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# EVALUATION OF PERFORMANCE PARAMETERS FOR TRACE ELEMENTS ANALYSIS IN PERENNIAL PLANTS USING ICP-OES TECHNIQUE

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**Abstract:** The aim of this paper is to present the validation of inductively coupled plasma optical emission spectrometry (ICP-OES) method used for metals determination from several perennial plant samples. The suitability of two digestion procedures using wet digestion with mineral acids mixture on hot plate and microwave digestion was investigated to determine As, Cd, Cu, Fe, Mn, Pb and Zn in plants samples. The LOD of the seven analysed elements in solid samples varied between 0.20µg $g^{-1}$ for Mn and 0.55µg $g^{-1}$ for Pb. The found values for metals determined by ICP-OES in a vegetable certified reference material digested using the two procedures were compared with the certified values and good agreements between these values were obtained. The proposed method indicated satisfactory recovery, detection limits and standard deviations for trace metal determination in perennial plants samples.

**Key words:** ICP-OES, plant analysis, method validation, multielemental analysis

## Introduction

For most of the analytical determinations from solid samples the sample digestion is required. The heavy metals like cadmium, copper, iron, manganese, lead and zinc in plants are determined after different digestion procedures including various mixtures of concentrated acids such as hydrofluoric acid (HF), hydrochloric acid (HCl), nitric acid ($HNO_3$), perchloric acid ($HClO_4$) and sulphuric acid ($H_2SO_4$). Different digestion equipment can be used: open beakers heated on hot plates, block digesters and digestion units placed in microwave ovens [MARGUI & al. 2005; GOMEZ & al. 2007].

The analytical techniques that can be used for metals determination from aqueous solutions obtained through plants digestion are mainly based on atomic spectrometry with mono-elemental detection, such as flame atomic absorption spectrometry (FAAS) [MENDIL, 2006], graphite furnace atomic absorption spectrometry (GF-AAS) [AYAR & al. 2009]. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) have the advantages of high samples throughput due to the multi-elemental determination and also, these methods have a wide working range [TORMEN & al. 2011].

Due to its advantages, ICP-OES has become one of the most used techniques for elemental determination, many studies being conducted to validate this method for metals analysis in a large variety of sample types. AYDIN (2008) has compared dry, wet and microwave digestion procedures for the determination of chemical elements in wool samples using ICP-OES technique, obtaining satisfactory recovery, detection limits and standard

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deviation for trace metal determination. BAKIRCIOGLU and co-workers [BAKIRCIOGLU & al. 2011] used ICP-OES technique for determination of some trace metals in cheese samples, packaged in plastic and tin containers, by ICP-OES after dry, wet and microwave digestion. ICP-OES and ICP-MS techniques were used for metals determination in vegetable seeds used in the production of biodiesel [CHAVES & al. 2010].

Dantas and co-workers [DANTAS & al. 2010] have measured the metals content in gum samples obtained from the deposits of internal combustion engines, using ICP-OES after microwave digestion. The heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout was also studied by ICP-OES [FALLAH & al. 2011]. The ICP-OES technique was validated for the determination of trace elements in basil powder [GHANJAOUI & al. 2011] and for the quality control of herbal medicines [GOMEZ & al. 2007]. MARGUI & al. (2005) has made a comparative study between EDXRF and ICP-OES after microwave digestion for element determination in some plant specimens. ICP-OES was also used for metals determination in biological samples: animal tissues [MATOS & al. 2009], nuts and seeds [NAOZUKA & al. 2011] or in different food samples [NARDI & al. 2009]. This method was successfully applied for the measurement of metals in environment (water, soil and sediment samples) used in the assessment of environmental quality [FRENTIU & al. 2007; LEVEI & al. 2010; ZOBRIST & al. 2009; SENILA & al. 2011; SIMA & al. 2011].

