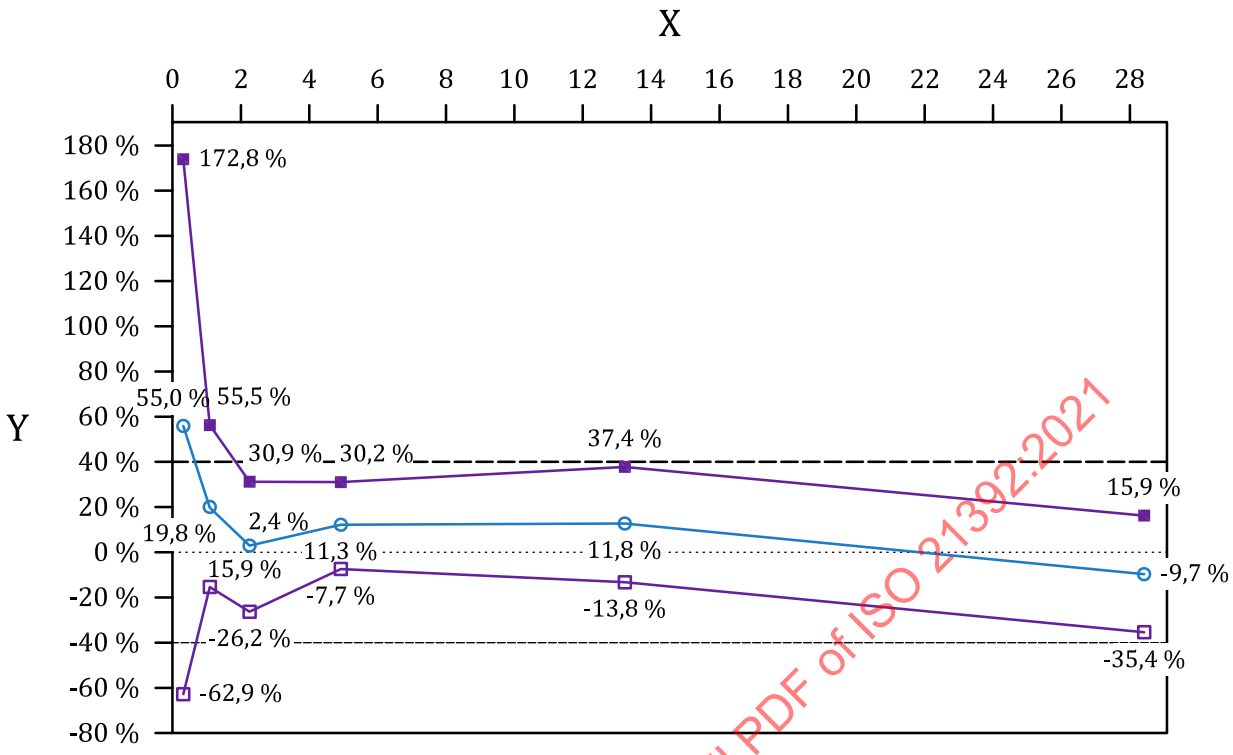
Figure A.1 — Accuracy profile for chromium validating the method in the range 8,6 mg/kg to 51 mg/kg



Key

X	cobalt concentration (mg/kg)
Y	recovery (%)
■—	relative high tolerance limit
—○—	mean recovery
- - - -	lower acceptance limit
—□—	relative low tolerance limit
— — —	upper acceptance limit
.....	zero level

