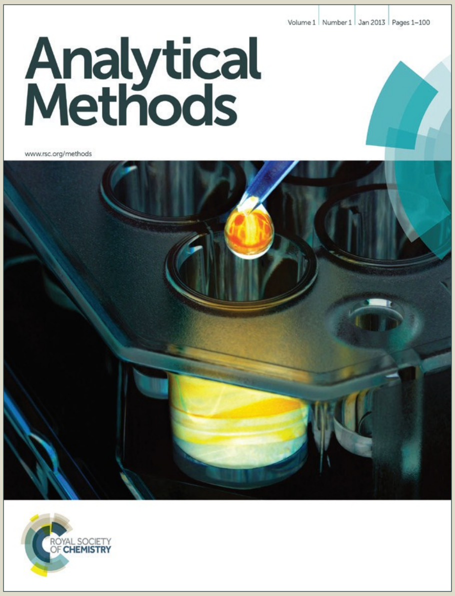

Analytical Methods

ARTICLE

Development of an ID ICP-MS Reference Method for Determination of Cd, Hg and Pb in Cosmetic Powder Certified Reference Material

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Sook Heun Kim, Youngran Lim, Euijin Hwang and Yong-Hyeon Yim*

Accurate measurement of the hazardous elements in cosmetics is considerably important in response to cosmetics safety issues. A sample preparation method using microwave-assisted acid digestion combined with double isotope dilution inductively coupled plasma/mass spectrometry (ID ICP-MS) was used for certification of the hazardous elements Cd, Hg and Pb in cosmetic powder samples. The HF-free sample preparation method was validated and applied to analyse Cd and Pb in cosmetic powder. The Hg content in the sample was determined by employing a fast valve sample introduction system to resolve the severe memory effect in Hg analysis. Homogeneity and stability were also evaluated. The certified values of Cd, Hg and Pb were (24.8 ± 1.3) mg/kg, (0.648 ± 0.065) mg/kg and (24.55 ± 0.31) mg/kg, respectively. The relative combined standard uncertainty was 2.7 % for Cd, 5.1 % for Hg and 0.62 % for Pb. The detailed procedure for certification and evaluation of the associated uncertainty is also presented. The proper use of the developed CRM for quality control and method validation in testing laboratories and the cosmetics industry is expected to significantly improve the reliability and quality of test results for hazardous elements in cosmetics, thereby contributing to cosmetics safety.

1. Introduction

Cosmetics are daily necessities commonly used by people of all ages throughout their lives. Prolonged exposure to hazardous elements from these products can cause chronic health problems, as well as acute health issues, depending on their levels of contamination.[1-3] Hazardous elements are occasionally included in cosmetics by adulteration but are more frequently introduced by raw ingredients that are naturally contaminated. In many different jurisdictions around the world, the presence of toxic elements, such as lead, arsenic, cadmium, mercury, antimony, and chromium in cosmetics is prohibited or restricted for consumer protection and public health. For instance, the EU cosmetics directive (76/768/EEC) allows the non-intended presence of prohibited elements at trace levels only if they are technologically inevitable and within the limit believed to be safe under normal conditions of the product's use.[4] These limits for As, Cd, Hg, and Pb in cosmetics are 2, 3, 1, and 10 mg/kg, respectively. In comparison, Canada sets limits below 3 mg/kg for As, Cd, and Hg, 5 mg/kg for Sb, and 10 mg/kg for Pb. In Korea, they are 10 mg/kg for As and Sb, 5 mg/kg for Cd, 1 mg/kg for Hg, and 20 mg/kg for Pb.

With increasing concerns over safety and the international trade of cosmetics, the SI-traceable and accurate measurement of hazardous elements in these products has become one of the essential requirements for consumer protection and related regulatory decision-making. The use of an appropriate certified reference material (CRM) for method validation and quality control purposes provides an excellent means to ensure the accuracy and comparability of results. Because the sample matrices of many cosmetics are rather complicated, obtaining reliable results for hazardous elements in cosmetics is quite challenging, and related CRM developments are demanding.[5] However, CRM developments for cosmetics analysis have been quite limited except for the cosmetic cream CRMs under development for toxic element analysis by the National Institute of Metrology of China and the Health Science Authority of Singapore. In the absence of the necessary CRM, many quality control analysts rely on reference materials (RMs) based on oil, milk powder, or sediment matrices as substitutes. Here, we have determined the content of hazardous elements in cosmetic powder samples and developed a cosmetic powder CRM, a powder-based cosmetic that, for the first time, can meet the needs of the cosmetics industry and regulatory bodies.

Double isotope dilution (ID) inductively coupled plasma mass spectrometry (ICP-MS) is a widely used method for certification of trace elements in candidate RMs by many National Metrology Institutes (NMIs) for highly accurate and SI-traceable determination of elemental contents in complex matrices. It is one of a few methods recognized as a potential primary method of analysis by the Comité Consultatif pour la

Center for Inorganic Analytical Chemistry, Metrology for Quality of Life, Korea Research Institute of Standards and Science, 267 Gajeong-ro, Yuseong-gu, Daejeon, 305-340 Republic of Korea. Email address: yhyim@kriss.re.kr, Tel: +82 42 868 5361, Fax: +82 42 868 5801

Quantité de Matière (CCQM). Because the double ID ICP-MS is based on isotope ratio measurements, loss of analyte during sample preparation, matrix effects, and instrumental drifts are drastically cancelled out provided that the spike isotope is completely equilibrated with the analyte in the sample through digestion.[6,7]

The application of double ID ICP-MS for the certification of hazardous elements in cosmetic powder samples imposes several challenges despite the robustness of the ID method. The cosmetic powder samples (baby powder here) contain talc as the major component. For complete recovery and equilibrium of the analytes, the use of HF is required in the digestion acid mixture, but its presence requires extreme precaution in sample handing because of its high toxicity and corrosiveness. In addition, it corrodes glass and quartz, materials typically found in ICP-MS sample introduction systems, thus requiring a special sample introduction system made of Teflon, ceramics, or platinum. Complete evaporation of the HF from the digest and reconstitution with more convenient acids is a potential solution to this issue, but this HF-free sample preparation method requires proper validation before its general application for ID ICP-MS of elements in a matrix requiring HF for its dissolution.