For multivariate optimization of ICP-OES technique used for the determination of microelements in fruit juice, Santos Froes and co-workers [SANTOS FROES & al. 2009] have been used the exploratory analysis (Hierarchical Cluster Analysis, HCA, and Principal Component Analysis, PCA), which evaluated the plasma conditions (nebulization gas flow rate, applied power, and sample flow rate). In other study, fractional factorial design was used for the optimization of the digestion procedures followed using ICP-OES technique for multi-elemental determination in nuts [MOMEN & al. 2007].

The aim of this work is the development of an analytical method for the determination of elements (As, Cd, Cu, Fe, Mn, Pb and Zn) in perennial plants grown in mining polluted areas by dual view inductively coupled plasma optical emission spectrometry (ICP-OES) using two types of wet digestion methods.

## Material and methods

A multi-elemental standard solution of 1000 $mgL^{-1}$ containing all analysed elements (As, Cd, Cu, Fe, Mn, Pb and Zn) supplied by Merck (Darmstadt, Germany) was used for calibration. $HNO_3$ 65% and $H_2O_2$ 30% from Merck (Darmstadt, Germany) analytical grade were used for samples digestion. Ultrapure water obtained by a Milli Q system (Millipore, France) was used for dilutions. A vegetable certified reference materials IAEA-359 Cabbage (Vienna, Austria) was used for the quality control of metals determination.

Determinations were carried out using a Perkin Elmer Model Optima 5300 DV spectrometer (Perkin Elmer, USA) ICP-OES equipped with a Ultrasonic Nebulizer CETAC U-6000AT+ (CETAC, USA) and an auto sampler AS 93-plus. Argon (purity higher than 99.995%) supplied by Linde Gas SRL (Cluj-Napoca, Romania) was used to sustain plasma and, as carrier gas. A closed-vessel microwave system Berghof MWS-3+ with temperature control mode, (Berghof, Germany) was used for wet digestion. All Teflon digestion vessels were previously cleaned in a bath of 10% (v/v) nitric solution for 48 h to avoid cross-contamination.

Four specimens of perennial plants samples (*Agrostis*, *Agropyrum repens*, *Trifolium repens*, *Urtica dioica*), collected from a mining affected area of Baia Mare (Romania), were analysed in this study. Samples were dried in an oven at 40˚C till constant weight was achieved. The dried samples were then grounded with a Mixer Grinder and sieved through a 100 microns mesh. All the samples were originally stored in closed plastics bags until analysis. The certified reference material were analysed in the same experimental conditions used for sample analyses in order to evaluate the accuracy of the method.

### Wet digestion on hot plate

For the wet digestion a mixture of $HNO_3/H_2O_2$ was used in this study. For this procedure, the temperature was maintained at 120˚C for 2 h during digestion of 1.0 g of plant sample with 16 mL of 6:2 $HNO_3/H_2O_2$ mixtures on the hot plate. After cooling, 10 mL of distilled water was added on the sample and mixed. The residue was filtered through filter paper and then the sample was diluted to 50 mL with distilled water. Metal contents of final solution were determined by ICP-OES.

### Microwave digestion

Approximately 1.0 g of sample was digested with 6 mL of HNO3 and 2 mL of $H_2O_2$ in microwave digestion system, according to the digestion program presented in Tab. 1. The resulting solutions were cooled and diluted to 50 mL with distilled water. The resulted solutions were analysed by ICP-OES.

### ICP-OES determination

The operating conditions employed for ICP-OES determination were 1300W RF power, 15 L $min^{-1}$ plasma flow, 2.0 L $min^{-1}$ auxiliary flow, 0.8 L $min^{-1}$ nebulizer flow, 1.5 mL $min^{-1}$ sample uptake rate. Axial view was used for metals determination, while 2-point background correction and 3 replicates were used to measure the analytical signal. The emission intensities were obtained for the most sensitive lines free of spectral interference.

The calibration standards were prepared by diluting the stock multi-elemental standard solution (1000 mg $L^{-1}$) in 0.5% (v/v) nitric acid. The calibration curves for all the studied elements were in the range of 0.01 to 1.0 mg $L^{-1}$.

