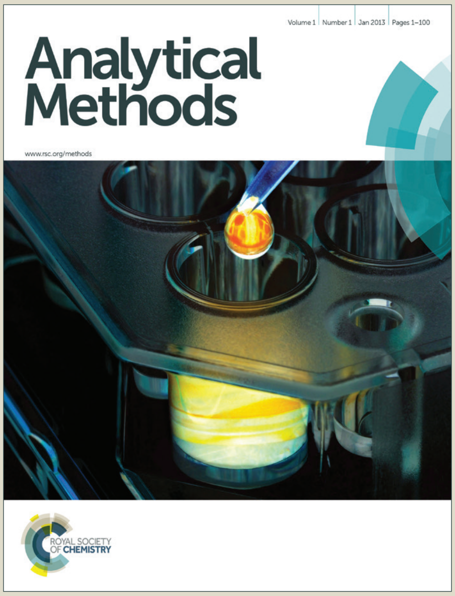
Analytical
Methods



ROYAL SOCIETY
OF CHEMISTRY

www.rsc.org/methods

High-throughput sugarcane leaf analysis using a low cost closed-vessel conductively heated digestion system and inductively coupled plasma optical emission spectroscopy

Kelber Miranda*, Alan Lima Vieira, José Anchieta Gomes Neto

Department of Analytical Chemistry, São Paulo State University,

P.O. Box 355, 14801-970, Araraquara - SP, Brazil

---

* Corresponding author. Tel: +55 16 33019738; Fax: +55 16 33019692.
E-mail address: kelbermiranda@hotmail.com (K. Miranda)

Abstract

Most small to medium-size laboratories involved in foliar diagnosis make use of digestion blocks with borosilicate glass tubes to decompose leaf samples. Then, the elements of interest can be simultaneously determined by ICP OES, except boron, because blank signals for this element are greater than the analytical signals. To determine boron by ICP OES, all samples are digested again using a dry ashing procedure, sometimes requiring up to 24 h per a small group of samples. In order to provide a low-cost alternative to prepare leaf samples for elemental determination, including boron, quartz digestion tubes were developed for the recently proposed closed-vessel conductively heated digestion system (CHDS). The performance of the CHDS with quartz tubes was evaluated by converting sugarcane leaf samples into solutions for subsequent determinations of macro (Ca, K, Mg, P, and S) and micronutrients (Al, B, Cu, Fe, Mn and Zn) by ICP OES. The analytical procedure was validated with three certified reference materials and applied to ten sugarcane leaf samples. Results for Al, B, Ca, Cu, Fe, K, Mg, Mn, P, S and Zn determined by ICP OES the conductively heated digestion system with quartz tubes were in fair agreement with those obtained after closed-vessel microwave-assisted digestion. For all elements determined, the relative standard deviation (n= 3) was usually below 5%. The use of quartz digestion tubes resulted in lower blank values for boron, allowing the determination of all elements of interest in one run.

Keywords: sugarcane, foliar diagnosis, closed-vessel, digestion, conductive heating, ICP OES.

1. Introduction

The environmental impact caused by the burning of fossil fuels has generated a considerable demand for the use of ethanol as a biofuel. Bio-ethanol production has increased significantly over the last decade and sugarcane is one of the most attractive feedstock options. Sugarcane is used in some countries (e.g. Brazil, India and China) to produce ethanol, making it a strategic crop for biofuels.[1-3] Brazil is the world's leading sugarcane producer (ca. 659 million tonnes) with 8.8 million hectares harvested for the 2013/2014 season. In the last season (2013/2014), Brazilian ethanol fuel production was around 27 billion liters which represents 40% of the world consumption.[4]

In order to improve the final production of sucrose during the harvest period, sugarcane foliar diagnosis is widely used to evaluate the nutritional status of the plant, helping the identification of fertilizer requirements.[5,6] For sugarcane leaf sampling, the cultivated area should be divided into plots not bigger than 10 ha. At least 20-30 leaves of 3-7 months old must be collected from each plot, mixed to make a sample and sent to the laboratory.[7] Considering that 10 ha generates one sample, 8.8 million hectares would yield 880 thousand sugarcane leaf samples to be analyzed per year in Brazil.

