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# Validation and measurement uncertainty evaluation of the ICP-OES method for the multi-elemental determination of essential and nonessential elements from medicinal plants and their aqueous extracts

Marin Senila[1*†], Andreja Drolc[2†], Albin Pintar[2], Lacrimioara Senila[1] and Erika Levei[1]

## Abstract

**Background:** The paper presents the development, validation, and evaluation of measurement uncertainty of a method for quantitative determination of essential and nonessential elements in medicinal plants and their aqueous extracts by using inductively coupled plasma optical emission spectrometry.

**Methods:** The detailed validation of the analytical procedure and calculation of the measurement uncertainty budget allowed the recognition of the methods' critical points.

**Results:** The obtained limit of quantification, repeatability, and measurement uncertainty were satisfactory. The trueness of the method was verified by recovery estimation using certified reference materials. The recovery rates of all metals were between 95% and 105%.

**Conclusions:** The paper presents for the first time all the steps needed to evaluate the measurement uncertainty and validate the determination method of selected elements in medicinal plants and their aqueous extracts. In summary, the obtained results demonstrate that the method can be applied effectively for the designed purpose.

**Keywords:** ICP-OES; Medicinal plants; Multi-elemental analysis; Validation; Measurement uncertainty

## Background

The inductively coupled plasma optical emission spectrometry (ICP-OES) is a strong tool for the determination of various elements in liquid and solid samples. Elevated concentrations of essential elements (e.g., Fe, Mn, Zn, Cr, Cu) and low concentrations of nonessential elements (e.g., Cd, Ni, As) may present a potential hazard for human health. When preparing tea by infusion of plants, metals can be leached into the water and consumed by humans. Therefore, the metal contents in the plant infusion should comply with the limit values set by the Drinking Water Directive (Council of the European Union 1998).

Numerous plant species used as remedies in traditional medicine are grown as spontaneous flora (Chuparina and Aisueva 2011). In Romania, among the most used medicinal plants in folk medicine are chamomile (*Matricaria recutita*), milfoil (*Achillea millefolium*), rattle (*Hypericum perforatum*), brotherwort (*Thymus serpyllum*), pot marigold (*Calendula officinalis*), linden (*Tilia platyphyllos*), and peppermint (*Mentha piperita*). Chamomile is used for its anxiolytic, antiseptic, and anti-inflammatory properties, while milfoil is used for its strong astringent effect and to treat a variety of illnesses and disorders from stomach aches to circulatory disorders. Rattle is usually used to treat digestive and neurological disorders; brotherwort has antiseptic properties and is used for acne and allergies treatment; pot marigold has anti-inflammatory and antitumor properties; linden is used for its calming effects and to ease

* Correspondence: marin.senila@icia.ro
[†]Equal contributors
[1]INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, Cluj-Napoca 400293, Romania
Full list of author information is available at the end of the article

cold and flu symptoms; while peppermint is used to relieve stomach aches, nausea, fever, stress, and to boost the immune system.

Since, all over the world, there are numerous metal-polluted sites (European Environment Agency 2007), metals from soil can be transferred to the plants (Moreno-Jimenez et al. 2009; Malandrino et al. 2011; Senila et al. 2012; Rodrigues et al. 2012) and may have adverse effects on consumers' health, as local residents use the plants in their diet, mainly for tea preparation. Consequently, there is a need to develop reliable methods for the determination of metals in medicinal plants and their aqueous extracts.

The ICP-OES method has become a routine analytical technique for metal determination; however, the information related to method validation are scarce, and research on this field is still needed (Mermet 2005). Several studies present the use of ICP-OES for metals determination in tea or other food samples (Mitic et al. 2012; Froes et al. 2014). For consistent interpretation of the measurement results, it is necessary to evaluate the confidence that can be placed in, therefore, the presentation of an analytical result which must be accompanied by indication of the data quality. This information is essential for the interpretation of the analytical result (Kessel 2002; Drolc and Pintar 2011). Method validation is an essential component of the measures that a laboratory should implement in order to produce reliable analytical data (EURACHEM 1998). Besides common method performance characteristics obtained in the validation process, testing laboratories shall have and apply procedures for estimating the uncertainty of measurements (International Organization for Standardization 2005). This clearly means that the analytical result cannot be viewed only as a separate value. The International Organization for Standardization (ISO) guide (International Organization for Standardization 1995) recommends the calculation of uncertainty using a model equation, based on its uncertainty components, and by using the law of propagation of uncertainty in order to combine them into uncertainty. It has subsequently been interpreted for analytical chemistry (Ellison et al. 2012). There are several possibilities to estimate the uncertainty, as reported in the literature (Ellison et al. 2012; International Organization for Standardization 1995; Magnusson et al. 2012; Baralkiewicz et al. 2013). The measurement uncertainty is estimated mainly by the top-down or bottom-up approaches. In the top-down approach, the major sources of uncertainty are identified and evaluated, while in the bottom-up approach, all the uncertainty sources are systematically evaluated and only those with significant contributions are used to derive the measurement uncertainty. The top-down approach is time-consuming and requires extensive knowledge of the analytical procedure, but it enables identification of major uncertainty sources and consequently reduction of total

measurement uncertainty. Another relatively quick and easy way of uncertainty estimation is the in-house validation that includes the determination of the method performance parameters (Baralkiewicz et al. 2013).

