High Throughput Multi-Elemental Profiling of Plant Samples with the 4200 MP-AES

Food & Agriculture

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Introduction

There is currently an increasing need for high-throughput, easy-to-use, multi-elemental analytical techniques, for two main reasons:

1. Genetic and genomic approaches require the analyses of several plant organs (roots, leaves, seeds/fruits) from multiple genotypes (natural accessions, mutant collections...) growing on different environmental conditions, generating large quantities of samples,
2. Plants often experience multiple stresses (water deficit and salt stress, Fe/ Zn/Mn deficiencies on calcareous soils) that will affect simultaneously several elements that, in some cases interact biologically, requiring systematic multi-elemental analyses.

Microwave Plasma-Atomic Emission Spectroscopy has rapidly emerged as a well-suited technology for analytical laboratories that typically do not have strong expertise and manpower for mass spectrometry-based techniques (ICP-MS for instance). This application note describes the sample preparation procedure and analytical method used to determine the concentration of up to 14 elements in several types of plant organs (roots, leaves, seeds) from different plant species.

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Experimental

Instrumentation

All measurements were performed using an Agilent 4200 MP-AES (see Table 1 for the method parameters). Nitrogen gas was supplied via an Agilent 4107 Nitrogen Generator. The samples were introduced via the OneNeb nebulizer and a double-pass cyclonic spray chamber.

An Agilent SPS 3 auto-sampler (loaded with 10 mL Agilent Polypropylene tubes) was used to deliver samples to the instrument.

Table 1. MP-AES method parameters

Parameter	Value
Replicates	3
Pump rate	15 rpm
Sample uptake delay	40 s
Rinse time	3 s
Stabilization time	10 s
Fast Pump during uptake and rinse	On (80 rpm)
Autosampler	Agilent SPS 3
Sample pump tubing	Orange/green
Waste pump tubing	Blue/blue

Samples

The different plant species (Arabidopsis thaliana, Oryza sativa, Triticum aestivum) were grown on soil or in hydroponic solutions, except the strawberry leaf powder reference that was purchased from LGC (reference LGC7162).

Sample preparation

Microwave digestion was used to prepare all samples, using a Berghof Speedwave 2 apparatus. A mixture of 750 µL HNO_3 (65%) and H_2O_2 (30%) was added to either 5 mg (seeds) or 20 mg (leaves) of dried material, using a method specially developed for plant samples (3 min ramp to 100 °C, 40 min at 180 °C, 2 min ramp to 140 °C, 38 min at 140 °C). Once cooled, the solutions were diluted to 5 mL with ultrapure water.

Table 2. Wavelength and working calibration concentration range (read time was 3 seconds for all elements).

Element and wavelength (nm)	Calibration range (ppm)
Cu 324.754	0.025–10
Fe 259.940	0.025–10
Mn 257.610	0.025–10
Zn 213.857	0.025–10
Na 568.820	0.025–10
K 766.491	0.1–110
Ca 445.478	0.025–10
Mg 383.829	0.025–10
Ba 455.403	0.025–10
P 213.618	1–100
Al 396.152	0.025–10
Cd 228.802	0.025–10
Sr 407.771	0.025–10
Cr 425.433	0.025–10

Wavelength selection and calibration parameters

Details of wavelength selection and calibration parameters are given in Table 2 and Figures 1–4.

