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# Evaluation of the Uncertainty in the Measurement of Nanoparticle Size and Concentration by Single Particle Inductively Coupled Plasma Mass Spectrometry Technique

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Single-particle inductively coupled plasma-mass spectrometry (sp-ICP-MS) is a powerful technique that enables the simultaneous measurement of nanoparticle size and the quantification of metal-containing nanoparticles at real concentrations. These measurements are critical for understanding the potential uses of nanoparticles in various fields. However, sp-ICP-MS is not yet considered a mature methodology. Standardization is necessary, particularly in assessing the reliability of size distribution measurements. This study aims to standardize sp-ICP-MS by assessing the reliability of size distribution measurements for polyvinylpyrrolidone-coated silver nanoparticles and quantifying the input quantities contributing to uncertainties. The uncertainties in calibration, repeatability, and trueness were evaluated based on the thresholds defined by International Organization for Standardization (ISO) standards. Calibration uncertainty was found to be 8.1%, while repeatability was 0.04%, both within the stipulated range of less than 10%. The expanded uncertainty was calculated to be 17%, with a 95% coverage probability for the reference material. Limits of detection (LOD) and quantification (LOQ) for dissolved concentration, particles *per* mL, and size were determined to be 0.37 μg $L^{-1}$, 97.5 particles $mL^{-1}$, and 24.6 nm, respectively. These results demonstrate that the reliability and repeatability of sp-ICP-MS meet ISO-defined thresholds, suggesting that with further standardization, sp-ICP-MS could become a reliable methodology for nanoparticle analysis.

**Keywords:** nanoparticles, reference material, sp-ICP-MS, uncertainty

## Introduction

Nanoparticle characterization has become increasingly significant as it provides essential analytical data that enables the identification and understanding of the physicochemical and biological properties of materials.[1] Accurate characterization is crucial for various applications in fields such as medicine, environmental science, and materials engineering. Metrology, the science of measurement, plays a fundamental role in ensuring the precision and reliability of these measurements. By integrating thorough metrological principles, including sampling, sample preparation, and rigorous measurement techniques, researchers can ensure the quality, traceability, and reliability of results.[2]

Despite advancements in nanoparticle characterization techniques, several open questions and limitations remain. These include the need for standardized methods not only to produce reference materials but also to ensure robust and reproducible measurement techniques across various types of analyses. Reliable calibration procedures for nanoscale measurements and methods tailored to specific nanoparticle properties are essential. Moreover, the accurate determination of size and size distribution-critical for assessing nanoparticle toxicity and biocompatibility-continues to present significant challenges.[3-6]

A recent research[7] has emphasized the potential of inductively coupled plasma mass spectrometry operating in single-particle mode (sp-ICP-MS) for nanoparticle analysis. This technique boasts high sensitivity, elemental specificity, and the capability to simultaneously measure particle number, concentration, and size. sp-ICP-MS is applicable to the analysis of metallic and composite nanoparticles. It has proven valuable not only for screening purposes but

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also for detailed characterization across various fields. In biology and medicine, particularly in cancer treatment, drug delivery systems, diagnostics, and therapeutic applications, sp-ICP-MS is essential for understanding nanoparticle behavior and efficacy. Additionally, environmental studies, materials science, food safety, cosmetics, and electronics benefit from this technique, as precise nanoparticle characterization is crucial for developing new products and technologies. This broad applicability emphasizes the importance of sp-ICP-MS in advancing scientific research and industrial applications.[7-12]

The fundamental principle of sp-ICP-MS is the individual detection of nanoparticles as they are sequentially introduced into the plasma in highly diluted suspensions. The detector reading frequency must be sufficiently high to capture these individual events. Each nanoparticle generates a discrete pulse of ions corresponding to its mass-to-charge ratio (*m/z*) over a period of a few hundred microseconds, which stands out against the continuous background signal. The intensity of each signal pulse is directly proportional to the mass of the nanoparticle, following careful calibration of the system. The size of the nanoparticles can then be determined from their mass, assuming a known element-specific density and particle geometry. The frequency of detected signal pulses corresponds to the number concentration of nanoparticles in the suspension.[7] Thus, sp-ICP-MS enables the rapid determination of average size, size distribution, and particle number concentration of nanoparticles, typically within a few minutes of measurement.

