Determination of macro and micronutrients in plants using the Agilent 4200 MP AES

Application note

Food and Agriculture

Authors

Clayton G. Liberato[1], Juan A. V. A. Barros[1], Alex Virgilio[1], Raquel C. Machado[2], Ana Rita A. Nogueira[2], Joaquim A. Nóbrega[1], Daniela Schiavo[3]

1. Group of Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, São Carlos, SP, Brazil

2. Embrapa Pecuária Sudeste, São Carlos, SP, Brazil

3. Agilent Technologies, São Paulo, SP, Brazil

Introduction

Appropriate plant nutrition is crucial to plant health and for adequate growth of the crops with maximal productivity. Among the nutrients consumed by the plants, N, P, K, Ca, Mg and S are essential, required in large quantities, while B, Cl, Co, Cu, Fe, Mn, Mo and Si are needed at lower levels [1,2].

The determination of macro and micronutrients in plant tissues is an important measure used to analyze plant nutritional status and to evaluate the possible need for fertilizer supplementation. The analysis of leaves provides the most information for the deficiency or excess of nutrients in plants [3]. This procedure is often performed using spectrochemical techniques such as Flame Atomic Absorption Spectroscopy (FAAS) and Inductively Coupled Plasma Optical Emission

Agilent Technologies

Spectroscopy (ICP-OES). However, the Agilent 4200/4210 Microwave Plasma Atomic Emission Spectrometer (MP-AES) is a suitable alternative technology for agricultural laboratories concerned with improving safety and looking to reduce costs. Running costs are significantly reduced through the use of nitrogen as the instrument plasma gas, which in turn, increases safety, due to the removal of flammable gases. Additionally, the MP-AES is able to achieve lower detection limits, particularly for phosphorus, compared to FAAS.

In this application note, the Agilent 4200 Microwave Plasma Atomic Emission Spectrometer (MP-AES) with nitrogen-based plasma generated from magnetically-coupled microwave energy was used for determination of P, K, Ca, Mg, B, Co, Mo, Cu, Fe, Mn, Si and Zn. This application is also applicable for Agilent's 4210 MP-AES.

Experimental

Instrumentation

All measurements were carried out using the Agilent 4200 MP-AES fitted with the Agilent 4107 Nitrogen Generator. The sample introduction system consisted of a double pass cyclonic spray chamber, OneNeb nebulizer, Solvaflex pump tube (orange/green) and Easy-fit torch to introduce the sample.

The intuitive MP Expert software features easy to use auto-optimization tools, which allow for quick optimization of the nebulization gas flow rate and viewing position for each element and wavelength in the method. In addition, MP Expert software allows for automatic background correction. Operating conditions and analyte settings are presented in Tables 1a and b, respectively.

Table 1a. Agilent 4200 MP-AES operating parameters for determination of macro and micronutrients in plants

Parameter	Value
Pump speed (rpm)	15
Sample uptake delay (s)	15
Stabilization time (s)	15
Read time (s)	15
Replicates	3
Rinse time (s)	30
Sample pump tubing	Orange/green Solvaflex
Waste pump tubing	Blue/blue Solvaflex
Background correction	Auto
Gas source	4107 Nitrogen generator

Table 1b. Agilent 4200 MP-AES method settings for each analyte

Element*	Emission wavelength (nm)	Viewing Position	Nebulizer gas flow rate (L/min)
P (I)	214.915	-10	0.65
K (I)	766.491	10	1.0
Ca (II)	396.847	0	0.8
Mg (II)	279.553	10	0.6
Si (I)	251.611	0	0.6
B (I)	249.772	0	0.55
Cu (I)	324.754	-10	0.85
Fe (II)	259.940	0	0.6
Mn (II)	257.610	0	0.65
Mo (I)	379.825	-10	1.0
Co (I)	350.228	-10	0.85
Zn (I)	213.857	0	0.65

* I: atomic line, II: ionic line

Standard and sample preparation

Multi-element calibration standards containing P, K, Ca, Mg and Si were prepared in concentrations of 5, 10, 25, 50 and 75 mg L^{-1}, and B, Cu, Fe, Mn, Mo, Co and Zn at 0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 2, 3 and 4 mg L^{-1}, for macro and micronutrients determination. All standards were prepared in 1% HNO_3 (v/v) medium.

Corn plant samples were analyzed and a certified reference material (apple leaves SRM 1515) from the National Institute of Standards and Technology, Gaithersburgh, MD, USA, was used to validate the method.