In spite of a several papers published on the topic, there is a lack of fully validated methods for metal determination in medicinal plants and their extracts. The purpose of the present work was to perform a detailed validation of the analytical procedure and estimate the measurement uncertainty budget for determination of some essential (Fe, Mn, Zn, Cr, Cu, Al, Mg) and toxic (Pb, Cd, Ni, As) elements in the medicinal plants and their aqueous extracts. The method was validated according to the international guidelines ISO/IEC 17025:2005 (International Organization for Standardization 2005). The assessment of uncertainty was carried out using modelling approach and a full combined uncertainty calculation, including possible sources of uncertainty.

## Methods
### Instrumentation
Analyses were carried out using a dual viewing inductively coupled plasma optical emission spectrometer Optima 5300DV (PerkinElmer, Waltham, MA, USA) coupled to an ultrasonic nebulizer CETAC 6000AT+ (CETAC, Omaha, NE, USA). The operating conditions employed for ICP-OES determination were 1,300 W RF power, 15 L min$^{-1}$ plasma flow, 2.0 L min$^{-1}$ auxiliary flow, 0.8 L min$^{-1}$ nebulizer flow, and 1.5 mL min$^{-1}$ sample uptake rate. Axial view was used for metals determination, while 2-point background correction and six replicates were used to measure the analytical signal. In order to eliminate the memory effect caused by the use of ultrasonic nebulization, the delay time for washing between samples and signal measurement was set to 180 s. The measurement of a blank solution after measuring 1 mg L$^{-1}$ calibration standard indicated the lack of memory effect. High-purity argon (99.995%) supplied by Linde Gas SRL (Timis, Timisoara, Romania) was used to sustain plasma and as carrier gas. A closed-vessel MWS-3+ microwave system (Berghof, Germany) with temperature control mode was used for sample digestion. All PTFE digestion vessels were previously cleaned in a bath of 10% (*v/v*) nitric solution for 48 h to avoid cross-contamination.

### Reagents and CRMs
Multi-elemental solutions of 1,000 mg L$^{-1}$ ICP Standard Certipur® (Merck, Darmstadt, Germany) containing the analysed elements (Fe, Mn, Al, Mg, Pb, Zn, Cr, Cu, Cd, Ni, and As) were used for calibration. Analytically graded 65% HNO$_3$ and 30% H$_2$O$_2$ (Merck, Germany) were used for sample digestion. Ultrapure water obtained by a Milli-Q system (Millipore, Molsheim, France) was used for all

**Table 1 Wavelengths for selected elements, LoD, and LoQ in aqueous extracts and in dry plants**

| Element | Wavelength (nm) | Plant aqueous extracts[a] | | | Plant dry mass[b] | | |
|---|---|---|---|---|---|---|---|
| | | LoD (mg L$^{-1}$) | LoQ (mg L$^{-1}$) | Target value[c] (mg L$^{-1}$) | LoD (mg kg$^{-1}$) | LoQ (mg kg$^{-1}$) | Target value[d] (mg kg$^{-1}$) |
| Cd | 228.805 | $0.13 \times 10^{-3}$ | $0.43 \times 10^{-3}$ | $0.50 \times 10^{-3}$ | 0.019 | 0.063 | $0.05 \times 10^{-3}$ |
| Cr | 267.713 | $0.60 \times 10^{-3}$ | $2.00 \times 10^{-3}$ | $5.00 \times 10^{-3}$ | 0.075 | 0.25 | - |
| Cu | 327.398 | $0.75 \times 10^{-3}$ | $2.50 \times 10^{-3}$ | $200 \times 10^{-3}$ | 0.12 | 0.40 | - |
| Fe | 238.205 | $2.24 \times 10^{-3}$ | $7.46 \times 10^{-3}$ | $20 \times 10^{-3}$ | 0.25 | 0.83 | - |
| Al | 396.153 | $1.59 \times 10^{-3}$ | $5.30 \times 10^{-3}$ | $20 \times 10^{-3}$ | 0.20 | 0.67 | - |
| Mg | 258.213 | $0.60 \times 10^{-3}$ | $2.00 \times 10^{-3}$ | - | 0.11 | 0.37 | - |
| Pb | 220.355 | $0.29 \times 10^{-3}$ | $0.97 \times 10^{-3}$ | $1.00 \times 10^{-3}$ | 0.043 | 0.14 | $0.5 \times 10^{-3}$ |
| Mn | 257.611 | $0.25 \times 10^{-3}$ | $0.83 \times 10^{-3}$ | $5.00 \times 10^{-3}$ | 0.030 | 0.10 | - |
| Ni | 231.604 | $0.57 \times 10^{-3}$ | $1.90 \times 10^{-3}$ | $2.00 \times 10^{-3}$ | 0.13 | 0.43 | - |
| Zn | 213.856 | $1.52 \times 10^{-3}$ | $5.06 \times 10^{-3}$ | - | 0.22 | 0.73 | - |
| As | 193.759 | $0.30 \times 10^{-3}$ | $1.00 \times 10^{-3}$ | $1.00 \times 10^{-3}$ | 0.045 | 0.15 | - |

[a]Calculated for the extraction method (1 g of plant digested with nitric acid and perhydrol in 100 volumetric flask); [b]calculated for the extraction method (0.5 g of plant extracted in water in 100 volumetric flask); [c]10% of the limit values according to Drinking Water Directive; [d]10% of the limit values according to the European Pharmacopeia.

dilutions and infusions. For metals' determination in plants and their aqueous extracts, the calibration standards were prepared by diluting the reference multielemental standard solution in 8% (*v*/*v*) nitric acid and 0.5% (*v*/*v*) nitric acid, respectively, in order to assure the similar concentration of nitric acid in samples and in calibration standards.