## Results and discussion

### Figures of merit

Method validation is an important requirement in the practice of chemical analysis and it is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. The estimation of the uncertainty associated to the analytical methods is necessary in order to establish the comparability of results, and it is an important parameter in reporting of analytical results.

The limit of detection (LOD) and limit of quantification (LOQ) of the method and also the main analytical characteristics of the calibration curves (slope and correlation coefficients) for the developed ICP-OES procedure are indicated in Tab 2. The instrumental detection and quantification limits were estimated by analysing ten blank solutions. The LOD is defined as three times the standard deviation of the ten measurements, while the LOQ are defined as ten times the standard deviation of the ten measurements. The LODs and LOQs were calculated for the original solid samples (µg $g^{-1}$) by taking into account the amount of sample digested and the final volume obtained by dilution. The LOD of the seven elements studied varied between 0.20 µg $g^{-1}$ for Mn and 0.55 µg $g^{-1}$ for Pb. These values are appropriate for the measurement with a good accuracy of maximum admitted limits of 1 µg $g^{-1}$for Cd, 2 µg $g^{-1}$ for As and 10 µg $g^{-1}$for Pb, established by the European Directive 2002/32/EC on undesirable substances in animal feed. Recovery percentage values found for the analysis of spiked plants samples varied between 89% and 113%.

As no certified reference materials for perennial plants were available, the accuracy of the proposed method was evaluated by analysing a vegetable certified reference material IAEA-359 Cabbage (Vienna, Austria). The use of this material is appropriate, because the CRM was digested and diluted in the same way as the perennial plants. The results are shown in Tab 3. The obtained results by both digestion methods show good agreement for all analysed elements between found and certified values, according to the t-test for a 95% confidence level, and the method is thus considered accurate.

Repeatability was established from the average relative standard deviation (RSD) of three independent analyses at the real samples. Standard addition curves obtained from a plant sample spiked at different concentration levels from 50 to 500 µg $L^{-1}$ were compared with external calibration lines established from multi-elemental standards in order to evaluate the matrix effect. The recovery values ranged between 86 and 119% of the spiked values. No matrix effect has been observed and therefore aqueous standards have been used for calibration.

### Analytical results

Shoots of the four different perennial plants samples were digested using the wet digestion on hot plate and the microwave digestion. After digestion, 7 elements were determined by ICP OES: As, Cd, Cu, Fe, Mn, Pb and Zn, using external calibration with aqueous standard solutions. The obtained results are shown in Tab. 4, reported with 95 % confidence limit (n = 3).

According to a Student's t-test, there was no difference between the concentrations of all elements for wet digestion on hot plate and microwave digestion at a 95% confidence level. Generally, lower standard deviations were obtained using the microwave digestion method. The results obtained reveal that the proposed digestion methods and measurement technique (ICP-OES) can be successfully applied to different kind of plants analysis. The measured values for As, Cd and Pb were generally below the maximum admitted limits established by the European Directive 2002/32/EC on undesirable substances in animal feed, while for the others analysed elements there are not established maximum admitted limits.

## Conclusions

The principal figures of merit for the determination of trace elements by ICP-OES from perennial plants were evaluated. Two methods of sample digestion: wet digestion on hot plate and microwave digestion were compared and no significant differences between the results obtained were observed. The obtained results using the two digestion methods show good agreement with the certified values of one analysed vegetable CRM, for all analysed elements. Standard addition curves obtained from a plant sample spiked at different concentration levels were compared with external calibration lines established from multi-elemental standards and no significant matrix effect has been observed. The results show that the proposed technique (ICP-OES) and also the two digestion methods are suitable for metals determination in perennial plants.