The analysis of Hg also imposes great challenges because it notoriously causes memory effects, which are attributed to a combination of the sample introduction, spray chamber, and nebulization effects.[8] It seems that Hg, even at relatively low concentrations, adheres to the walls of the introduction system, causing contamination of subsequent samples.[9] This carry-over can cause substantial bias in the measurement and also results in non-linear calibration graphs, long washout times (> 10 min with a double-pass nebulizer chamber) and dependence of signals on the matrix,[10] which affects the accuracy and reliability of the analytical procedure. There have been attempts to minimize these memory effects. One successful attempt used an Au solution as a stabilizing agent.[11] Another used a combination of flow injection sample introduction and either Triton X-100/ammonia/ ethylenediamine tetraacetic acid[8,12,13] or 2-mercaptoethanol[14] as the carrier solution. Though the use of these additives has been more or less successful in improving the memory effect, development of a more complete solution to the problem remains desirable.

This article describes an analytical methodology to determine Cd, Hg, and Pb in cosmetic powder samples and reports the development of a cosmetic powder CRM. The candidate cosmetic powder RM was prepared following the established protocol for fortified powdered materials. For certification of the analyte elements in the candidate RM, double ID ICP-MS was used with the assessment of specific challenges encountered in the analysis of Cd, Hg, and Pb. A sample preparation method that includes the removal of HF from the sample digest was developed, optimized, and validated for Cd and Pb. To certify Hg, the memory effect was addressed using a fast valve sample introduction system. This article also describes the complete process of certification, homogeneity testing, stability monitoring of the cosmetic powder CRM and uncertainty evaluation. The cosmetic powder CRM developed here will be useful for validating analytical methods and ensuring the quality of results, particularly for the cosmetics industry and regulatory bodies.

2. Experimental

2.1. Preparation of the candidate RMs

Approximately 3.6 kg of commercially available baby powder was obtained from a local market in Korea. Because the level of contamination from endogenous hazardous elements was negligible in the raw material, appropriate amounts of 1000-mg/kg Cd, Hg, and Pb standard solutions were added to form a paste that was then mixed in a Teflon-coated mixing bowl for 7.5 hours. The amounts of standard solutions spiked were 91 g, 3.7 g and 90 g, respectively. The paste was freeze-dried (PVRFD 100R; Ilshin Lab, Korea) and passed through a 50-µm mesh nylon sieve to remove particles larger than 50 µm. The smaller-sized particles were homogenized using a V-blender (Daega Powder, Korea) at 30 rpm for more than 10 hours, yielding approximately 3.5 kg of cosmetic powder, which was divided into 142 pre-cleaned amber glass bottles (60-mL capacity). Each bottle received 20 g of powder and was sealed and sterilized by irradiation with ^{60}Co gamma rays at a dose of approximately 25 kGy. They were vacuum-sealed in Mylar bags and stored at room temperature until use.

2.2. Instrumentation

All isotope ratio measurements for ID ICP-MS were performed with an Element 2™ double focusing high resolution ICP-MS (Thermo Fisher Scientific, Germany) in low-resolution mode. The HF-resistant sample introduction system, consisting of a PFA microflow nebulizer, a PFA cyclonic spray chamber, a platinum injector, and a standard quartz torch (Elemental Scientific, USA), was used for measuring solutions containing HF. When the Hg isotope ratio was measured, the memory effect was controlled by using a fast valve sample introduction system (Elemental Scientific, USA) and 5 % (v/v) HNO_3 as the carrier. The operating conditions of the ICP-MS were optimized, as summarized in Table 1. Microwave (MW)-assisted acid digestion of the prepared cosmetic powder was performed in a closed vessel using an Ethos PLUS MW digestion system (Milestone, Inc., USA) with temperature control. Evaporation of the digested samples was carried out using a DigiPREP graphite block digestion system (SCP SCIENCE, Canada).

2.3. Chemicals and standards

All reagents used were of the highest purity grade. Ultrapure deionized water (resistivity > 18 MΩ·cm) from a Milli-Q system (Millipore, USA) was used throughout the experiment. High-purity concentrated HNO_3 and HCl were prepared by subboiling distillation of commercially available acids obtained from Dongwoo Fine-chem (Iksan, Korea).