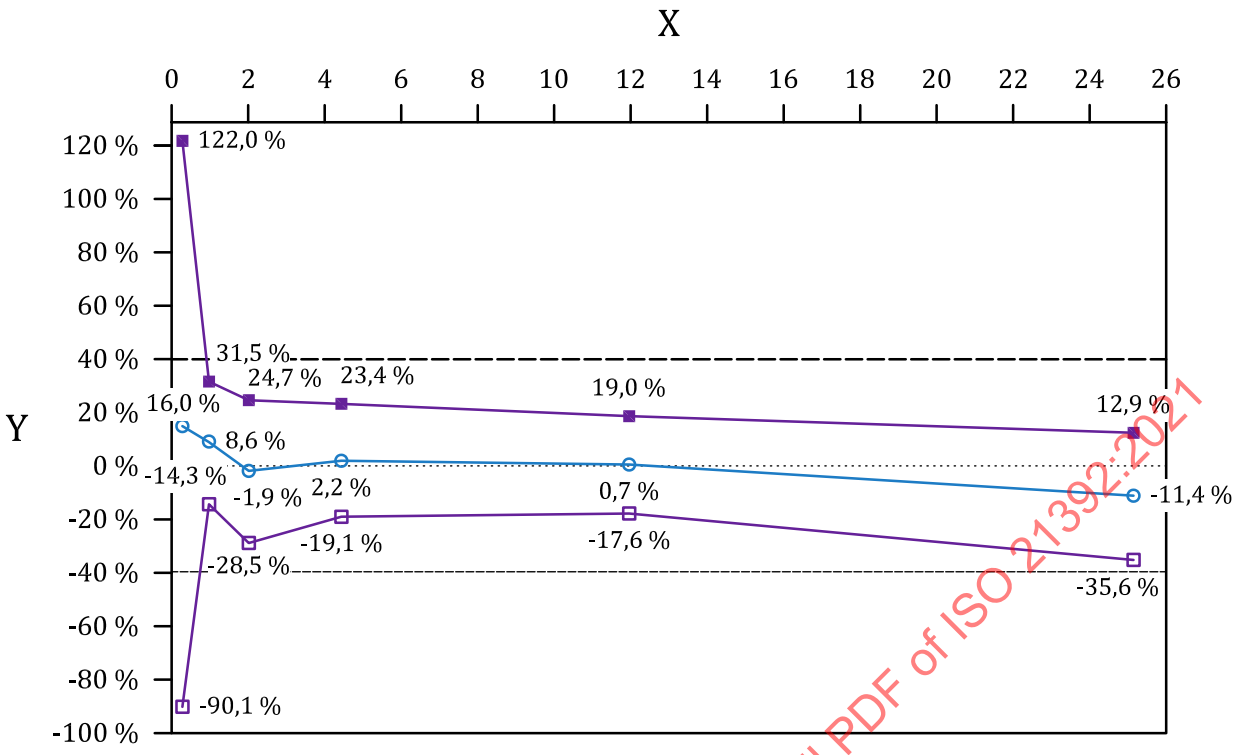
Figure A.2 — Accuracy profile for cobalt validating the method in the range 0,4 mg/kg to 22,3 mg/kg



Key

X	nickel concentration (mg/kg)
Y	recovery (%)
——■——	relative high tolerance limit
——○——	mean recovery
- - - -	lower acceptance limit
——□——	relative low tolerance limit
— — —	upper acceptance limit
.....	zero level