A vegetable certified reference material (CRM) IAEA-359 Cabbage (IAEA, Austria) and a water CRM trace metals 1-WP QC11132 (Sigma Aldrich, Steinheim, Germany) were used for the quality control of metals' determination.

### Plant samples

Seven medicinal plants (chamomile, milfoil, rattle, brotherwort, pot marigold, linden, peppermint) were randomly collected from spontaneous flora grown in NW Romania. Three specimens of each plant species were intensely rinsed with tap water and distilled water and dried in an oven at 40°C until weight is constant. In order to accelerate the digestion process, samples were grinded to powder with a kitchen mixer grinder and sieved through a 100-μm mesh. Five sub-samples from each plant species were used for analysis.

### Microwave digestion procedure

An amount of 0.5 g of plant powder was weighted into dry, clean PTFE vessels then 6 mL of $HNO_3$ and 2 mL of $H_2O_2$ were added. Vessels content were mixed and kept at room temperature for 12 h, then the vessels were introduced in the microwave digestion system and digested using a four-step digestion program: (1) 5 min at 280 W, (2) 5 min at 700 W, (3) 10 min at 1,050 W, (4) 1 min at 0 W. The resulting solutions were cooled, diluted to 50 mL with distilled water, filtered, and then analysed by ICP-OES. In order to evaluate the accuracy of the method, the vegetable CRM was analysed in the same experimental conditions as the samples.

### Aqueous extracts

An amount of 1 g of plant powder was prepared for infusion in 200 mL of boiling ultrapure water for approximately 10 min. The obtained infusions were filtered, evaporated to approximately 10 mL on a hot plate, then 1 mL of $HNO_3$ was added and the samples were digested in the microwave digestion system using the same digestion program as for solid samples. After cooling, the obtained solutions were filtered and diluted with ultrapure water in 100 mL volumetric flasks and analysed by ICP-OES.

**Table 2 Confirmation of LoQ in aqueous extracts and in plant dry mass**

| Element | Plant aqueous extracts | | Plant dry mass | |
|---|---|---|---|---|
| | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) |
| Cd | 9.61 | 95.6 | 10.5 | 98.9 |
| Cr | 11.8 | 91.2 | 10.2 | 95.6 |
| Cu | 8.86 | 104 | 9.95 | 101 |
| Fe | 15.2 | 112 | 12.6 | 115 |
| Al | 9.12 | 104 | 9.05 | 110 |
| Mg | 8.24 | 98.6 | 8.87 | 96.6 |
| Pb | 11.6 | 94.6 | 12.2 | 97.2 |
| Mn | 10.1 | 108 | 8.96 | 110 |
| Ni | 14.2 | 114 | 15.1 | 104 |
| Zn | 8.89 | 98.8 | 10.4 | 96.3 |
| As | 14.5 | 106 | 12.8 | 112 |

**Table 3 Calibration curves for working range LoQ to 1.00 mg L$^{-1}$**

| Element | a value | b value | r$^2$ value | PG |
|---|---|---|---|---|
| Cd | 22,300 | 901,200 | 0.9999 | 1.96 |
| Cr | 12,090 | 868,000 | 0.9999 | 2.88 |
| Cu | 5,345 | 1,045,000 | 0.9997 | 4.33 |
| Fe | 23,700 | 1,503,900 | 0.9999 | 4.18 |
| Al | −11,100 | 1,922,000 | 0.9999 | 3.12 |
| Mg | −5,600 | 3,459,000 | 1.0000 | 1.72 |
| Pb | 129 | 184,800 | 0.9998 | 4.56 |
| Mn | 141,500 | 8,094,000 | 1.0000 | 1.96 |
| Ni | 15,400 | 111,300 | 0.9999 | 4.06 |
| Zn | 56,500 | 1,159,000 | 0.9999 | 3.36 |
| As | −33 | 32,400 | 0.9999 | 4.67 |

## Results and discussion

### Method validation

The validation of the analytical procedure for quantitative determination of elements in medicinal plants and their aqueous extracts was performed by evaluating selectivity, working and linear ranges, limit of detection (LoD), limit of quantification (LoQ), trueness, and precision (repeatability and reproducibility) (EURACHEM 1998).

### Selectivity

Selectivity is the ability of a method to accurately quantify the analyte in the presence of interferences, under the stated conditions of the assay for the sample matrix being studied (EURACHEM 1998). The selectivity in the case of ICP-OES method is related to possible interferences of the emission spectrum at specific wavelengths. The emission lines used for quantitation of each element, based on known interferences and baseline signal at selected wavelengths observed empirically during the measurements, are presented in Table 1. Matrix effects were studied by standard addition method, by adding a spike of 1 mg L$^{-1}$

of each element to the original samples. The recoveries were within 90% and 110% for all the studied elements.