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**Tab. 1.** Operating conditions for the microwave digestion system

| | Stage | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Temperature / ˚C | 160 | 120 | 100 | 100 |
| Ramp time / min | 5 | 1 | 1 | 1 |
| Hold time / min | 25 | 5 | 1 | 1 |
| Power / %* | 60 | 20 | 10 | 10 |

* 100 % power corresponds to 1400 W

**Tab. 2.** Analytical parameters for metals determination using ICP-OES

| Element | Wavelength (nm) | LOD µg g$^{-1}$ | LOQ µg g$^{-1}$ | Slope | Correlation coefficient |
|---|---|---|---|---|---|
| As | 188.979 | 0.30 | 0.90 | 13496 | 0.9996 |
| Cd | 228.802 | 0.25 | 0.75 | 108844 | 0.9998 |
| Cu | 327.393 | 0.30 | 0.90 | 245987 | 0.9995 |
| Fe | 238.204 | 0.35 | 1.05 | 203903 | 0.9991 |
| Mn | 257.610 | 0.20 | 0.60 | 1094626 | 0.9999 |
| Pb | 220.345 | 0.55 | 1.65 | 23734 | 0.9997 |
| Zn | 213.856 | 0.30 | 0.90 | 259230 | 0.9995 |

**Tab. 3.** Analytical determination of metals in reference material. Values are expressed in µg $g^{-1}$ and reported as average±ts; n=3; 95% confidence level

| Element | Certified content | Found content | |
|---|---|---|---|
| | | *Hot plate digestion* | *Microwave digestion* |
| As | 0.096 - 0.104 | 0.094±0.012 | 0.103±0.011 |
| Cd | 0.115 - 0.125 | 0.120±0.018 | 0.119±0.012 |
| Cu | 5.49 - 5.85 | 5.39±0.48 | 5.28±0.51 |
| Fe | 144.1 - 151.9 | 146±5.32 | 143±4.45 |
| Mn | 31.3 - 32.5 | 31.7±2.48 | 32.5±2.23 |
| Zn | 37.9 - 39.3 | 39.5±3.07 | 38.8±2.19 |

**Tab. 4.** Concentrations (average±ts; n=3; 95% confidence level) for perennial plants obtained by ICP OES (dry weight), expressed in µg $g^{-1}$

| Sample/ element | Digestion procedure | *Agrostis* | *Agropyrum repens* | *Trifolium repens* | *Urtica dioica* |
|---|---|---|---|---|---|
| **As** | HP* | 0.55±0.06 | 0.46±0.08 | 0.35±0.06 | 0.47±0.07 |
| | MW** | 0.51±0.05 | 0.44±0.06 | 0.37±0.05 | 0.43±0.06 |
| **Cd** | HP | 0.89±0.09 | 0.69±0.07 | 0.55±0.06 | 0.96±0.12 |
| | MW | 0.93±0.08 | 0.66±0.06 | 0.54±0.07 | 0.93±0.10 |
| **Cu** | HP | 4.51±0.45 | 3.25±0.39 | 2.23±0.25 | 4.62±0.30 |
| | MW | 4.22±0.36 | 3.53±0.39 | 2.11±0.20 | 4.72±0.41 |
| **Fe** | HP | 79.2±4.6 | 65.1±4.8 | 41.4±3.5 | 72.9±5.5 |
| | MW | 75.5±3.8 | 64.2±2.2 | 42.9±2.1 | 70.1±4.0 |
| **Mn** | HP | 5.65±0.48 | 4.34±0.59 | 3.05±0.38 | 5.11±0.52 |
| | MW | 5.44±0.25 | 4.55±0.28 | 3.55±0.39 | 4.96±0.23 |
| **Pb** | HP | 3.51±0.22 | 2.08±0.33 | 5.18±0.42 | 6.41±0.58 |
| | MW | 3.09±0.25 | 2.21±0.24 | 4.96±0.34 | 6.77±0.36 |
| **Zn** | HP | 47.7±2.5 | 66.9±5.3 | 44.8±3.7 | 84.4±6.3 |
| | MW | 44.8±2.4 | 67.5±3.1 | 46.1±1.9 | 84.0±3.3 |

* Hot plate; **Microwave

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