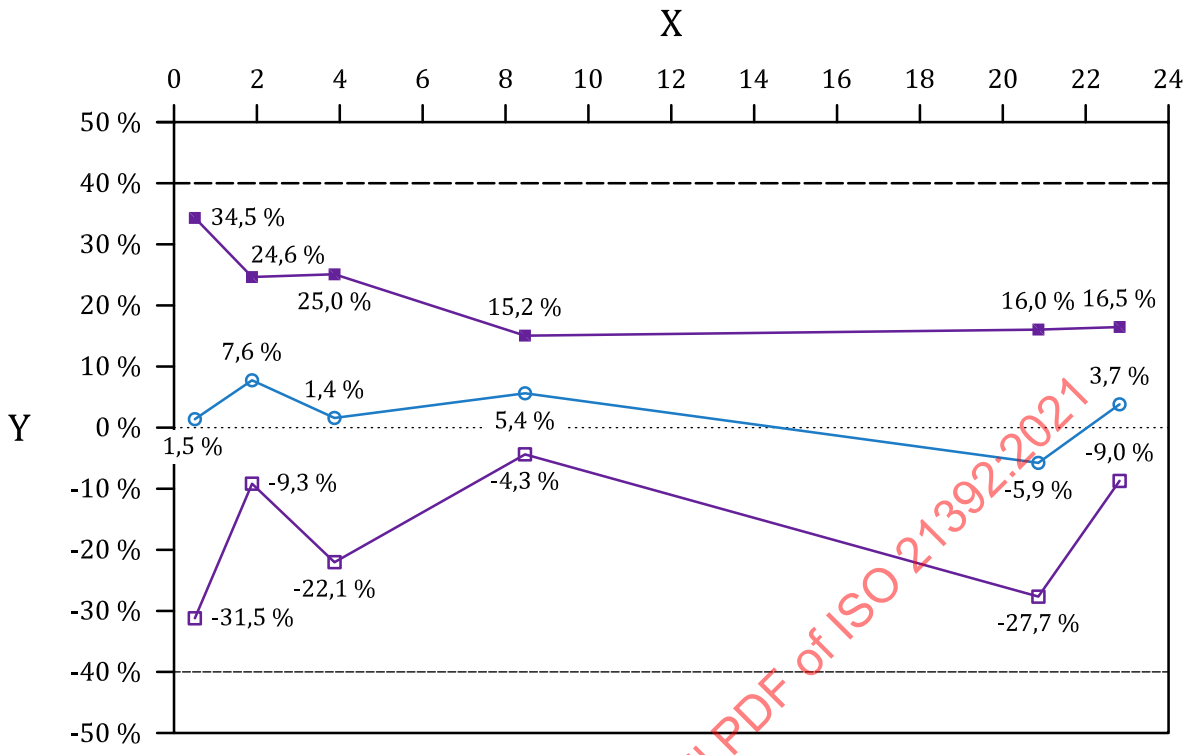
Figure A.3 — Accuracy profile for nickel validating the method in the range 1,8 mg/kg to 28,3 mg/kg



Key

X	arsenic concentration (mg/kg)
Y	recovery (%)
■—■	relative high tolerance limit
○—○	mean recovery
- - - -	lower acceptance limit
□—□	relative low tolerance limit
— — —	upper acceptance limit
.....	zero level

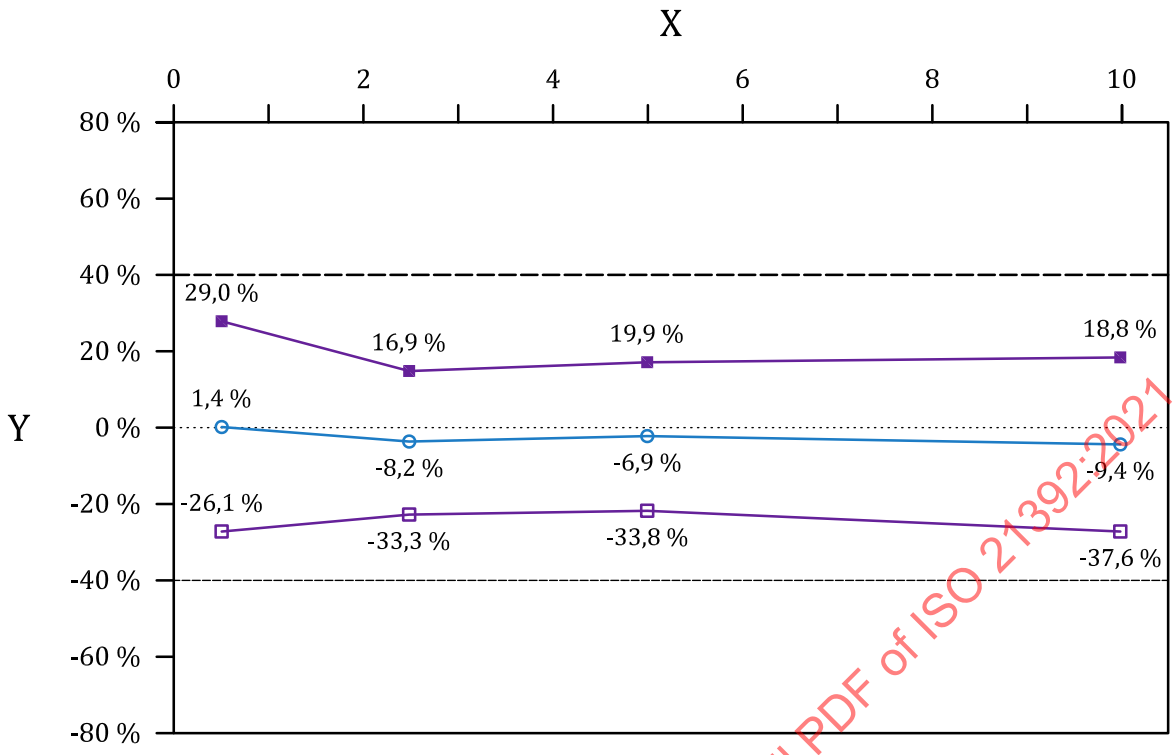
Figure A.4 — Accuracy profile for arsenic validating the method in the range 0,9 mg/kg to 25,1 mg/kg