Measurement uncertainties are fundamental parameters that define the quality of metrological results.[13] In this context, the method validation process involves establishing the limits of detection (LOD) and quantification (LOQ), as well as evaluating selectivity and recovery. These parameters are essential for assessing analytical method performance and ensuring reliable, reproducible measurements. Proper assessment and control of these uncertainties enable the production of high-quality, traceable, and comparable results across different laboratories and studies, which is essential for advancing scientific knowledge and technological applications.[14-17]

Laborda *et al.*[14] studied the metrological criteria for implementing the sp-ICP-MS methodology applied to nanoparticle size characterization and quantification. The standard uncertainty associated with determining number concentrations was 5%. The uncertainty for determining nanoparticle diameters was also studied, varying from 3 to 10% for diameters in the range of 100-40 nm, respectively. Reliable average number concentrations and sizes were obtained, although the number size distributions showed a significant broadening contribution due to the sp-ICP-MS measurement process.

Waegeneers *et al.*[17] developed and validated an sp-ICP-MS method for determining sizing and quantifying the fraction of AgNP in confectionery. For E174 (silver), a food additive that may contain silver nanoparticles (AgNP), the expanded measurement uncertainty for AgNP sizing was calculated to be 16% in E174-containing food products, increasing to 23% in E174 itself.

Yamashita *et al.*[18] evaluated sp-ICP-MS uncertainties in size analysis based on two calibration approaches: (*i*) using particle size standards and (*ii*) using ion standard solutions. For the particle size standard approach, the uncertainty in determining the target NP diameter was related to the variation in signal intensities of both target NPs and the particle size standard, and to the size distribution of the particle size standard. The relative uncertainties of the 50 nm silver NP as the target were 15.0, 9.9, and 10.8% when particle size standards of 30, 60, and 100 nm silver NPs were used, respectively. In the ion standard solution approach, the sources of uncertainty were the concentration of the working standard solution, sample flow rate, transport efficiency, slope of the calibration curve, and variation in signal intensity of both the ion standard solution and the target NPs. The relative uncertainties for the 50 nm silver NP were 18.5% for 1 ng $g^{-1}$, 7.6% for 10 ng $g^{-1}$, and 4.7% for 100 ng $g^{-1}$ solutions.

This study aims to evaluate and validate sp-ICP-MS as a method for detecting nanoparticles in suspensions. By applying metrological criteria and concepts related to detectability, this research seeks to establish robust, reliable measurement protocols that minimize uncertainties. These protocols aim to ensure the accuracy of sp-ICP-MS measurements, facilitating high-quality, reproducible results essential for scientific advancement and industrial applications.

## Experimental

### Materials

A Master System Ultra Purifier MS3000 (GEHAKA - Kaufmann Group, Brazil) was used to provide ultrapure water (Type I) for the synthesis and dilutions in this study.

The selectivity test was performed using fluoridated alkaline earth mineral water supplied by Olímpica as a matrix for the preparation of silver nanoparticle solutions. For quality control, reproducibility, and reliability of sp-ICP-MS results, reference material RM 8017 - polyvinylpyrrolidone-coated silver nanoparticles with a nominal diameter of 75 nm, supplied by National Institute of

Standards and Technology (NIST, Gaithersburg, USA), was used. This reference material consists of a vial containing a lyophilized polyvinylpyrrolidone (PVP)-coated silver (Ag) nanoparticle cake. The sealed vial nominally contains 2 mg of Ag and 20 mg of PVP (molar mass approximately 40 kDa). The RM was reconstituted with 2 mL of deionized water before use, with the reconstituted Ag concentration being nominally 1 mg $mL^{-1}$. According to the certificate, the diameter was 74.6 ± 3.8 nm (coverage factor, k = 2.1/95%) as determined by transmission electron microscopy (TEM) using a JEM3010 (JEOL, Peabody, USA). The mean diameter was 69.2 nm with a standard deviation of 0.9 nm, determined by quadrupole ICP-MS operating in single-particle mode (the model of the device used were not provided).

For the size analytical curve in the sp-ICP-MS analysis, standard materials of 60 and 80 nm silver-shelled gold nanospheres (Au@AgNP), supplied by nanoComposix, were used. For the 60 nm Au@AgNP standard, the certificate reported a total diameter of 59 ± 6 nm (TEM). For the 80 nm Au@AgNP standard, the total diameter was 79 ± 9 nm (TEM).