Samples and CRM were microwave-assisted acid digested by adding 5 mL of sub-boiling 7 mol L^{-1} HNO_3 and 5 mL 30 % m m^{-1} H_2O_2 to 0.5 g of material. All samples were microwave digested using the Speedwave Four microwave system (Berghof, GmbH, Eningen, Germany), in TFM-PTFE vessels (40 mL, 230 °C and 40 bar of pressure), according to the following heating program: (i) heating ramp: 120 °C, 5 min, (ii) heating plateau: 120 °C, 5 min, (iii) heating ramp: 160 °C, 5 min, (iv) heating plateau: 160 °C, 5 min, (v) heating ramp: 20 °C, 5 min, (vi) heating plateau: 200 °C, 5 min, (vii) cooling step: 3 min.

Results and discussion

Calibration linearity

The calibration curves obtained for macronutrients (Ca and Si) and micronutrients (B and Fe) showed good linearity across the concentration range. Dynamic linear range were 0-4 mg L^{-1} for all micronutrients and 0 – 75 mg L^{-1} for all macronutrients analyzed.

All calibration curves were obtained using linear calibration fit with the exception of K (I) 766.491 nm, for which a rational fit was used. The calibration curves for Ca, Si, B and Fe are displayed in Figure 1. One of the advantages of the MP-AES is the wider linear dynamic range when compared to FAAS. This allows users to select emission lines with appropriate sensitivities eliminating the need to make successive dilutions, which simplifies analysis, increasing productivity and reduces errors from contamination due to multiple dilutions.

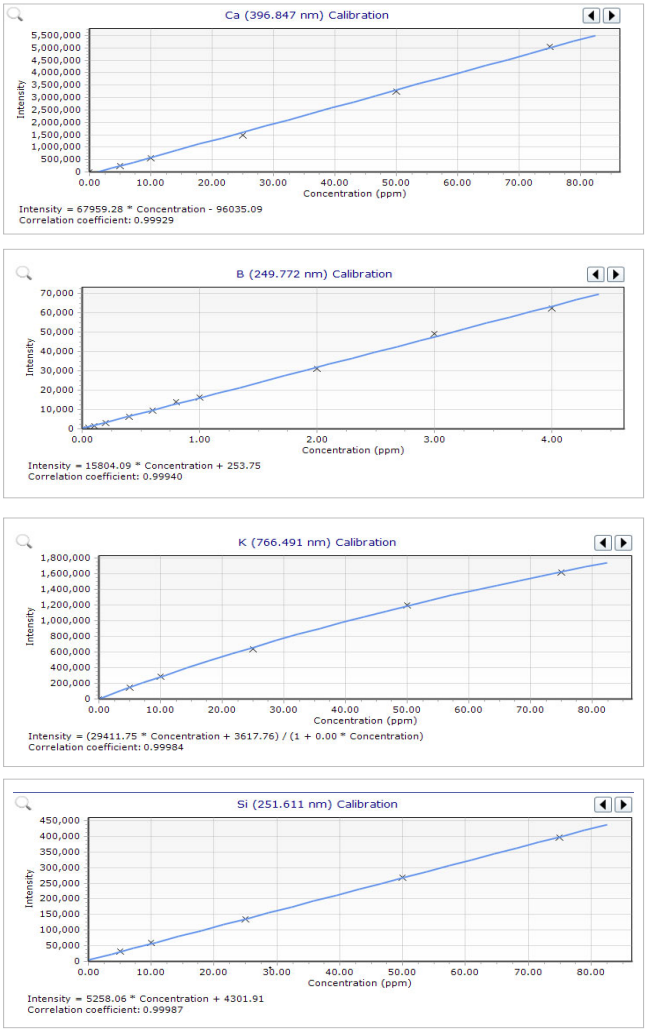

Figure 1. Calibration curves for B, Ca, Fe, Si and Zn

Method detection limits

Method Detection Limits (MDLs) and Limit of Quantification (LOQ) were calculated using the background equivalent concentrations (BEC), the signal-to-background ratios (SBR) and the relative standard deviations (RSD) for 10 consecutive blank measurements for each analyte. The values of the BEC, MDL and LOQ for all elements are presented in Table 2.

Table 2. BEC, MDL and LOQ for plant materials analyzed by MP-AES (n = 10).