Results and Discussion

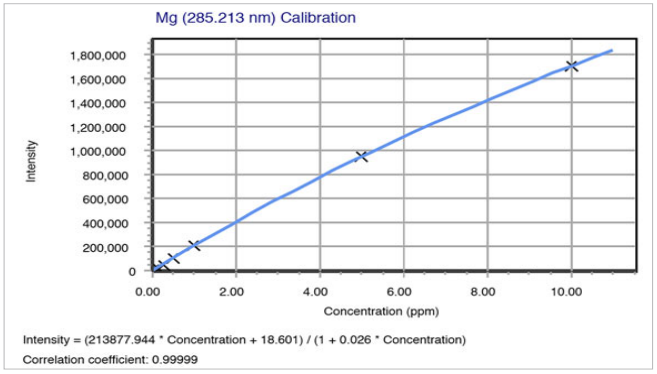

Figure 1. Calibration curve for Magnesium

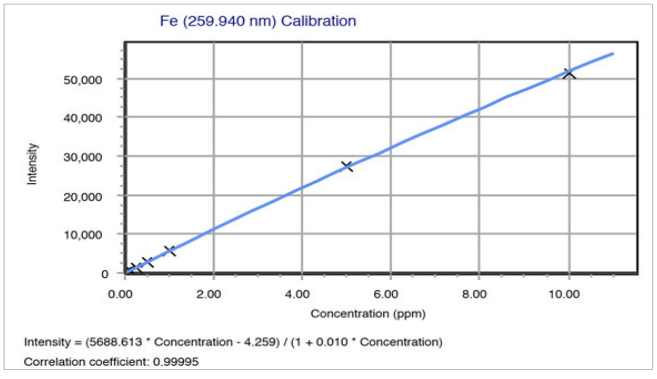

Figure 2. Calibration curve for Iron

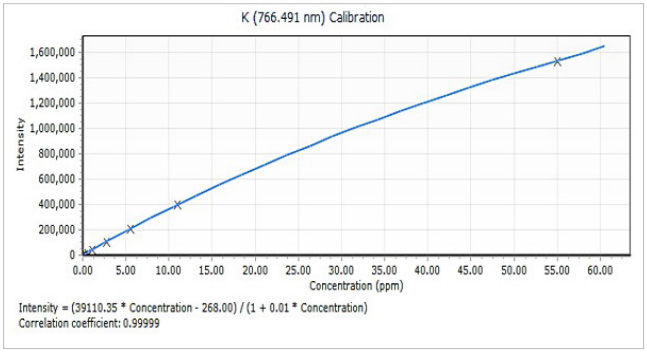

Figure 3. Calibration curve for Potassium

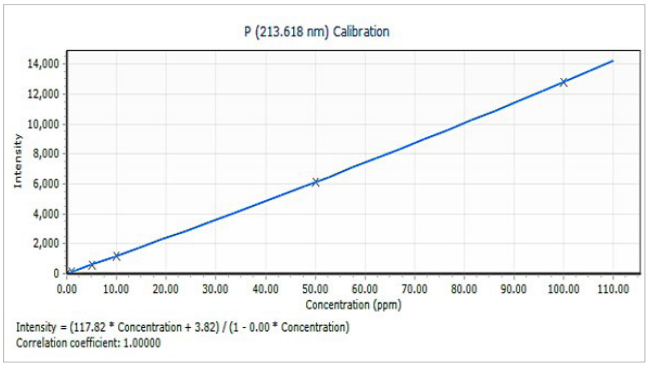

Figure 4. Calibration curve for Phosphorus

Typical calibration curves for a macro-element (Mg), a micro-element (Fe), an "easily ionisable" element (K) and a more "refractory" element (P) are shown in Figures 1–4. The calibration fit chosen was "rational weighted". The dynamic range (commonly between 0.025 and 10 ppm) allows a great versatility in sample measurement, compared with flame atomic absorption spectrometry (FAAS). This dynamic range covers the concentration variability encountered in plant samples and thus eliminates the need to proceed to further dilutions of specific samples.

Table 3. MP-AES results for total elemental analysis of strawberry leaf powder (ref LGC7162)

Element	Wavelength (nm)	Measured Value (µg/g)	Reference Value (µg/g)	Accuracy (%)
Mg	285.213	3682	3770 ± 170	97.7
K	766.491	19778	19600 ± 1000	100.9
Ba	455.403	112	107 ± 10	104.7
Fe	259.940	663	818 ± 48 (a)	81.1
Mn	403.076	164.4	171 ± 10	96.2
Sr	407.771	70	64 ± 6	109
Zn	213.857	23.74	24 ± 5	98.9
Na	588.995	75	65 (b)	114.7
Cu	324.754	11	10 (b)	106.3
Al	396.152	581	600 (b)	96.8

(a) data from a single analytical laboratory
(b) indicative values, not certified by the manufacturer

From the Certified Reference Materials available on the market, we chose the leaf strawberry powder (LGC7162) as it corresponded perfectly to the type of matrices we usually handle. The signal recovery (expressed as Accuracy in %) for 10 elements is presented in Table 3. Except for Fe, the accuracy obtained was highly acceptable (calculated average accuracy: 100%). The reason of the discrepancy for Fe quantification is likely due to the fact that the value given by the manufacturer originates from a single analytical laboratory.