Table 1 Optimized instrumental setup for the ID ICP-MS experiment

Plasma conditions	
Forward power	1250 W
Plasma gas flow rate	16 L/min
Auxiliary gas flow rate	0.8 L/min
Nebulization gas flow rate	0.99 L/min
Spray Chamber	
Cyclonic spray chamber	
Nebulizer	
Micromist nebulizer	
Sample uptake rate	0.4 mL/min
Fast valve introduction system	
Sample loop	500 µL
Carrier solution	5 % HNO_3
Data acquisition	
Mass Resolution, *m/Δm*	300
Mass window	5
Integration window	50
Sample per peak	200
Integration time (Runs × Passes)	5 × 200 5 × 70 for Hg determination
Take-up time	20 s
Wash time	1.5 min
Isotopes monitored	^{110}Cd, ^{111}Cd, ^{113}Cd ^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb ^{199}Hg, ^{200}Hg, ^{202}Hg

Hydrogen peroxide and HF for digesting the samples were obtained from Sigma Aldrich (USA). For the purpose of calibration, elemental standard of Cd, Pb, and Hg, prepared by the Korea Research Institute of Standards and Science (KRISS), were used. The elemental standards were prepared gravimetrically from a commercially available pure material, whose purity was assessed for more than 60 elements by KRISS. The gravimetric value corrected by purity was used as the certified value. The enriched isotopes ^{111}Cd, ^{206}Pb (NIST SRM 991), and ^{202}Hg (IRMM 640) were obtained from U.S. Services Inc. (USA), the National Institute of Standards and Technology (NIST, USA), and the Institute for Reference Materials and Measurements (IRMM, EU), respectively, and used as internal standards for ID analysis. A natural Hg isotope standard, IRMM 639 (IRMM, EU), was used for the validation of the analytical method that employed the fast valve sample introduction system as well as for the correction of mass bias in the isotope ratio measurements of Hg. The NIST SRMs 981 and 982 were used to correct the mass bias in the Pb measurements.

2.4. Cleaning sample bottles and plastic ware

Trace analysis requires a clean environment during sample treatment, storage, and measurement for accurate and precise results. Therefore, all sample preparations and analyses were carried out in a clean room environment. Only new polyethylene bottles, pipet tips, and autosampler tubes were used after they were cleaned using the following procedure. Plasticware was cleaned by soaking in a 2.5-N HCl bath, then in a 2 N HNO_3 bath, and finally in ultrapure water for 24 hours each. They were rinsed three times with deionized water after each step described above. Acids, water, and spiked or non-spiked digested materials were stored in pre-cleaned bottles. Teflon vessels used for digestion were cleaned using a TraceClean system (Milestone, Italy), followed by MW heating in the Ethos PLUS MW digestion system using concentrated HNO_3 (approximately 65%) and the same temperature program used for sample digestion. Clean Teflon vessels were soaked in ultrapure water for 24 hours, then rinsed with fresh ultrapure water. They were dried in the oven at 50 °C for 12 hours and used for sample preparation after re-equilibrating them to the laboratory temperature.

2.5. Process of certification

2.5.1. Preliminary analysis of samples using ICP-MS

Approximate levels of Cd, Hg, and Pb in the cosmetic powder were measured using ICP-MS with external calibration. For this purpose, approximately 0.2 g of cosmetic powder was weighed exactly in a pre-cleaned Teflon MW digestion vessel and decomposed using the experimental protocol (Section 2.5.2). The result was used to design optimal sampling and ID for the exact matching approach in double ID ICP-MS. It also provided a means to determine whether the digestion conditions were sufficient for complete dissolution of the material and if there were potential biases in isotope ratios from spectral interferences. These interferences, when they are large enough, typically appear as differences in isotopic ratios between digested samples and those of pure elemental standards beyond the isotopic abundance variations listed by the International Union of Pure and Applied Chemistry (IUPAC).[15,16]

2.5.2. Sample preparation for double ID ICP-MS

For representative sampling and ID ICP-MS analysis from the batch of cosmetic powder, ten to twelve bottles were selected systematically at regular intervals, including the first and last bottles of the batch. Approximately 0.2 g of subsample (x) was taken from each selected bottle and accurately weighed in a pre-cleaned Teflon digestion vessel. For each element to be analysed, a spike solution of the corresponding enriched isotope was prepared. The isotopes ^{111}Cd, ^{202}Hg and ^{206}Pb were used as internal standards for the analysis of Cd, Hg and Pb, respectively. Preliminary analysis was used to prepare a working solution of a spike isotope (y) so that the isotope ratios of a subsample blended with the spike isotope (sample blend, b) were close to 0.25, 0.3, and 1.0, respectively, for ^{110}Cd/^{111}Cd (and ^{113}Cd/^{111}Cd), ^{199}Hg/^{202}Hg, and ^{208}Pb/^{206}Pb. The isotope ratios of the sample blends were designed to compromise between the limited dynamic range of the isotope ratios of the ICP-MS and the uncertainty magnification in the ID analysis. The isotope ratio measurement has optimum precision and accuracy when it is close to 1.0, but the error magnification factor of the ID analysis is frequently at its minimum at an isotope ratio different from 1.0 [6]. The ID ICP-

MS protocol of KRISS typically specifies an optimum isotope ratio between 0.25 and 4 while maintaining the error magnification factor at less than 2.5. Error magnification factors for the $^{110}Cd/^{111}Cd$ (and $^{113}Cd/^{111}Cd$), $^{208}Pb/^{206}Pb$, and $^{199}Hg/^{202}Hg$ ratios adopted here were 1.36 (and 1.38), 1.87, and 2.13, respectively. At least three procedure blanks were also prepared using pre-cleaned Teflon digestion vessels by adding known amounts of gravimetrically diluted (typically 1/100) spike solutions of ^{111}Cd, ^{202}Hg, or ^{206}Pb and treating them with the same procedure used for the cosmetic powder samples. Subsamples not spiked with enriched isotopes were also prepared to measure the natural isotope ratios in the cosmetic powder and to check for potential isobaric interferences in the ICP-MS analysis.