Key

X	cadmium concentration (mg/kg)
Y	recovery (%)
——■——	relative high tolerance limit
——○——	mean recovery
- - - - -	lower acceptance limit
——□——	relative low tolerance limit
— — — —	upper acceptance limit
.....	zero level

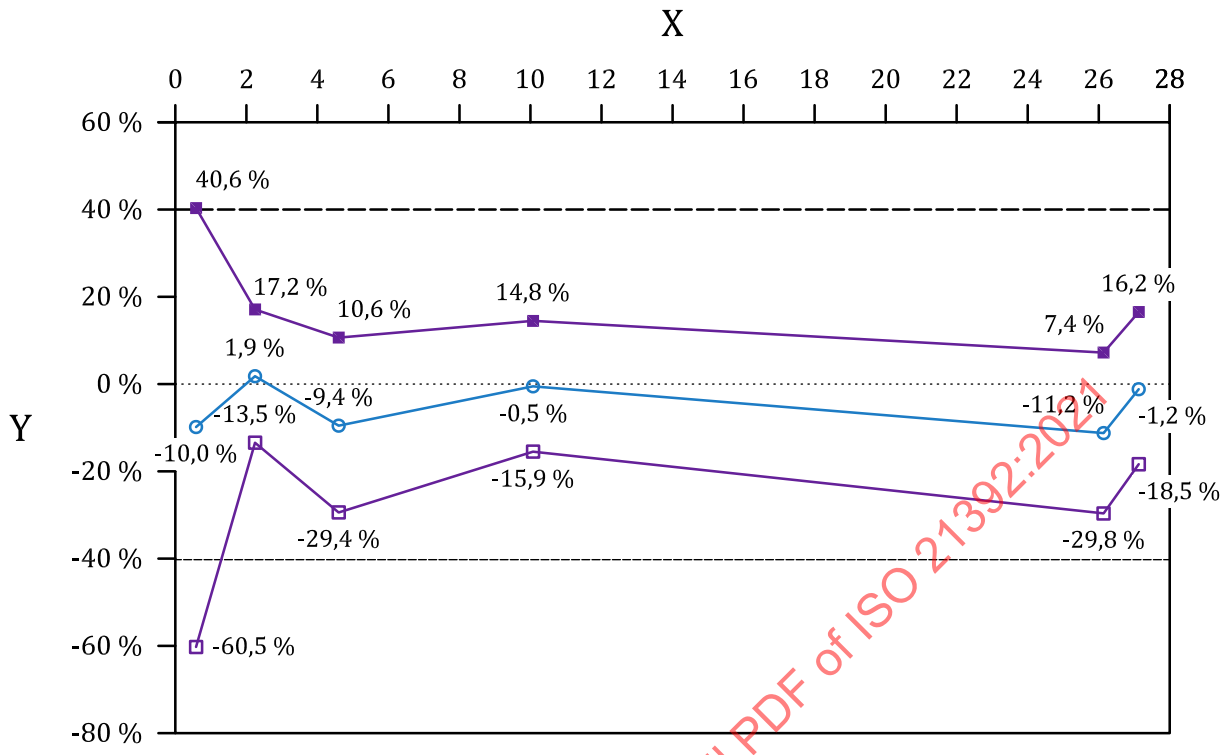
Figure A.5 — Accuracy profile for cadmium validating the method in the range 0,5 mg/kg to 23 mg/kg