## Methods

The single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS) analysis was carried out using a NexION 300D (PerkinElmer, USA), an instrument designed for elemental analysis of various samples. The Syngistix™ Nano Application Module,[19] software developed by PerkinElmer, was used for all determinations, providing real-time single-particle acquisition and fast data processing. A quadrupole ICP-MS was utilized to quantify the $^{107}Ag$ intensity in time-resolved analysis mode to obtain sp-ICP-MS data on reconstituted RM 8017.

Before sp-ICP-MS analysis, small aliquots of the samples were diluted to Ag mass fractions in the range of 0.1-1 ng $mL^{-1}$ by weighing.[8] Two types of calibration were necessary: a particle calibration to determine transport efficiency and a mass calibration to relate ICP-MS signal intensity to particle mass.[17] Transport efficiency (TE) was measured daily before the analysis of the particle size using 5 and 10 μg $g^{-1}$ Ag as dissolved standards and the particle size method with Au@AgNP 60 and 80 nm standards. Silver (Ag) standards were prepared daily in water by gravimetric dilution. The dissolved Ag fraction was determined using an intensity-*versus*-mass calibration curve, established daily from a dissolved Ag SPEX Plasma Standard (SPEX Chemical). The Au@AgNP 60 and 80 nm standard solutions were used to establish the intensity-*versus*-mass-*per*-event calibration curve. Working suspensions were analyzed within 1 h of dilution to minimize Ag nanoparticle oxidation. Particle pulses were distinguished from the background using a 3σ criterion. Assuming spherical shape, individual pulse intensities were converted to mass using the calibration curve, and then the diameter was calculated using the bulk density of Ag. The dwell time was 50 μs, scan time was 100 s, and the sample flow rate was 0.346 ± 0.001 mL $min^{-1}$, determined by weighing.

The calculated transport efficiency was 9.2%, and the room temperature was 21 °C. The SPEX standard was prepared in the range of 2 to 10 ng $mL^{-1}$ by weighing. The RM was suspended in ultrapure water. Three replicates of each dilution were prepared (0.1:14 m/m dilution, followed by a second 0.2:20 m/m dilution). The samples were diluted with ultrapure water and analyzed in five runs on the NexION 300D device. Dilution control was achieved by weighing the introduced quantity of solution and dispersant when preparing the diluted samples. For the selectivity test, fluoridated alkaline earth mineral water supplied by Olímpica was used for dilutions.

### Limits of detection (LOD) and quantification (LOQ)

The limit of detection (LOD) is the lowest particle size and/or concentration at which the method can detect the analyte within the matrix with a certain degree of confidence. The limit of quantification (LOQ) is the lowest particle size and/or concentration at which the analyte can not only be reliably detected but also meet predefined goals for bias and precision. According to the Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO), DOQ-CGCRE-008,[20] the basic equations for calculating LOD and LOQ are given in equations 1 and 2:

$$LOD_x = y_x + 3.3 \times s_x \quad (1)$$

$$LOQ_x = y_x + 10 \times s_x \quad (2)$$

where x is dissolved concentration or particle concentration or size; $y_x$ is blank signal (ultrapure water) and $s_x$ is standard deviation. To estimate the limits of detection of dissolved concentration ($LOD_{diss}$), particle concentration ($LOD_{part}$) and size ($LOD_{size}$), equation 1 was used. To estimate the limits of quantification of dissolved concentration ($LOQ_{diss}$), particle concentration ($LOQ_{part}$), and size ($LOQ_{size}$) equation 2 was used.

### Recovery (Re)

The recovery of the analytical method was calculated using the equation 3:

$$\mathrm{Re}(\%) = \frac{\text{experimental size}}{\text{standard size}} \times 100 \quad (3)$$

### Uncertainties evaluation

The estimation of uncertainty in particle size measurements by sp-ICP-MS followed the guidelines outlined in the Guide to the Expression of Uncertainty in Measurement (GUM).[21] The sources of uncertainty and their relative contributions were investigated. Uncertainty levels were primarily evaluated using Type A methods, where uncertainty was quantified by the standard deviation of replicated measurements and counting statistics derived from signal intensity. Measurement parameters such as uncertainty in dissolved concentration and particle concentration related to device calibration were established. The relative standard uncertainty of calibration ($u_{cal}/d$), relative uncertainty of trueness ($u_{trueness}$), relative uncertainty of repeatability ($u_{repe}$), combined uncertainty ($u_{c_{size}}$), and expanded uncertainty (U) were also estimated. All uncertainty sources were identified in the Ishikawa diagram (Figure 1).