Four calibration blends (b´) were prepared by gravimetrically adding the spike isotope (y) to an appropriately diluted primary standard (z) of the element to be analysed. For exact matching one-point calibration, the calibration blends were prepared so that their isotopic ratios and ICP-MS responses were almost the same as those of the sample blends (b). For this purpose, the dilution of the primary standard and the amount of the spike isotope (y) and primary standard (z) were carefully planned.

The moisture content of the cosmetic powder was obtained for dry-mass correction of the subsample mass taken for analysis. From each bottle, three subsamples of approximately 0.5 g were taken in parallel with the samples for ID ICP-MS and placed over P_2O_5 in a desiccator at room temperature for 7 days (168 hours). The loss of weight was measured and used to obtain a dry mass correction factor.

Spiked and non-spiked cosmetic powder samples were subjected to MW-assisted acid digestion to obtain the sample blend solutions (b) and un-spiked sample solution. 6 mL HNO_3 (approximately 65 %) was added to each digestion vessel prepared above. After immediate sealing, they were left overnight, then 2 mL HF (approximately 50 %) and 2 mL H_2O_2 (approximately 30 %) were added. After 2 hours of equilibration, the closed vessels were heated to 200 °C over 10 minutes and then subjected to MW irradiation for 20 min at 200 °C. The digested sample solutions for Pb analysis were transferred to conical centrifuge tubes and heated on a hot plate at 90 °C for 11 hours in a clean bench to evaporate to dryness so that all acids were removed, particularly HF. Then, 3.5 mL HNO_3 (approximately 65%) and 46.5 mL deionized water were added to re-dissolve the analytes. The solutions were centrifuged at 4,500 rpm for 40 min then filtered through 0.45-µm nylon filters (Whatman, USA). Filtrates were collected in low density polyethylene bottles and further diluted properly for ICP-MS measurement. The samples prepared for Cd analysis were halved; one half was processed the same way as those prepared for Pb analysis, and the other was used to validate the preparation method. They were appropriately diluted with deionized water and used directly in the ICP-MS analysis. Because HF was used in the sample digest, analysis by ICP-MS was carried out using the HF-resistant sample introduction system and compared with the result obtained using the new procedure, in which sample solutions were evaporated, reconstituted, centrifuged, and then filtered. Because there was a potential for loss of Hg in this evaporation procedure, the digested sample solutions for Hg analysis were diluted with deionized water without removing HF.

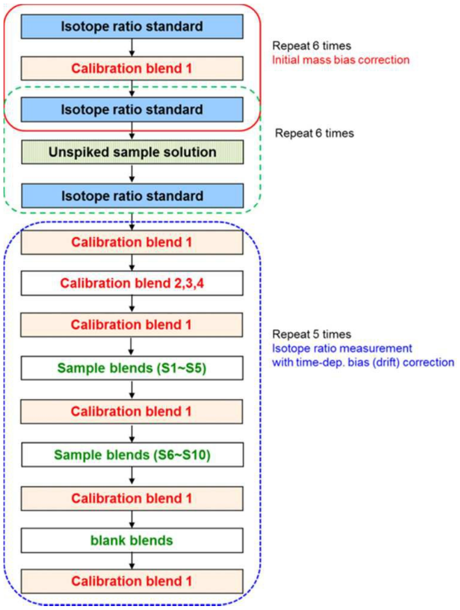

Figure 1 Schematic diagram of the bias/drift corrected isotope ratio measurement by ICP-MS.

2.5.3. ID ICP-MS procedure

For the determination of Cd, Hg, and Pb in the prepared cosmetic powder samples using double ID ICP-MS, the $^{110}Cd/^{111}Cd$ (or $^{113}Cd/^{111}Cd$), $^{199}Hg/^{202}Hg$, and $^{208}Pb/^{206}Pb$ isotope ratios were selected. The isotope ratio measurement sequence used here is described in Figure 1. Corrections for mass bias were carried out using the following as isotope ratio standards: the standard solution with the natural isotope ratio for Cd, IRMM 639 for Hg, and NIST SRM 981 for Pb. Drifts in isotope ratios were corrected using isotopic standards with ratios similar to those of the sample blends when available, as in the case of NIST SRM 982 for Pb. Otherwise, one of the calibration blends was used as the standard for drift correction. All the corrections for mass bias and drift were performed by bracketing measurements of every five or six sample or calibration blends with isotope ratio standards. ICP-MS measurements were carried out in the signal range where the detector dead-time effect was negligible. Furthermore, the uncertainties due to mass bias correction and detector dead-time effects were minimized by preparing the sample blends and calibration blends so that measured signal intensities and isotope ratios closely matched each other.

From the isotope ratio of the sample blends (b), calibration blend (b´), sample (x), spike (y), and primary elemental standards (z), the elemental content of the sample was calculated using the following equation.[17]

$$c_\mathrm{x} = D\cdot c_\mathrm{y}\cdot\frac{m_\mathrm{y}}{w\cdot m_\mathrm{x}}\cdot\frac{R_\mathrm{y}-R_\mathrm{b}}{R_\mathrm{b}-R_\mathrm{x}}\cdot\frac{\sum R_\mathrm{xi}}{\sum R_\mathrm{yi}} - c_\mathrm{blank}$$

$$= D\cdot c_\mathrm{z}\cdot\frac{m_\mathrm{y}\cdot m_\mathrm{z}}{w\cdot m_\mathrm{x}\cdot m'_\mathrm{y}}\cdot\frac{R_\mathrm{y}-R_\mathrm{b}}{R_\mathrm{b}-R_\mathrm{x}}\cdot\frac{R_\mathrm{b'}-R_\mathrm{z}}{R_\mathrm{y}-R_\mathrm{b'}}\cdot\frac{\sum R_\mathrm{xi}}{\sum R_\mathrm{zi}} - c_\mathrm{blank} \qquad (1)$$