Key

X	antimony concentration (mg/kg)
Y	recovery (%)
■—	relative high tolerance limit
—○—	mean recovery
_____	lower acceptance limit
—□—	relative low tolerance limit
— — —	upper acceptance limit
.....	zero level

Figure A.6 — Accuracy profile for antimony validating the method in the range 0,5 mg/kg to 10 mg/kg



Key

X	lead concentration (mg/kg)
Y	recovery (%)
——■——	relative high tolerance limit
——○——	mean recovery
- - - -	lower acceptance limit
——□——	relative low tolerance limit
— — —	upper acceptance limit
.....	zero level

Figure A.7 — Accuracy profile for lead validating the method in the range 1,3 mg/kg to 27,1 mg/kg

Annex B
(informative)

Evaluation of the method via ISO 5725 statistical approach

NOTE As the interlaboratory tests were performed prior to the development of this document:

— some elements such as Chromium and cobalt are not included in the interlaboratory test;

— some existing results on elements (such as baryum) or non-cosmetic matrices (such as tattoo colorants) are presented because they can be of interest for the user.

The procedure has been verified in 2015 by means of interlaboratory tests according to ISO 5725-2[1] for the elements nickel, arsenic, cadmium, antimony and lead. In total, seven samples (lipstick, tattoo colorant, body lotion, tooth paste, eyeshadow and water make-up) with varying contents of the elements specified above were analysed. Two of these seven samples were obtained from the same sample material (body lotion) and thus constitute blind duplicate samples. For each sample, the determination of the respective analyte content was carried out on the same day in duplicate, under repeatability conditions. The analysis had been done within 3 days to 7 days after digestion of the respective sample. The statistical analyses were performed using the statistical approaches according to ISO 5725-2. The two body lotion samples 3 and 6 were also evaluated using the statistical approaches according to ISO 5725-3[2] for a nested interlaboratory test design.

The statistical evaluation according to ISO 5725-2 is based on the data after outlier elimination. In addition to the overall mean value, the results of this evaluation are the reproducibility standard deviation s_R and the repeatability standard deviation s_r.

The reproducibility standard deviation s_R characterizes the total variability of the measurement values, taking into account the variability between different laboratories. The repeatability standard deviation s_r describes the variability within one laboratory and under constant measuring conditions (repeatability conditions).

From the reproducibility and repeatability standard deviation, the reproducibility limit, R, and the repeatability limit, r, are calculated. The reproducibility limit, R, describes the maximum expected deviation between two measured values from different laboratories for the same sample. For larger deviations, it can be assumed that either different samples were used, or an error occurred during the measurement. The repeatability limit, r, describes the maximum expected deviation between two measured values under repeatability conditions, which were thus realized in the same laboratory shortly after each other.

The Horwitz function is often used to calculate the theoretically expected reproducibility standard deviation. Together with the resulting Horwitz ratio (HORRAT) value they are often used as a general criterion for the efficiency of a method as a function of the measured concentration. Here, a HORRAT value significantly larger (or smaller) than 1 means that the reproducibility standard deviation achieved in the ring trial is significantly greater (or smaller) than the expected theoretical standard deviation (Horwitz standard deviation). In interlaboratory tests, HORRAT values up to 2 are generally considered inconspicuous.

The combined evaluation of the body lotion samples 3 and 6 is carried out according to ISO 5725-3. In addition to the repeatability and reproducibility standard deviation, the intermediate standard deviation s_I is determined, which characterizes the variability of the measured values between the two subsamples.