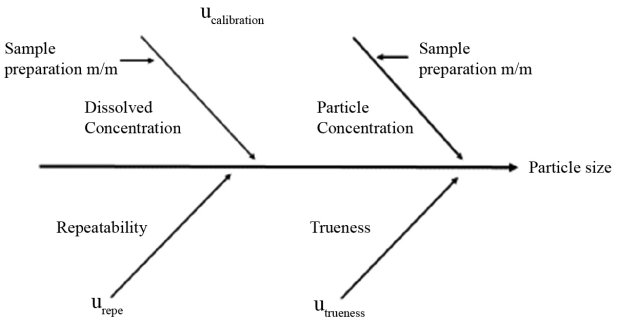


**Figure 1.** Ishikawa diagram. Sources of uncertainty considered in this study.

### Uncertainty contribution of dissolved silver concentration ($u_{diss}$) and particle concentration ($u_{part}$)

The uncertainty in the dissolved silver concentration ($u_{diss}$) was estimated based on the dissolved background signal concentration (ng $mL^{-1}$), provided by the software,[19] which was in turn based on the dissolved intensity and calibration with a dissolved silver standard. The uncertainty in silver particle concentration ($u_{part}$) was estimated from the calculated particle concentration (particles $mL^{-1}$), as determined by the software, considering transport efficiency and the number of detected peaks. The equation used for calculating $u_{diss}$ and $u_{part}$ is:[17]

$$u_x = \frac{S\text{replicates}}{Cx} \quad (4)$$

where x represents dissolved concentration or particle concentration; $s_{replicates}$ is the standard deviation; and *C*x is the concentration or mass.

### Relative standard uncertainty of calibration ($u_{cal}/d$)

The relative standard uncertainty of calibration ($u_{cal}$) was calculated as the square root of the sum[13] of $u_{diss}$ and $u_{part}$, providing the relative standard uncertainty ($u_{cal}/d$) of the mean effective diameter of nanoparticles in the sample. This is expressed in equation 5, concerning equipment calibration:

$$\frac{u_{cal}^2}{d^2} = (u_{diss})^2 + (u_{part})^2 \quad (5)$$

### Evaluation of relative standard uncertainty of trueness

The relative standard uncertainty of trueness ($u_{trueness}$) was estimated using equation 6:

$$u_{trueness} = \sqrt{\left(\frac{s_{replicates}}{\sqrt{n}}\right)^2 + (u_{RM})^2} \quad (6)$$

where $s_{replicates}$ is the standard deviation, n is the number of measurements, and $u_{RM}$ is the uncertainty of the certified value obtained using equation 7, where k is the coverage factor (2.1 for a confidence level of 95%):

$$u_{RM} = \frac{U_{RM}}{k} \quad (7)$$

### Evaluation of relative uncertainty of repeatability ($u_{repe}$)

The relative uncertainty of repeatability ($u_{repe}$) was evaluated under single-operator conditions, requiring consistent sample preparation through precise weighing on a semi-analytical balance and adherence to operator competence. This assessment involved five repetitions across three RM samples (totaling 15 readings). Certain components of uncertainty were evaluated experimentally based on the variability observed in repeated measurements. The repeatability uncertainty was then estimated using equation 8:

$$u_{repe} = \frac{S\text{replicates}}{\sqrt{n}} \quad (8)$$

where n is the square root of the number of measurements.