Having set up the MP-AES analytical conditions, we quantified several macro- and micro-, essential and non-essential elements in seeds of the model plant Arabidopsis thaliana as well as two crops, rice and wheat. The main difference between species concerns the concentration in micro-elements (Fe, Mn, Zn, Cu) that is consistently lower in cereals (rice, wheat) than in dicotyledonous plants like Arabidopsis (Table 4).

The elemental content was also quantified in the soil pot used to grow the Arabidopsis plants and produce the seeds (Table 5). Finally, we initiated a Genome Wide Association Study (GWAS), intended to identify loci in the Arabidopsis genome that could be linked to the accumulation of Fe in the leaves, in conditions of Fe deficiency (plants grown on a calcareous soil).

For this pilot experiment, 322 accessions of Arabidopsis, covering most of the genetic and geographic variation of this species, were grown, leaves harvested and their Fe content quantified with our high throughput analytical device (Figure 5). It was clear from this first experiment that a high variability in Fe content existed within Arabidopsis accessions, which was a promising result for future identification of genes controlling the accumulation of this essential metal in leaves in conditions of Fe scarcity.

Table 4. MP-AES results for total elemental content of seeds from different species. Each value is the average of 3 different analyses. Values are expressed in ppm ($\mu g.g^{-1}$ dry weight).

Element	Seed type		
	Arabidopsis thaliana	Triticum aestivum	Rice Oriza sativa
Al	21.43	1752.12	nd
Ba	28.57	nd	nd
Ca	4746	656	243
Cd	7.14	9.55	7.44
Cr	3.33	4.45	7.74
Cu	3.33	1.91	6.99
Fe	414	19.1	33.5
K	28052	4009	8320
Mg	3983	105	1552
Mn	261.7	14	30.4
Na	157	38	34
P	10238	1209	4464
Sr	11.4	1.91	1.34
Zn	212	33.4	48.2

Table 5. MP-AES results for total elemental content from the soil substrate used to grow the plants. Each value is the average of 3 different analyses. Values are expressed in ppm (µg.g-1 dry weight).

Element	Concentration
Al	762.5
Ba	9.87
Ca	2248
Cd	4.59
Cr	2.17
Cu	15.42
Fe	740.8
K	2447
Mg	3809
Mn	72.58
Na	146
P	260
Sr	64.7
Zn	15.68

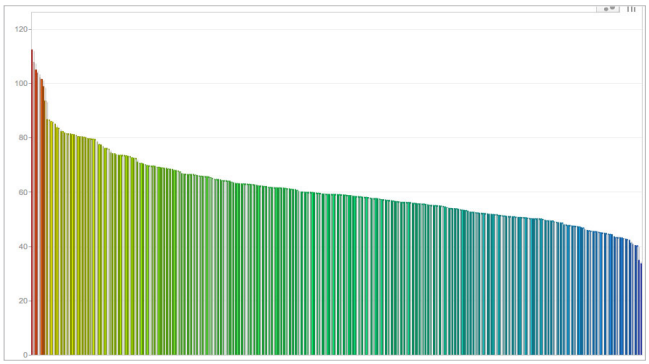

Figure 5. Fe content (ppm) from leaves of 322 accessions of Arabidopsis thaliana grown on alkaline soil (each colored bar represents one accession).

Conclusions

The analytical equipment set up in our laboratory, which includes a microwave oven, the N_2 generator 4107, the MP-AES and the SPS 3 auto-sampler corresponds to the ideal equipment for an analytical laboratory.

The accuracy, estimated with the strawberry leaf powder standard, was satisfactory (average accuracy close to 100%).

The MP-AES appeared to be user-friendly, inexpensive and highly efficient when coupled to the auto-sampler.

The analysis rate was *ca* 100 samples per day with up to 15 elements measured for each sample.

The sample volume obtained after mineralization (5 mL) was more than sufficient for the quantification of the 15 elements. The analyses actually only consumed 2 mL of sample, which implies that complementary analyses could be performed on the same samples if required.

The set up was also versatile in the way that very diverse types of samples (seeds, lignified roots, leaves, soil samples etc) could be handled and analyzed (Tables 4-5).

The high-throughput capacity of the SPS 3-MP-AES device is compatible with large-scale genetic approaches such as genome wide association studies (GWAS) where it is often necessary to analyze several hundreds of individuals (Figure 5) in order to link the phenotype (ie the ionome) with the genotype (molecular markers) to potentially identify genes controlling a specific process of mineral nutrition.

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Results presented in this document were obtained using the 4200 instrument, but performance is also verified for the 4210 MP-AES

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