The quantification of antimony and cadmium in sample 7 (water make-up) was only possible for a minority of laboratories. For this reason, it was necessary to restrict the method's working range. As for the remaining sample-element combinations - except for the determination of antimony in sample

5 (eyeshadow) – the relative reproducibility and repeatability standard deviation lay below 30 % the results are considered as acceptable. This is also shown by the fact that the HORRAT values are always below 2 (except for antimony in sample 5). Based on the results of the homogeneity tests it is likely that homogeneity problems are responsible for the reproducibility standard deviations exceeding 30 % and the HORRAT above 2 for antimony in sample 5.

A comparison of the two blind duplicates of the body lotion samples shows that the obtained reproducibility standard deviations for antimony, arsenic, cadmium and nickel represent the actual standard deviations under repeatability conditions.

The interlaboratory test has been performed in the following working ranges:

— nickel in a range from 1,30 mg/kg to 30,45 mg/kg;

— arsenic in a range from 0,47 mg/kg to 8,55 mg/kg;

— cadmium in a range from 0,11 mg/kg to 4,12 mg/kg;

— antimony in a range from 0,45 mg/kg to 13,77 mg/kg;

— lead in a range from 0,56 mg/kg to 17,36 mg/kg.

in the following cosmetic matrices:

Lipstick – Eyeshadow – Water make-up - Body lotion - Toothpaste

On the basis of all the data obtained, the procedure shows that it fits for purpose in various cosmetic matrices and for a range of concentration of interest.

The statistical characteristics of the interlaboratory tests are listed in Tables B.1 to B.6.

Table B.1 — Statistical characteristics (based on ISO 5725-2) for determination of nickel in cosmetic products by means of ICP-MS

Parameter	Lipstick	Tattoo colorant	Toothpaste	Eyeshadow	Water make-up	Body lotion – identical sampling material	
	Sample 1	Sample 2	Sample 4	Sample 5[a]	Sample 7[a]	Sample 3	Sample 6
Number of participating laboratories	10	10	10	7	10	10	10
Number of laboratories with quantitative values	10	10	10	7	6	10	10
Number of outliers (laboratories)	0	2	1	0	0	0	0
Number of laboratories for determination of characteristics	10	8	9	7	6	10	10
Mean value, mg/kg	10,46	5,50	4,87	30,45	1,30	25,81	26,22
± confidence interval, mg/kg	±0,77	±0,27	±0,37	±0,71	±0,09	±0,61	±0,92
Reproducibility standard deviation s_R, mg/kg	1,27	0,39	0,56	1,05	0,12	1,15	1,50
Relative reproducibility standard deviation $s_{R,rel}$, %	12,13	7,12	11,42	3,46	9,56	4,44	5,73
Reproducibility limit R, mg/kg	3,55	1,10	1,56	2,95	0,35	3,21	4,21
Relative reproducibility limit, R_{rel}, %	33,96	19,94	31,97	9,69	26,77	12,42	16,05
Repeatability standard deviation s_r, mg/kg	0,53	0,12	0,06	0,68	0,07	0,87	0,56
Relative repeatability standard deviation $s_{r,rel}$, %	5,05	2,24	1,28	2,23	5,73	3,38	2,13
Repeatability limit r, mg/kg	1,48	0,35	0,17	1,90	0,21	2,45	1,56
Relative repeatability limit r_{rel}, %	14,15	6,28	3,59	6,23	16,05	9,47	5,96
Relative Horwitz standard deviation, %	11,24	12,38	12,61	9,57	15,38	9,81	9,78
HORRAT	1,08	0,58	0,91	0,36	0,62	0,45	0,59

[a] Due to the low number of laboratories (<8) with quantitative values, the precision values determined are accompanied by greater insecurities. It cannot be ruled out that the actual precision values can be only two thirds, on the one hand or twice as high as the specified value, on the other hand. In such cases, it is still ensured that the relative comparative standard deviation is less than 30 % so that the method can be considered successfully validated, even under consideration of these element contents.