### LoD and LoQ in aqueous extracts and in dry plants

The LoD indicates the level at which detection becomes problematic, while LoQ is the lowest concentration of the analyte that can be determined with an acceptable level of repeatability, precision, and trueness. LoD was estimated from the calibration function for a signal equal to the net signal of blank and three times its standard deviation, while LoQ was estimated from the calibration function for a signal equal to the net signal of blank and ten times its standard deviation (EURACHEM 1998; Miller and Miller 2000). Standard deviation of the blank resulted from the analysis of ten independent reagent blank solutions, each measured once on the same day. As the metal content in tea is not legislated, the performance criteria targeted for the LoD for aqueous plant extracts were 10% of the limit values (µg L$^{-1}$) for drinking water: As - 10; Cd - 5; Cr - 50; Cu - 2,000; Pb - 10; Ni - 20; Fe - 200; Al - 200; and Mn - 50 (Council of the European Union 1998). The European Pharmacopeia (Council of Europe 2011) proposed a limit of 5 mg kg$^{-1}$ for Pb and 0.5 mg kg$^{-1}$ for Cd in herbal drugs. For plant samples, the performance criteria targeted for the LoD were 10% of these values. Data in Table 1 showed that the performance targets were achieved by our methods. In order to experimentally confirm LoQ, six standard solutions with concentrations close to the LoQ were prepared and analysed. The targeted repeatability expressed as relative standard deviation (RSD) and targeted recovery were 20% and 90% to 115%, respectively. The measured RSD and recovery are presented in Table 2.

### Working and linear range

Working range is the range of analyte concentrations over which the method is linear. At the lower end of the concentration range, the limiting factor is LoQ, while at the upper end limitations are imposed by various effects

**Table 4 Results of analysis of water CRM trace metals 1-WP QC11132 (Sigma Aldrich)**

| Element | Found value (µg L$^{-1}$) | s (µg L$^{-1}$) | Certified value (µg L$^{-1}$) | $U_{CRM}$ (µg L$^{-1}$) | Recovery (%) |
|---|---|---|---|---|---|
| Cd | 390 | 3.10 | 385 | 5.07 | 101 |
| Cr | 862 | 25.7 | 864 | 11.6 | 99.8 |
| Cu | 592 | 8.00 | 603 | 7.80 | 98.2 |
| Fe | 1,350 | 27.0 | 1,340 | 24.4 | 101 |
| Al | 452 | 12.9 | 463 | 11.4 | 97.6 |
| Pb | 908 | 30.2 | 929 | 14.4 | 97.7 |
| Mn | 1,200 | 7.15 | 1,200 | 15.5 | 100 |
| Ni | 1,730 | 21.1 | 1,710 | 20.4 | 101 |
| Zn | 918 | 8.04 | 915 | 16.2 | 100 |
| As | 414 | 7.25 | 416 | 8.25 | 99.5 |

**Table 5 Results of analysis of IAEA-359 cabbage CRM (IAEA)**

| Element | Certified content, $\mu g\ g^{-1}$ | Found content[a], $\mu g\ g^{-1}$ |
|---------|------------------------------------|-----------------------------------|
| Cd | 0.115 to 0.125 | 0.116 ± 0.014 |
| Cr | 1.24 to 1.36 | 1.25 ± 0.088 |
| Cu | 5.49 to 5.85 | 5.44 ± 0.41 |
| Fe | 144.1 to 151.9 | 147 ± 4.53 |
| Mn | 31.3 to 32.5 | 32.4 ± 2.15 |
| Ni | 1.00 to 1.10 | 1.03 ± 0.094 |
| Zn | 37.9 to 39.3 | 37.8 ± 1.89 |
| As | 0.096 to 0.104 | 0.100 ± 0.012 |
| Mg | 2,110 to 2,210 | 2,114 ± 23.6 |

[a]Values are expressed in microgram per gram dry weight and reported as average ± s; n = 5; 95% confidence level.

depending on the instrument response. Although generally, three or four calibration standards are used to evaluate the linear range of ICP-OES method in order to evaluate the appropriate measurement uncertainty budget; in our study, linearity was evaluated from the regression function of calibration using eight standards, the lowest concentration close to the LoQ, while the others were 0.05, 0.10, 0.20, 0.40, 0.60, 0.80, and 1.00 mg L$^{-1}$ for each element. The fit for purpose working range was selected to be between LoQ of each element and 1.00 mg L$^{-1}$.

Ten replicates of the lowest and ten at the highest concentration of the working range were measured. To check the homogeneity of variances, the standard deviations ($s_1$) and ($s_2$) of the lowest and the highest concentrations from calibration curves and the PG ratios ($s_1^2/s_2^2$ or $s_2^2/s_1^2$) were calculated and compared with the critical value $F_{9;9;0.99} = 5.35$. The values for intercept ($a$), slope ($b$), correlation coefficient ($r^2$), and PG ratio are presented in Table 3. The experimental data showed that the variances are homogenous; therefore, linear regression curve can be used (International Organization for Standardization 1990).

## Trueness

The most frequent approach to estimate trueness of the method is CRM analysis. Six parallel samples of water and vegetable CRMs were analysed in order to determine the method's trueness (Tables 4 and 5). These results showed that the recoveries for all elements were generally within ±5% of the certified values. The Student's $t$ test confirmed that the obtained recoveries are not significantly different from 100%.

## Precision

The most common measures of precision are repeatability and reproducibility (Tables 6 and 7), which were estimated considering within and between days variation, respectively. The results obtained in repeatability were conducted on six parallel samples by a single operator using the same equipment. The set targets for concentrations lower than 100 μg L$^{-1}$ were standard deviation of repeatability ($s_r$) below 10% and limit of repeatability ($r$) below 28%, while for concentrations higher than 100 μg L$^{-1}$, $s_r$ below 7% and $r$ below 20%.