Elements/ Emission line (nm)	BEC (mg L^{-1})	MDL (µg g^{-1})	LOQ (µg g^{-1})
B (249.772)	0.0028	0.009	0.03
Ca (396.847)	0.11	0.01	0.04
Co (350.228)	0.038	0.07	0.2
Cu (324.754)	0.010	0.005	0.02
Fe (259.940)	0.0093	0.02	0.05
K (766.491)	0.039	0.005	0.02
Mg (279.553)	0.041	0.002	0.008
Mn (257.610)	0.013	0.02	0.06
Mo (379.825)	0.026	0.01	0.03
P (214.915)	3.7	1.8	5.9
Si (251.611)	1.4	0.2	0.6
Zn (213.857)	0.0097	0.002	0.006

The accuracy of the method was evaluated by analysing an apple leaves CRM (NIST 1515). The t-student test was applied to compare measured and certified values at 95% confidence. Recovery results displayed in Table 3, showed good accuracy for all elements, with recoveries within ±10 % of certified values. For easily ionizable elements, such as K and Mg, excellent results were obtained without the need of an ionization buffer, such as Cesium Chloride. The t-student test demonstrated good agreement between the measured and certified values at 95% level of confidence. The corn plant samples were analyzed on the 4200 MP-AES with results presented in Table 4.

Table 3. Recovery results obtained for CRM (NIST 1515 - apple leaves) using MP-AES (mean ± standard deviation, n = 2) .

Element	Measured value	Certified value	Recovery (%)
Major (%)			
Ca	1.507 ± 0.016	1.526 ± 0.015	99
K	1.61 ± 0.11	1.68 ± 0.02	96
Mg	0.258 ± 0.003	0.271 ± 0.008	95
P	0.159 ± 0.006	0.159 ± 0.011	100
Minor (µg g^{-1})			
B	25 ± 0.5	27 ± 2	93
Cu	6.1 ± 0.10	5.64± 0.24	108
Fe	76 ± 1.8	83 ± 5	92
Mn	49 ± 0.6	54 ± 3	91
Zn	12 ± 0.8	12.5 ± 0.3	96

Table 4. Determination of macro and micronutrients in corn plants (mean ± standard deviation, n = 3)

Element	Emission line (nm)	Concentration
Major (g kg^{-1})		
Ca	396.847	3.54 ± 0.01
K	766.491	39.1 ± 0.8
P	214.915	3.05 ± 0.11
Mg	279.553	2.68 ± 0.06
Minor (mg kg^{-1})		
B	249.772	4.0 ± 0.3
Co	350.228	< 0.2
Cu	324.754	10.1 ± 0.8
Fe	259.940	156.5 ± 9.2
Mn	257.610	29.3 ± 1.7
Mo	379.825	<0.03
Si	251.611	2.83 ± 0.24
Zn	213.857	29.2 ± 1.5

Conclusion

The analytical performance of the 4200 MP-AES for this application demonstrated that the unique nitrogen plasma technology is a suitable spectrochemical tool for the determination of macro and micronutrients in plants. The instrument eliminates hazardous gases, lowering running costs due to the use of the nitrogen, making it an ideal alternative for agricultural labs over traditional techniques. Excellent MDL results were obtained for all elements, including B, P and Si, which are difficult to analyze using FAAS. Excellent linearity for most elements was achieved, across a wide linear dynamic range compared to FAAS, minimizing dilution steps and contamination, and increasing productivity. The accuracy of the procedure was evaluated and demonstrated with analysis of the CRM apple leaves, with all recoveries within ±10 % of certified values.

References

1. DECREE 4954/2004. January, 14th, 2004. (Regulation of Law 6894). 16/December/1980. BRASIL – MINISTÉRIO DA AGRICULTURA. PECUÁRIA E DESENVOLVIMENTO.

2. AGRIBOOK. Agrichem do Brasil Ltda.. 2010.

3. NOGUEIRA. A. R. A. Manual de Laboratório: Solo. Água. Nutrição Vegetal. Nutrição Animal e Alimentos. São Carlos. 2005. p. 334.

Acknowledgments

The authors are grateful to grants 2013/26672-5 and 2014/18393-1 of São Paulo Research Foundation (FAPESP) for the scholarships provided to R.C.M. and A.V. We also would like to thank Agilent Technologies for its support.

Verified for Agilent
4210 MP-AES

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

www.agilent.com/chem

Published March 8, 2017
Publication number: 5991-7856EN

Agilent Technologies