Here, the subscripts x, y, z, b, and b´ represent the sample, enriched spiked isotope, elemental standard, sample blend (blend of *x* and *y*), and calibration blend (blend of *y* and *z*), respectively. The meaning of the other symbols are as follows: c_x, c_y, and c_z are the mass fractions of the element in x, y, and z, respectively; m_x and m_y are the masses of x and y in blend b; m'_y and m_z are the masses of y´ and z in blend b´; *w* is the dry mass correction factor; c_{blank} is the procedure blank; *D* is the dilution factor for *z*; R_x, R_y, R_z, R_b, and $R_{b'}$ are the mass bias corrected isotope ratios of x, y, z, b, and b´, respectively; and $\sum R_{xi}$, $\sum R_{yi}$, and $\sum R_{zi}$ are the sums of all the isotope ratios for x, y, and z, respectively. Isotope ratios for Pb in the sample and in the standard solution (R_x and R_z) were directly measured by ICP-MS. In contrast, the isotope ratios for Cd and Hg were assumed to be natural (R_x = R_z), and the IUPAC values were used.[15,16] The isotope ratio of the enriched isotope, R_y, was obtained from the certificate of the enriched isotope. Combined standard uncertainties were calculated according to the guides from ISO/GUM[18] and Eurachem[17] by applying the uncertainty propagation procedure to individual uncertainty components. The expanded uncertainty was calculated by multiplying the combined standard uncertainty by a coverage factor, which was determined by the effective degrees of freedom postulating the normal distribution at a 95 % confidence level. When the degree of freedom is sufficiently high, *k* is usually approximated as 2.

2.5.4. Homogeneity and stability test

The homogeneity and stability of the sample batch were evaluated according to ISO Guide 35:2006.[19] For homogeneity assessment, subsample from ten to twelve units was taken for double ID ICP-MS analysis. The measurement results were used for homogeneity assessment.

The long-term stability of the cosmetic powder CRM was evaluated. A 65- and 24-month stability tests were carried out for Pb and Cd, respectively. In the case of Hg, long-term stability was monitored every 2 years up to 4 years after initial certification. The short-term stability of the CRM at 50 °C for 1 week was also tested, particularly for Hg. The element contents were monitored by analysing four or five subsamples using ID ICP-MS.

3. Results and discussion

3.1. Determination of Cd content in cosmetic powder

Preliminary Cd analysis was used to investigate isobaric interferences in the Cd isotope ratio. In ID ICP-MS of Cd, pairs of Cd isotopes from ^{110}Cd, ^{111}Cd, and ^{113}Cd are typically selected to be measured depending on the availability of spike isotopes and potential isobaric interferences. Direct comparison of the measured isotope ratios with the natural isotopic ratio cited by IUPAC, the so-called IUPAC reference value, usually provides a good indication of potential interferences.[7] In the digested cosmetic powder samples, the ^{110}Cd/^{111}Cd ratio was 1.001 ± 0.002, which is different from the IUPAC reference value of 0.976 ± 0.017. This indicates non-negligible isobaric interference in the ^{110}Cd/^{111}Cd isotope ratio measurement, which can cause bias in the Cd analysis of the cosmetic powder. The possible sources of isobaric interferences to ^{110}Cd are Pd$^+$, ZnAr$^+$, MoO$^+$, and ZrO$^+$. The ^{113}Cd/^{111}Cd isotope ratio, in contrast, shows very good agreement between the measured Cd isotope ratio (0.957 ± 0.001) and the IUPAC reference value (0.955 ± 0.013). A brief ICP-MS analysis of In, Mo, Sn, Pd, Zn and Zr isotopes showed that the presence of Zn in the sample generated a non-negligible interference from ^{70}Zn^{40}Ar$^+$ in the ^{110}Cd signal, while those potential interfering isotopes did not affect the ^{111}Cd and ^{113}Cd measurements significantly. Therefore, the ^{111}Cd and ^{113}Cd isotopes were chosen for ID ICP-MS analysis of Cd in the cosmetic powder.

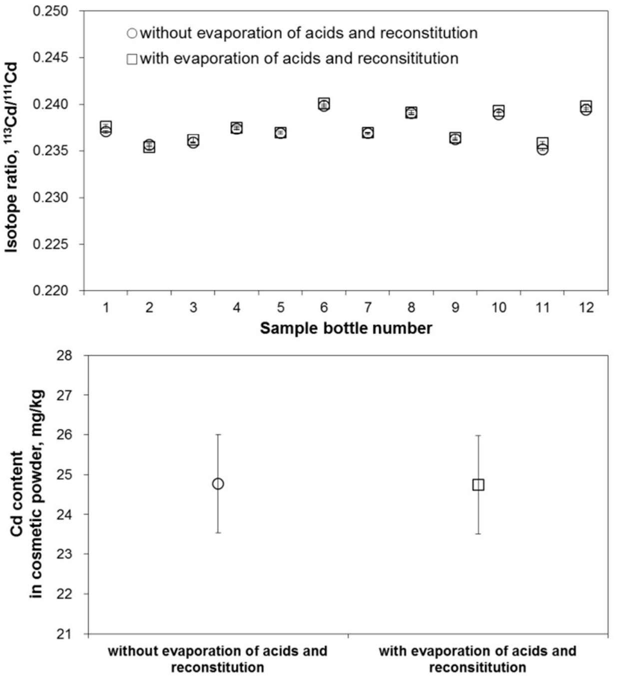

Figure 2 Comparison of the isotope ratio measurement (top) and Cd content measured (bottom) using sample solutions prepared by simple dilution of spiked sample blends after MW digestion with that of the sample solutions prepared with evaporation of acids from MW digests of the spiked sample blends followed by reconstitution with HNO$_3$.