## Measurement uncertainty

Measurement uncertainty was evaluated based on the bottom-up approach (International Organization for Standardization 1995). All the contributions were obtained from calibration certificates and from statistical analysis of repeated measurements. Trueness of the method was calculated from results of CRM analysis, while repeatability was evaluated from precision experiments. The uncertainty of volumetric operations (volumetric flasks, pipettes) was calculated by using manufacturer data on calibration uncertainty (from certificates), the uncertainty associated with the use of glassware at a temperature different from that of calibration, and the repeatability of volumetric deliveries. Uncertainty of balances was calculated from data obtained from

**Table 6 Results from the repeatability study for two levels of concentration**

| Element | Average (μg L$^{-1}$) | $s_r$ (%) | $r$ (%) | Average (μg L$^{-1}$) | $s_r$ (%) | $r$ (%) |
|---------|-----------------------|-----------|---------|-----------------------|-----------|---------|
| Cd | 23.6 | 6.8 | 19 | 211 | 3.6 | 10 |
| Cr | 24.6 | 7.1 | 20 | 208 | 3.7 | 10 |
| Cu | 26.1 | 6.4 | 18 | 198 | 4.4 | 12 |
| Fe | 25.5 | 9.5 | 27 | 221 | 6.3 | 18 |
| Al | 25.0 | 6.2 | 17 | 213 | 3.8 | 11 |
| Mg | 25.3 | 4.1 | 11 | 200 | 2.9 | 8.1 |
| Pb | 24.6 | 7.1 | 20 | 222 | 3.2 | 9.0 |
| Mn | 24.8 | 7.4 | 21 | 232 | 4.1 | 11 |
| Ni | 26.3 | 7.8 | 22 | 215 | 5.2 | 15 |
| Zn | 25.1 | 6.9 | 19 | 225 | 4.7 | 13 |
| As | 24.3 | 8.8 | 25 | 208 | 6.5 | 18 |

$s_r$, standard deviation of repeatability; $r$, limit of repeatability ($s_r \times 2.8$).

**Table 7 Results obtained for the reproducibility by ICP-OES**

| Element | Average ($\mu g\ L^{-1}$) | $s_R$ (%) | $R$ (%) |
|---|---|---|---|
| Cd | 107 | 9.8 | 27 |
| Cr | 98.5 | 10 | 28 |
| Cu | 101 | 12 | 34 |
| Fe | 94.8 | 14 | 39 |
| Al | 96.8 | 6.9 | 19 |
| Mg | 101 | 5.8 | 16 |
| Pb | 105 | 8.6 | 24 |
| Mn | 111 | 11 | 31 |
| Ni | 105 | 14 | 39 |
| Zn | 114 | 8.8 | 25 |
| As | 95.6 | 16 | 45 |

$s_R$, standard deviation of reproducibility; $R$, limit of reproducibility ($s_R \times 2.8$).

calibration certificates (declared uncertainty) and the repeatability of weighing. After estimation, all sources of uncertainty were combined according to the law of propagation of uncertainties, obtaining the combined standard uncertainty ($u(C_a)$). The final result was reported as expanded uncertainty ($U(C_a)$), calculated as $U(C_a) = k \times u(C_a)$, where $k$ is the coverage factor, corresponding to a 95% confidence level.

The identified main sources of measurement uncertainty were uncertainty of calibration reference materials ($C_i$), uncertainty of delivered volumes, uncertainty of measured intensities of the reference solutions ($A_i$), and recovery of the method (Figure 1).

The contributions of repeatability to the measurement uncertainty were combined into one contribution for the overall experiment and were obtained from the method validation study performed in the laboratory. Recovery accounts for possible interferences in the method when samples of selected matrix are analysed. With these corrections, the concentration of each element ($C$) in a sample was expressed by the model:

$$C = \frac{A-a}{b}\frac{1}{R}F_{\text{rep}}F_{\text{dil}} \tag{1}$$

where $R$ is the method recovery, $F_{\text{dil}}$ is the dilution factor, $F_{\text{rep}}$ is the repeatability factor, $A$ is the area of the sample, while $a$ and $b$ are the linear regression coefficients. The sources of uncertainty and uncertainty components in determining elements are schematically presented in the cause and effects diagram (Figure 1). Calculations were made by using GUM Workbench software version 1.3 (Metrodata GmbH, Grenzach-Wyhlen, Germany) which is a standard application program with the possibility that user can define any model equation in order to enable various uncertainty calculations. The software was checked and validated before use in order to demonstrate that it is suitable for intended use. The results of the measurement uncertainty are listed in Table 8. Results revealed that for all the metals tested in plant aqueous extracts, extended measurement uncertainty is lower than 10% and therefore fulfills the requirements stated in the Drinking Water Directive (Council of the European Union 1998).
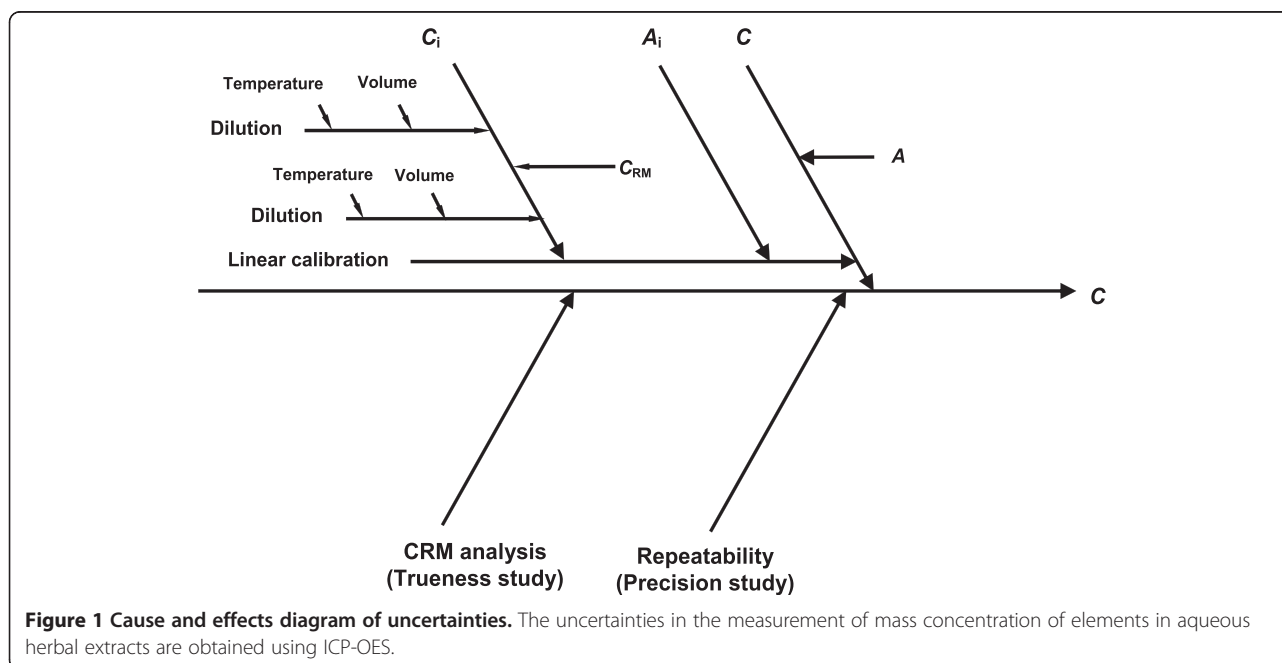


**Figure 1 Cause and effects diagram of uncertainties.** The uncertainties in the measurement of mass concentration of elements in aqueous herbal extracts are obtained using ICP-OES.

**Table 8 Measurement uncertainty of elements determination in plant aqueous extracts and plant dry mass**

| Element | Measurement uncertainty, % ($k = 2$) | |
|---|---|---|
| | Plant aqueous extracts | Plant dry mass |
| Cd | 5.8 | 8.7 |
| Cr | 7.5 | 8.8 |
| Cu | 5.7 | 8.9 |
| Fe | 8.2 | 8.3 |
| Mn | 6.2 | 6.4 |
| Ni | 6.6 | 8.8 |
| Zn | 5.9 | 6.3 |
| As | 7.7 | 9.8 |

The uncertainty components of Cr concentration in plant aqueous extracts and in plant dry mass as a case study are presented in Table 9.

The relative uncertainty variance contributions are used to illustrate the relative impact of different uncertainty components. The relative contribution ($r_i$) of an

uncertainty component $x_i$ to the combined standard uncertainty is defined as follows:

$$r_i = \frac{\left(\frac{\delta y}{\delta x_i}\right)^2 \cdot u(x_i)^2}{u(y)^2} \tag{2}$$

where $u(x_i)$ is the standard uncertainties of the input parameters, and $\partial y/\partial x_i$ is the sensitivity coefficient.

The importance of uncertainty sources is determined by their quantitative effect on the measurement result. In case of Cr both for extracts and dry plants, the largest contribution comes from $u(R)$ and from repeatability ($u(F_{rep})$), while uncertainty contributions from other input quantities are of minor importance.

**Results on real samples (aqueous plant extract and dry plant)**

The concentrations of essential and nonessential elements in the dry mass of the analysed plant samples are presented in Table 10. The As concentrations were, in all cases, below the LoQ, while Pb and Cd concentrations

**Table 9 Uncertainty components of Cr in plant aqueous extracts and mass fraction in plant dry mass**

| Symbol | Unit | Plant aqueous extracts | | | Plant dry mass | | |
|---|---|---|---|---|---|---|---|
| | | Value | Standard uncertainty | $r_i$ | Value | Standard uncertainty | $r_i$ |
| $C_1$ | mg L$^{-1}$ | 0.00 | 0.004 | 0.5 | 0.00 | 0.004 | 0.5 |
| $C_2$ | mg L$^{-1}$ | 0.05 | 0.004 | 0.5 | 0.05 | 0.004 | 0.5 |
| $C_3$ | mg L$^{-1}$ | 0.10 | 0.004 | 0.4 | 0.10 | 0.004 | 0.5 |
| $C_4$ | mg L$^{-1}$ | 0.20 | 0.004 | 0.4 | 0.20 | 0.004 | 0.4 |
| $C_5$ | mg L$^{-1}$ | 0.40 | 0.004 | 0.3 | 0.40 | 0.004 | 0.3 |
| $C_6$ | mg L$^{-1}$ | 0.60 | 0.004 | 0.3 | 0.60 | 0.004 | 0.3 |
| $C_7$ | mg L$^{-1}$ | 0.80 | 0.004 | 0.0 | 0.80 | 0.004 | 0 |
| $C_8$ | mg L$^{-1}$ | 1.00 | 0.004 | 0.1 | 1.00 | 0.004 | 0.1 |
| $A_1$ | - | 20,190 | 3,028 | 0.4 | 20,190 | 3,028 | 0.4 |
| $A_2$ | - | 55,030 | 1,045 | 0.0 | 55,030 | 1,045 | 0 |
| $A_3$ | - | 95,540 | 840 | 0.0 | 95,540 | 840 | 0 |
| $A_4$ | - | 181,800 | 1,181 | 0.0 | 181,800 | 1,181 | 0 |
| $A_5$ | - | 355,180 | 1,882 | 0.0 | 355,180 | 1,882 | 0 |
| $A_6$ | - | 529,800 | 3,284 | 0.2 | 529,800 | 3,284 | 0.2 |
| $A_7$ | - | 703,882 | 3,097 | 0.1 | 703,882 | 3,097 | 0.1 |
| $A_8$ | - | 880,011 | 5,104 | 0.1 | 880,011 | 5,104 | 0.1 |
| $A$ | - | 255,400 | 1,127 | 0.1 | 31,140 | 4,670 | 0.1 |
| $R$ | - | 1.00 | 0.015 | 20.4 | 1.00 | 0.027 | 35.8 |
| $F_{rep}$ | - | 1.00 | 0.029 | 76.2 | 1.00 | 0.029 | 60.7 |
| $C_m$ | g L$^{-1}$ | - | - | - | 10 | 0.001 | 0.0 |
| Result | | 0.283 mg L$^{-1}$ | 0.019 mg L$^{-1}$ (6.7%, $k = 2$) | | 2.53 mg kg$^{-1}$ | 0.202 mg kg$^{-1}$ (9.0%, $k = 2$) | |