### Combined uncertainty ($u_{c_{size}}$)

The combined uncertainty ($u_{c_{size}}$) was estimated by combining the standard uncertainties for calibration ($u_{cal}$), trueness ($u_{trueness}$), and repeatability ($u_{repe}$) according to equation 9:

$$u_{c_{size}} = \sqrt{(u_{cal})^2 + (u_{trueness})^2 + (u_{repe})^2} \quad (9)$$

### Coverage factor (k) and expanded uncertainty (U)

The coverage factor (k) is a numerical multiplier applied to the combined uncertainty ($u_{c_{size}}$) to obtain the expanded uncertainty of measurement, as shown in equation 10. For a 95% confidence level,[22] the coverage factor is k = 2:

$$U_{measurement} = u_{c_{size}} \times k \quad (10)$$

## Results and Discussion

The limit of detection for dissolved concentration ($LOD_{diss}$), particle concentration ($LOD_{part}$), and size ($LOD_{size}$) were obtained from the standard deviations calculated for the seven blanks (water) analyzed using equation 1. The Syngistix™ Nano Application Module software[19] provides the limit of detection for size ($LOD_{size}$). The limit of quantification for dissolved concentration ($LOQ_{diss}$), particle concentration ($LOQ_{part}$), and size ($LOQ_{size}$) were estimated using equation 2. Table 1 presents the obtained limits.

**Table 1.** Limits of detection and quantification for dissolved concentration, particle concentration and size

| | ave ± s | LOD | LOQ |
|---|---|---|---|
| Dissolved concentration / (ng $mL^{-1}$) | 0.30 ± 0.02 | 0.37 | 0.50 |
| Particle concentration / (particles $mL^{-1}$) | 54.3 ± 13.1 | 97.5 | 185.3 |
| Size / nm | 20.0 ± 1.4 | 24.6 | 34.0 |

ave ± s: average ± standard deviation; LOD: limit of detection; LOQ: limit of quantification.

The limits of detection and quantification represent the smallest values capable of generating a signal above the noise. Thus, under the conditions of the analyses, the method is unable to detect nanoparticles smaller than 25 nm and quantify nanoparticles smaller than 34 nm. The ISO/TS 19590:2017[23] standard states that nanoparticles with sizes smaller than the limit of detection for the sp-ICP-MS method can be quantified as ionic (i.e., dissolved). The limits of detection and quantification for dissolved particle concentration ($LOD_{diss}$ and $LOQ_{diss}$) were estimated using the concentration of the background signal of dissolved silver (ng $mL^{-1}$) provided by the software, which takes into account the signal intensity of the dissolved silver, calibrated using a dissolved silver standard. Therefore, the method is incapable of detecting dissolved silver nanoparticles at concentrations below 0.37 ng $mL^{-1}$.

On the other hand, the limits of detection and quantification for silver particle concentration ($LOD_{part}$ and $LOQ_{part}$) were estimated based on the calculated particle concentration, also provided by the software, considering the transport efficiency and the number of detected peaks. Once again, the method cannot detect nanoparticle concentrations below 97.5 particles $mL^{-1}$. Bazilio *et al.*[24] obtained $LOD_{size}$, $LOD_{diss}$, and $LOD_{part}$ values of 17.5 nm, 0.736 ng $mL^{-1}$, and 146 particles $mL^{-1}$, respectively, which are comparable to the results presented in this paper.

To evaluate the method selectivity, RM 8017 was diluted with mineral water (fluoridated alkaline earth mineral water supplied by Olímpica) according to the procedure described in the "Methods" sub-section. The mean effective nanoparticle size from three samples and five independent analyses for each sample, as well as the RM size, were 71.3 ± 0.1 and 69.2 ± 0.9 nm, respectively. The response was 103% of the RM value, indicating the method ability to determine particle size even in the presence of a matrix.

Additionally, samples were prepared with 60 and 80 nm standards, as well as a mixture of both standards, using ultrapure water for dilutions (always with concentrations below 1 ng $mL^{-1}$). The total diameters of the 60 and 80 nm standards, as indicated in the NanoComposix report and obtained by TEM, were 59 and 79 nm, respectively.

The most frequent sizes identified were 56.1 nm for the 60 nm standard and 83 nm for the 80 nm standard, resulting in recoveries of 95 and 105%, respectively, as calculated using equation 3. The histogram of the 60 and 80 nm standard mixture, along with the deconvolution analysis, is shown in Figure 2. This analysis reveals two primary peaks corresponding to distinct nanoparticle sizes, with peaks at 56 and 82.6 nm. Additionally, two smaller, broader peaks were observed in the histogram, likely representing nanoparticle aggregates. Based on these identified peaks, method recoveries of 95 and 104% were achieved, closely matching those of the individual solutions and demonstrating the selectivity of the method in distinguishing nanoparticles of different sizes.