The main ingredient of cosmetic powder is talc, which is a mineral composed of hydrated magnesium silicate. Therefore, HF is required for complete dissolution and equilibration of the sample matrix with spike isotopes, which will necessitate use of the special sample introduction system in the ICP-MS. The most important pre-requisite of ID analysis is complete equilibrium between the analyte element in a sample and the spike isotope used as an internal standard. Hence, removal of the acids, including HF, followed by reconstitution with an acid easily dissolves the element without affecting the isotope ratio provided that full chemical equilibration was achieved in the sample blends and that recovery is not too low. To investigate this possibility, the digested sample blend for Cd analysis containing HF was divided into two equal fractions and subsequently treated using one method with and one without HF removal, and the isotope ratios (^{113}Cd/^{111}Cd) were compared. In one method, the digested sample blend was diluted, but the HF was not removed. In the other, it was prepared by evaporation of the acids to dryness followed by reconstitution with HNO_3, further dilution with deionized water, centrifugation, and filtering. A one-by-one comparison of isotope ratios for each subsample showed near perfect agreement with each other, as shown in Figure 2 (top).

The Cd contents obtained from the two sample preparation methods were also the same regarding the stated uncertainties, as shown in Figure 2 (bottom). The certified value of the Cd mass fraction in the cosmetic powder was (24.8 ± 1.3) mg/kg with a coverage factor of k = 1.96, which corresponds to an estimated confidence interval of 95%. The moisture content of the sample was less than 0.4%. The homogeneity and stability of the sample batch were evaluated as in Section 3.4, and the uncertainty evaluation is described in Section 3.5.

3.2. Determination of Pb content in cosmetic powder

The sample blends for the certification of Pb in a cosmetic powder candidate CRM were prepared by the same procedure used for Cd measurements. That is, sample blends were evaporated to dryness after MW digestion, re-dissolved in HNO_3, diluted, and then centrifuged, followed by filtration. For double ID ICP-MS, the ^{208}Pb/^{206}Pb isotope ratio was selected. Corrections for mass discrimination and drift were carried out by bracketing measurements using isotopic standards with natural (NIST SRM 981) and equimolar (NIST SRM 982) isotope ratios. In the case of Pb measurement, isotope ratios of the Pb primary standard as well as the unspiked sample (R_{zi} and R_{xi}) were determined by measuring the ratios of all isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb). Mass bias corrected isotope ratios of the sample blends (R_b) and calibration blends ($R_{b'}$) were also measured, as shown in Table 2. The certified value of Pb with dry mass correction was (24.55 ± 0.31) mg/kg. The details of the homogeneity and stability test results and the uncertainty evaluation are described in Sections 3.4 and 3.5, respectively.

3.3. Determination of Hg content in cosmetic powder

3.3.1. The mercury memory effect

The pronounced memory effect of Hg in ICP-MS is well-known. It introduces bias to Hg measurements that use ID ICP-MS even though it is a robust isotope ratio-based measurement. A severe memory effect was also observed here when a typical ICP-MS setup was used. The background signals of Hg for deionized water and 5 % nitric acid were increased significantly after running the sequence of repetitive isotope ratio measurements, as in Figure 1. The intensity of ^{199}Hg and ^{202}Hg for deionized water increased from 330 cps and 740 cps to 12400 cps and 48000 cps after the sequential measurements, while those for 5 % nitric acid also increased significantly from 950 cps and 1900 cps to 8400 cps and 34000 cps, respectively. Because the ID ICP-MS protocol used here involves bracketing the isotope ratios of natural isotopic standards, natural sample digests, sample blends, and calibration blends with different isotope ratios, as shown in Figure 1, the measured isotope ratios are prone to interference from the residual backgrounds of previous sample runs. For example, the isotope ratios of a sample blend gradually changed over repeated measurements, and the relative standard deviation (RSD) became significantly larger than usual. The RSD was 1.3 %, while it is typically less than 0.4 %. This severe memory effect causes contamination of subsequent samples even with a long washout time, which affects the accuracy and reliability of the analytical results.

3.3.2. Verification of the fast valve method

To eliminate the memory effect described in Section 3.3.1, a fast valve method was optimized. This method uses a fast sample injection system that allows for significant improvements in sample throughput by effectively washing out residual Hg in the sample introduction system of the ICP-MS. It consists of a high-flow vacuum pump and a 6-port valve. The sample solution is loaded in the sample loop by the vacuum pump as the spray chamber, nebulizer and tubing are flushed by the carrier solution. Then, the autosampler probe and tubing are rinsed while the sample is analysed. This approach virtually eliminates sample uptake and wash times by utilizing dual rinse stations. The additional cleaning in a given time by employing the fast valve sample introduction system can help to reduce the memory effect associated with Hg. The efficiency of the present fast valve method was demonstrated by confirming that the blank level of the Hg signal returned to the initial level before the next sample injection without a noticeable increase of RSD in the isotope ratio measurement. In addition, the validation of the present fast valve method was also confirmed by comparing the isotope ratio standard solution of Hg (IRMM 639) measured with and without applying the fast valve method. There was great consistency between the results of the isotope ratio (^{199}Hg/^{202}Hg) with and without applying the fast valve method, and they quite agree with the corresponding certified value within their uncertainty.