$C_1$ to $C_8$ are the concentrations of calibration standard solutions; $A_1$ to $A_8$ are the respective standard solutions; $A$ is the emission intensity of the sample; $R$ is the recovery from CRM; and $F_{rep}$ is repeatability factor; $C_m$ is the concentration of measured sample in digested solution.

**Table 10 Contents of metals in dry plant mass**

|  | Mg | Al | Cd | Cr | Cu | Fe | Pb | Mn | Ni | Zn | As |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chamomile | 2,390 ± 18.5 | 152 ± 8.25 | 0.084 ± 0.011 | 2.50 0.22 | 11.2 ± 0.078 | 144 ± 5.56 | 0.90 ± 0.11 | 219 ± 5.95 | 3.05 ± 0.23 | 22.4 ± 1.36 | <0.15 |
| Milfoil | 1,620 ± 12.6 | 41.1 ± 3.12 | 0.25 ± 0.022 | 0.88 ± 0.08 | 15.7 ± 0.12 | 42.1 ± 3.27 | 0.68 ± 0.063 | 83.2 ± 4.12 | 5.14 ± 0.48 | 46.2 ± 3.17 | <0.15 |
| Rattle | 1,380 ± 11.9 | 126 ± 4.88 | 0.14 ± 0.015 | 1.55 ± 0.16 | 19.1 ± 0.15 | 189 ± 8.48 | 0.69 ± 0.077 | 120 ± 9.26 | 2.87 ± 0.22 | 55.9 ± 3.38 | <0.15 |
| Brotherwort | 2,230 ± 12.5 | 186 ± 10.2 | 0.35 ± 0.029 | 3.18 ± 0.31 | 21.9 ± 0.18 | 224 ± 11.4 | 2.23 ± 0.17 | 296 ± 11.3 | 6.02 ± 0.23 | 25.8 ± 1.57 | <0.15 |
| Pot marigold | 3,120 ± 20.8 | 172 ± 11.4 | 0.11 ± 0.010 | 2.88 ± 0.23 | 29.1 ± 0.19 | 234 ± 20.7 | 1.33 ± 0.13 | 178 ± 9.59 | 4.08 ± 0.24 | 51.1 ± 2.96 | <0.15 |
| Linden | 1,450 ± 11.8 | 72.6 ± 3.33 | 0.071 ± 0.008 | 1.50 ± 0.11 | 9.22 ± 0.066 | 64.9 ± 6.23 | 0.44 ± 0.042 | 71.9 ± 3.77 | 0.63 ± 0.071 | 18.8 ± 1.07 | <0.15 |
| Peppermint | 2,660 ± 24.1 | 144 ± 6.03 | 0.41 ± 0.036 | 3.61 ± 0.30 | 19.9 ± 0.18 | 306 ± 12.2 | 2.53 ± 0.21 | 255 ± 14.4 | 2.11 ± 0.11 | 64.4 ± 3.22 | <0.15 |

Values are expressed as milligrams per kilogram (mean ± standard deviation of five replicates).

were below the proposed limits by European Pharmacopoeia of 5 and 0.5 mg $kg^{-1}$, respectively. Ni concentrations varied between 0.63 and 6.02 mg $kg^{-1}$. These results were in the same order of magnitude with those reported by other authors for herbal drugs collected in Europe (Razic et al. 2006; Basgel and Erdemoglu 2006; Gentscheva et al. 2010).

The contents of essential elements such as Mg (1,450 to 3,120 mg $kg^{-1}$), Al (41.1 to 186 mg $kg^{-1}$), Cr (0.88 to 3.61 mg $kg^{-1}$), Cu (9.22 to 29.1 mg $kg^{-1}$), Fe (42.1 to 306 mg $kg^{-1}$), Mn (71.9 to 296 mg $kg^{-1}$), and Zn (22.4 to 64.4 mg $kg^{-1}$) were similar with those reported in the literature (Gentscheva et. al. 2010; Maharia et al. 2010; Chuparina and Aisueva 2011; Miranda and Pereira-Filho 2013).