To evaluate whether the results obtained from sp-ICP-MS are reproducible and reliable, certain parameters must be established. First, the evaluation follows the procedure applied to a reference material, which ensures particle size accuracy (d), suspension stability, and a narrow size

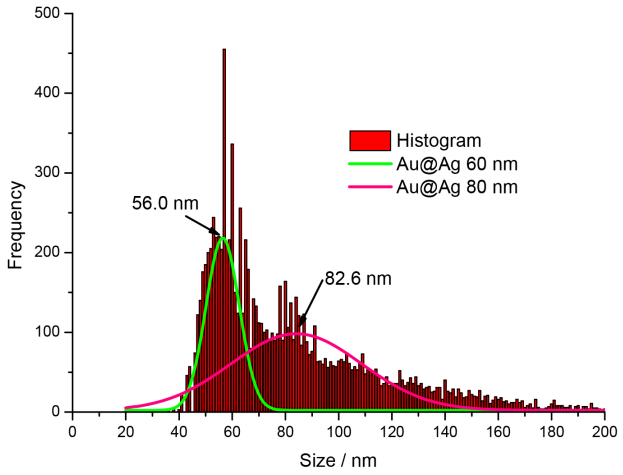


**Figure 2.** Particle size distribution histogram for the mixed solution of 60 and 80 nm standards. Peaks deconvolution estimated by OriginPro 8.5 (OriginLab, Northampton, MA, USA).[25]

distribution. Second, the diluted standards must remain stable during analysis,[13] and samples were analyzed within 1 h of dilution to minimize Ag nanoparticle oxidation. Successive dilutions were prepared by weighing (m/m). The means and standard deviations of dissolved and particle concentrations, as well as particle size determined by sp-ICP-MS, are summarized in Table 2. The table also includes the RM 8017 size with expanded uncertainty from TEM and size standard deviation determined by sp-ICP-MS.

**Table 2.** Means and standard deviations for dissolved and particle concentrations and particle size determined by sp-ICP-MS

| | Mean and standard deviation |
|---|---|
| Dissolved / (μg $g^{-1}$) | 100 ± 7.7 |
| Particle / (part $mL^{-1}$) | $2.79 \times 10^{11} \pm 3.23 \times 10^{9}$ |
| Size (d) / nm | 70.80 ± 0.12 |
| Size RM 8017 ($d_{RM}$) / nm | 74.6 ± 3.8[a] |
| | 69.2 ± 0.9[b] |

[a]k = 2.1 with 95% confidence; [b]standard deviation not uncertainty.

The uncertainty contributions for dissolved silver concentration ($u_{diss}$) and particle concentration ($u_{part}$) were calculated using equation 4 and estimated at 0.08 ng $g^{-1}$ and 0.012 particles $mL^{-1}$, respectively. The relative standard uncertainty of calibration ($u_{cal}$), concerning equipment calibration, was then calculated using equation 5. The square root of the sum of these components ($6.5 \times 10^{-3}$) gives the relative standard uncertainty of the nanoparticle diameter in the sample, resulting in $u_{cal}/d = 0.081$. With a mean size of 70.8 nm, the standard uncertainty is $u_{cal}$ = 5.73 nm (calibration), corresponding to 8.1% of the diameter.

Since certified reference materials with certified size distribution for sp-ICP-MS are unavailable, the trueness was calculated using the expanded uncertainty from TEM and the standard deviation of nanoparticle size determined by sp-ICP-MS, as reported in RM 8017. Using equation 6, $u_{trueness}$ = 1.81 nm (2.5%) was obtained. The $u_{trueness}$ based on the sp-ICP-MS measurements was 0.9 nm (1.3%).

Method precision was defined by the closeness among independent test results under defined conditions. Repeatability (same analyst, method, and instrument in the same laboratory)[13,26,27] was evaluated, with a mean diameter of 70.80 ± 0.12 nm over 15 readings. The relative uncertainty of repeatability ($u_{repe}$) was 0.031 nm (0.04%). Intermediate precision was not evaluated.

The ISO/TS 19590:2017[23] standard defines methods for measuring and characterizing nanoparticles using sp-ICP-MS. The uncertainties for repeatability and trueness should be within 5 and 10%, respectively. These limits are essential to ensure the consistency and validity of the results obtained with sp-ICP-MS, and the results presented in this study are in agreement with these standards.