3.3.3. Hg measurements

The validated fast valve method was applied for the certification of Hg in the cosmetic powder candidate CRM using exact matching double ID ICP-MS. Sample blends for the certification of Hg were prepared by the direct dilution of sample blends after the MW-assisted acid digestion of spiked samples. The certified value ($c_{x,\text{ mean}}$) of Hg was (0.648 ± 0.065) mg/kg. The details of the homogeneity and stability test results and the uncertainty evaluation are described in Sections 3.4 and 3.5, respectively.

Although almost 99 % of the Cd and Pb spiked in the raw materials was recovered in the final candidate RM, only 64 % of the Hg spiked was found in the final material. This effect is most likely due to the loss of volatile Hg during the freeze-drying process under vacuum conditions.

3.4. Homogeneity and stability test

The homogeneity study of the cosmetic powder sample was carried out using ID ICP-MS analysis. The relative standard deviations of Cd, Pb and Hg contents due to between-sample inhomogeneity were less than 0.13 %, 0.42 % and 1.14 % of the certified value, respectively. The standard deviation corresponds to the maximum estimate of between-bottle homogeneity because the repeatability of the measurement method is also included in it. This type of homogeneity study scheme is described in Section 7.8 of ISO Guide 35[19] and tested by Kim et al.[20]

The long-term and short-term stabilities of certified values of the cosmetic powder CRM were also evaluated. The long-term stability test results, (24.72 ± 1.25) mg/kg for Cd and (24.62 ± 0.16) mg/kg for Pb, showed good agreement with the initial certified values within the stated uncertainties. The stabilities were also evaluated statistically following the criteria in Section 8.4.2 of ISO Guide 35: 2006.[19] The statistical evaluation results demonstrate that Cd and Pb are stable for 2 and 5 years here, respectively.

Long-term stability as well as short-term stability was tested for Hg. The 24- and 52-month stability of Hg in the cosmetic powder samples was investigated. The Hg results were (0.635 ± 0.050) mg/kg and (0.630 ± 0.030) mg/kg, respectively, which is matched with the initial certified value within the stated uncertainties. The short-term stability of the CRM at 50 °C for 1 week was also tested to verify the stability under extreme transport conditions. Compared with the certification values, the short-term stability test results, (0.641 ± 0.032) mg/kg, showed no noticeable difference.

3.5. Uncertainty evaluation

The overall combined standard uncertainty, $u(c_{x,\text{mean}})$, was estimated according to the guides from ISO/GUM[17] and Eurachem[16] by combining the uncertainty contribution from the systematic effects and the standard deviations of measured values from systematically selected bottles.

$$u(c_{x,\text{mean}})^2 = u^2_{\text{char,sys}} + (SD_{\text{bb}})^2 \qquad (2)$$

The symbols $u_{\text{char,sys}}$ and SD_{bb} represent the standard uncertainty for characterization of the sample contributed by systematic effects and the standard deviation of results obtained from selected sample bottles, respectively. The theoretical background of the evaluation of uncertainty in this study is described in the previous study,[21,22]

Table 2 Uncertainty budget for the determination of Cd, Pb and Hg in the cosmetic powder candidate reference material (cf. Equation 1) and measurement results. The sources of uncertainty due to systematic effects and their typical values, standard uncertainties and contributions are also listed.

Source of uncertainty	Cd			Pb			Hg		
	Typical value (x_i)	Standard uncertainty ($u(x_i)$)	Contribution (%)	Typical value (x_i)	Standard uncertainty ($u(x_i)$)	Contribution (%)	Typical value (x_i)	Standard uncertainty ($u(x_i)$)	Contribution (%)
Concentration of each elemental standard, c_z (mg/kg)	1039.277	0.394	0.02	1002.581	0.360	0.90	998.983	1.880	0.2
Mass of the sample in a sample blend, m_x (g)	0.2	0.0005	0.83	0.2	0.0005	43.42	0.2	0.0005	0.27
Mass of the enriched spike in a sample blend, m_y (g)	1	0.0005	0.03	1	0.0005	1.75	2	0.0005	0
Mass of the enriched spike in a calibration blend, $m_{y'}$ (g)	1	0.0005	0.03	1	0.0005	1.74	2	0.0005	0
Mass of the standard in a calibration blend, m_z (g)	1	0.0005	0.03	1	0.0005	1.75	1	0.0005	0.01
Dilution factor of the elemental standard, D	0.0048	0.00000098	0.01	0.0049	0.00000086	0.21	0.00016	0.000000052	0
Concentration of the blank, c_{blank} (mg/kg)	0.0018	0.0003	0	0.18163	0.00017	0	0.00047	0.00005	0
Bias corrected isotope ratio of the sample, R_x	0.955	0.013	**42.25**	2.0669	0.0012	7.63	0.565	0.0089	**41.75**
Bias corrected isotope ratio of the enriched isotope, R_y	0.0052	0.0005	0	0.00013	0.00001	0	0.001579	0.000016	0
Bias corrected isotope ratio of the standard, R_z	0.955	0.013	**44.22**	2.0772	0.0016	12.33	0.565	0.0089	**54.72**
Sum of the isotopic ratios divided by the enriched isotope for an element in the sample, ΣR_{xi}	7.8125	0.0530	6.16	3.9624	0.0013	0.79	3.349	0.016	1.03
Sum of the isotopic ratios divided by the enriched isotope for an element in the standard, ΣR_{zi}	7.8125	0.0530	6.08	3.9765	0.0017	1.26	3.349	0.016	1.02
Bias corrected isotope ratio of a sample blend, R_b	0.238	0.00022	0.21	0.9592	0.0010	24.04	0.2902	0.0007	0.95
Bias corrected isotope ratio of a calibration blend, $R_{b'}$	0.241	0.00009	0.04	0.8878	0.0004	3.83	0.3172	0.0002	0.08
Mean concentration of element, $c_{x,\text{mean}}$ (mg/kg)	24.746			24.550			0.648		
Standard uncertainty due to systemic effects, $u_{\text{char,sys}}$ (mg/kg)	0.672			0.109			0.032		
Standard deviation of results from selected bottles, SD_{bb} (mg/kg)	0.032			0.104			0.007		
Combined standard uncertainty, $u(c_{x,\text{mean}})$ (mg/kg)	0.672 (k=1.96)			0.151 (k=2.02)			0.033 (k=1.96)		
Expanded uncertainty, $U(c_{x,\text{mean}})$ (mg/kg)	1.318			0.306			0.065		