The content of metals in the aqueous plant extracts offers information about the uptake of these elements by drinking of a cup of tea. The concentration of essential and nonessential elements in the aqueous extracts (Table 11) offers information about the uptake of these elements following tea consumption. As and Cd concentrations were lower that the LoQ in all the analysed samples. Also, Pb concentrations were generally below the LoQ, while Ni concentrations ranged between 2.70 and 18.2 µg $L^{-1}$, below the maximum value of 20 µg $L^{-1}$ established for this element by EU Drinking Water Directive 98/83/EC. Also, the concentrations of essential elements that have established maximum values for drinking water were generally below these limits, except manganese extracted from brotherwort which slightly exceeded 50 µg $L^{-1}$.

The higher metal concentrations in plant extracts were found for Mg (1,590 to 7,800 µg $L^{-1}$), but this element have no maximum admitted limit for drinking water. Our results for the concentrations of Al, Cu, Mg, and Fe are in line with those reported by Froes et al. (2014), but Mn concentrations were generally lower, in our case.

By comparing the results presented in Tables 10 and 11, taking into account the mass of dry plants and final volume of infusion, it can be observed that among the analysed elements, Zn, Cu, and Ni were highly extracted in the aqueous extracts (31% to 64%, 23% to 71%, and respectively, 19% to 73%), in function of the plant species, while Pb and Fe had low solubility (below 10%).

## Conclusions

A fully validated method for metal analysis in medicinal dry plant mass and its extracts is presented. The fast and accurate ICP-OES method enables the quantification of selected metals in aqueous and dry samples.

The validation results are presented and organized in tables in order to provide an easy overview of the method's performance. The experimentally determined validation parameters for medicinal extracts were then compared to the criteria stated in the Drinking Water Directive. Measurement uncertainty was determined on basis of modelling approach. Detailed uncertainty budget is presented for Cr in aqueous and dry samples. Systematic uncertainty budgets such as these in the design presented facilitate the uncertainty evaluation process and make it easier to compare the contributions of uncertainty

**Table 11 Contents of metals in the aqueous extracts**

|  | Mg | Al | Cd | Cr | Cu | Fe | Pb | Mn | Ni | Zn | As |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chamomile | 6,010 ± 110 | 115 ± 5.03 | <0.43 | 2.20 ± 0.21 | 12.8 ± 0.80 | 45.5 ± 1.64 | <0.33 | 49.0 ± 3.15 | 11.1 ± 1.10 | 70.0 ± 8.58 | <1.00 |
| Milfoil | 1,060 ± 86.2 | 91.2 ± 6.22 | <0.43 | <2.00 | 27.0 ± 1.56 | 18.8 ± 1.10 | <0.33 | 39.2 ± 3.24 | 7.15 ± 0.61 | 103 ± 7.12 | <1.00 |
| Rattle | 2,220 ± 103 | 109 ± 4.10 | <0.43 | 2.21 ± 0.22 | 64.1 ± 2.90 | 44.2 ± 1.30 | <0.33 | 35.6 ± 2.27 | 2.70 ± 0.11 | 185 ± 10.1 | <1.00 |
| Brotherwort | 4,600 ± 185 | 190 ± 8.85 | <0.43 | 3.15 ± 0.30 | 76.2 ± 3.95 | 27.0 ± 1.81 | 0.66 ± 0.08 | 51.5 ± 4.03 | 18.2 ± 1.20 | 68.8 ± 5.22 | <1.00 |
| Pot marigold | 7,800 ± 230 | 95.6 ± 5.22 | <0.43 | 3.32 ± 0.34 | 70.0 ± 3.65 | 34.5 ± 2.16 | 0.53 ± 0.08 | 28.6 ± 2.05 | 9.58 ± 0.74 | 163 ± 6.96 | <1.00 |
| Linden | 1,590 ± 81.6 | 53.9 ± 2.76 | <0.43 | <2.00 | 10.6 ± 0.96 | 22.2 ± 1.31 | <0.33 | 25.9 ± 1.89 | 3.55 ± 0.24 | 29.3 ± 2.11 | <1.00 |
| Peppermint | 5,650 ± 211 | 127 ± 3.05 | <0.43 | 4.25 ± 0.39 | 70.7 ± 3.62 | 51.0 ± 3.10 | 0.83 ± 0.09 | 45.5 ± 2.63 | 6.21 ± 0.53 | 196 ± 11.2 | <1.00 |

Values are expressed as micrograms per liter (mean ± standard deviation of five replicates) per 1 g of plant extracted in 200 mL water.

components to the total uncertainty budget and offer a tool for improvement of the method performance. In addition, the use of commercial software can facilitate the calculations in order to make the entire process more user-friendly.

In dry plants, the concentrations of Pb and Cd were below the proposed limits by European Pharmacopoeia of 5 and 0.5 mg kg$^{-1}$, respectively. The concentrations of essential and nonessential elements in tea infusion of the analysed samples were generally lower than the maximum values established by EU Drinking Water Directive 98/83/EC; thus, these tea can be considered safely for consumption. However, depending on the metal pollution in the sites where the medicinal plants are grown and the uptake of metals in these plants, the concentrations of metals in water extracts can determine the exceeding of the limits for drinking water due to the relatively high extractability of metals like Zn, Cu, and Ni.

**Author details**
[1]INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, Cluj-Napoca 400293, Romania. [2]Laboratory for Environmental Sciences and Engineering, National Institute of Chemistry, 19 Hajdrihova, Ljubljana SI-1000, Slovenia.

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