The combined uncertainty ($u_{c_{size}}$ = 5.99 nm) was calculated by combining $u_{cal}$ (5.73 nm), $u_{trueness}$ (1.81 nm), and $u_{repe}$ (0.031 nm) using equation 9. The expanded uncertainty (U) at a 95% confidence level (k = 2) was U = 12 nm (17%) and U = 11.6 nm (16%) using standard deviation. Therefore, the mean size determined by sp-ICP-MS was 70.8 ± 12 nm.

Waegeneers *et al.*[17] found expanded uncertainties of 16% in E174-containing food products and up to 23% in E174 itself, corroborating our findings.

Figure 3 compiles the uncertainty estimated from the validation data (calibration equipment, trueness and repeatability) as well as expanded uncertainty.

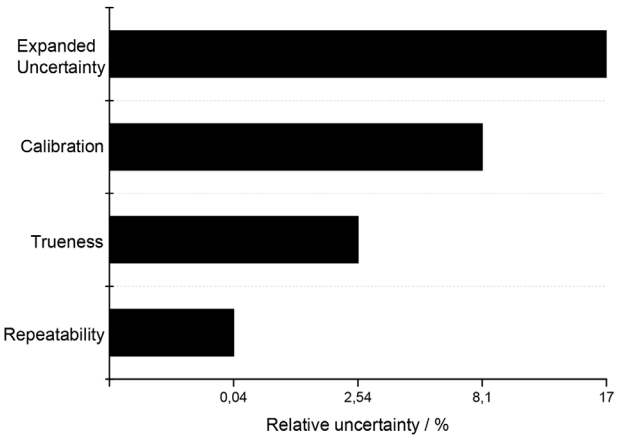


**Figure 3.** Relative standard and expanded measurement uncertainties (%) estimated for size measurements by sp-ICP-MS.

The quality of uncertainty assessment depends on the critical analysis of those contributing factors. As shown in Figure 3, the relative uncertainty from calibration

equipment was 8.1%, while the estimate of trueness was 2.5%. The low uncertainty in repeatability (0.04%) can be attributed to proper sample preparation and the skill of the researcher. Geraldes *et al.*[13] found similar results, emphasizing good laboratory practices. These observations suggest that calibration uncertainty dominates. However, the trueness and repeatability uncertainties proved to be irrelevant. In this paper, this means that any improvement in expanded uncertainty related to sp-ICP-MS equipment requires a reduction in the calibration standard uncertainty value and can be achieved by studying plasma parameters, dwell time, and/or transport efficiency.

Single-particle ICP-MS is often viewed as a promising technique to characterize metallic nanoparticles,[7,9,15,18] and the current study corroborates this point of view. Overall, the study confirms that sp-ICP-MS is a promising technique for characterizing metallic nanoparticles. The method, with a measurement uncertainty of 70.8 ± 12 nm, is fit for purpose, providing high confidence in the results.

## Conclusions

The validation of the methodology, based on the experimental conditions for determining particle size using ICP-MS in single-particle mode, enabled the identification of uncertainties both globally and at each stage of the measurement process. This approach ensured that the entire process remained under control, providing robust and traceable results while enabling preventive control throughout the measurement process. By establishing the total uncertainty level, the reliability limit for each data point obtained was clearly defined.

The validated protocol proved effective for both mono- and bimetallic nanoparticles, demonstrating that the method, along with its resulting particle sizes and expanded uncertainties, is fit for purpose. This provides a high level of confidence in the reliability and accuracy of the measurements and represents a significant advancement, confirming that sp-ICP-MS can be applied across various fields.

Moreover, this protocol opens new opportunities for research, especially in critical areas such as medicine, where it can support studies on drug delivery, regenerative process modeling, and disease treatment, including cancer. Beyond medicine, the method shows broad applicability in environmental science, materials science, food safety, cosmetics, and electronics. It also presents promising potential for detecting and quantifying micro- and nanoplastics, which is increasingly relevant in environmental monitoring.

This validation reinforces sp-ICP-MS as a powerful tool for nanoparticle characterization. With further standardization, it can become a key technique for a wide range of scientific and industrial applications.

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