The standard uncertainty for characterization of the sample ($u_{\text{char,sys}}$) was calculated by applying the uncertainty propagation procedure to an individual uncertainty component. The sources, typical values, and their standard uncertainties contributing to $u_{\text{char,sys}}$ are listed in Table 2. It must be noted that the uncertainties for the IUPAC values[15] of natural Cd or Hg isotopic compositions (R_x and R_z) are responsible for more than 85% of the overall combined uncertainty in the determination of Cd or Hg, as shown in Table 2 (bolded entries). Hence, there is room to reduce the uncertainty of certification of Cd and Hg by measuring the isotope ratios of samples and standards directly. The standard deviation, instead of the standard deviation of the mean, of the results obtained from selected sample bottles was used to consider the between-bottle homogeneity. The overall combined uncertainties estimated using equation (2) were 2.7 %, 0.6 % and 5.1 %, respectively, for Cd, Pb, and Hg. The results of stability analysis were not used to re-evaluate the uncertainty of certified values. Instead, the material was considered sufficiently stable and the certified value and its associated uncertainty that was initially assigned were confirmed. The cosmetic powder CRM (KRISS CRM 114-01-001) developed here for the first time by KRISS will provide an excellent tool for validation and quality control in challenging cosmetics analysis, and it is expected to be used for evaluation of the reliability of analytical methods in determining these elements in powder-type cosmetics and similar materials.

4. Conclusions

The hazardous elements Cd, Hg, and Pb in cosmetic powder were determined. An accurate analytical method for certification of Cd, Hg, and Pb in cosmetic powder was developed and validated by a method using optimized sample preparation and ID ICP-MS. The ID ICP-MS method and HF-free sample preparation established here were applied to certification of Cd and Pb in the cosmetic powder CRM. The method using a fast valve sample introduction system was also validated and used to certify Hg in the candidate cosmetic powder CRM. The homogeneity and stability of the material were assessed and found to be sufficient for its use as an RM.

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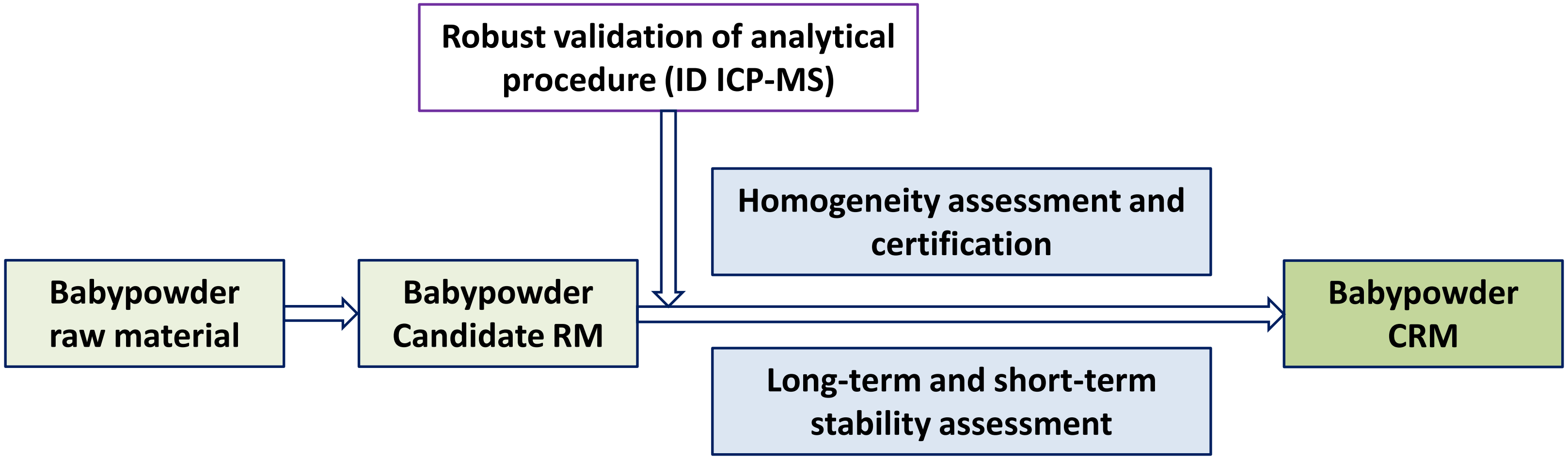
Robust validation of analytical procedure (ID ICP-MS)
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Homogeneity assessment and certification
Long-term and short-term stability assessment
Babypowder CRM