Most small to medium-size laboratories involved in foliar diagnosis make use of digestion blocks with borosilicate glass tubes to decompose leaf samples. Then, the elements of interest can be simultaneously determined by ICP OES, except boron, because blank signals for this element are greater than the analytical signals. In order to determine boron by ICP OES, all samples are decomposed using a dry ashing procedure, sometimes requiring up to 24 h per a small group of samples. Microwave-assisted digestion systems can be an option to overcome these shortcomings, but this technology has not been implemented to any considerable extent in routine analysis due to high costs involved in acquisition and maintenance.[8] In order to provide a low cost and fast alternative to digest leaf samples for the determination of all elements of interest, including boron, quartz digestion tubes were developed for the recently proposed closed-vessel conductively heated digestion system[9] (CHDS) and evaluated for sugarcane leaf analysis.

2. Experimental

2.1. Instrumentation

Macro and micronutrients were determined in digests by ICP OES (iCAP 6500 Duo, Thermo Scientific, Waltham, MA, USA). Operating conditions are described in Table 1.

Samples were digested using the CHDS. Details about this system are described elsewhere.[9]

A closed vessel microwave (MW) digestion system (Multiwave, Anton Paar, Graz, Austria) equipped with a rotor for 6 reaction PFA vessels (internal volume of 50 mL, minimum filling volume of 6 mL) and temperature sensor was employed for comparison of digestion efficiency.

Sugarcane leaves were ground using a Wiley type cutting mill (TE-648, Tecnal, Piracicaba, SP, Brazil).

2.2. Reagents, analytical solutions and samples

Deionized water (18 ΩM cm resistivity) obtained with a Milli-Q® water purification system (Millipore, Bedford, MA, USA) was used to prepare all solutions. All glassware and polypropylene flasks were washed with Extran® laboratory detergent and soaked in 10% (v/v) HNO_3 for 24 h and rinsed with deionized water prior to use. Nitric acid (69%, J.T. Baker, Deventer, Holland) and H_2O_2 (30%, Merck, Darmstadt, Germany) were used for sample digestion. Aluminum, B, Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn multielement analytical calibration solutions were prepared by appropriate dilution of individual 10,000 mg L^{-1} Spex plasma standards. Since no certified sugarcane leaves reference material was available, three plant certified reference materials were used to estimate accuracy and precision: Apple Leaves (SRM 1515), Trace Elements in Pine Needles (SRM 1575a) and White Cabbage (BCR 679). Sugarcane leaves were collected according to the recommended procedure for sugarcane diagnosis.[11]

2.3. Quartz digestion tubes

The aluminum heating block was designed with 28 positions to accommodate quartz digestion tubes. Making external screw threads around the necks of quartz tubes is a technological challenge for many countries (e.g. Brazil). Therefore, we have dedicated considerable time in developing a low-cost sealing system for the quartz digestion tubes.

The quartz digestion tube (15 mm internal diameter, 18 mm outside diameter, 200 mm height and internal volume of 34 mL) depicted in Figure 1 was developed with a rounded U-shaped bottom and a lip at the top. The tube receives on its opening a Teflon hollow rod (1). This hollow rod has a perfluorinated (FFKM) o´ring on its lower end that seals the quartz tube wall and an external screw thread on its upper end that takes a Bakelite (plastic) screw cap (5). A Teflon cover (4) containing a perfluorinated (FFKM) o´ring is pressed by the screw cap and seals the Teflon hollow rod. Since the screw cap is more fragile than the tube, it also works as a safety seal, allowing the integrity of the quartz vessel in case of overpressure. An aluminum hollow rod with embedded Teflon ring (2) and an aluminum screw cap (3) both keep the Teflon hollow rod inserted into the quartz tube, preventing it to move upward when the pressure inside the quartz tube increases during digestion.

In the first clogging system designed for borosilicate digestion tubes,[9] the o´ring sealed the top of the tube. In the clogging system of the quartz digestion tubes developed in this study, the o´ring seals the internal wall of the Teflon capsule that was designed with an inclination of 10 º. The clogging system of the quartz digestion tube is presented in Figure 2.

Quartz tubes, Teflon and aluminum parts, FFKM o´rings and Bakelite caps are all re-usable.

2.4. Improving safety conditions

In order to allow the analyst to vent the tube safely, a Teflon capsule was developed. After cooling the samples, the tube was inserted in the Teflon capsule and vented behind the cabinet safety shield by opening the screw cap with a short turn using only finger force. The Teflon capsule is depicted in Figure 3.

2.5. CHDS digestion procedure

Three certified reference materials and ten sugarcane leaf samples were digested in triplicate using the CHDS. Samples of 200 mg were accurately weighted in quartz digestion tubes followed by addition of 2.0 mL of concentrated (69%) HNO_3. Samples were left 15 min at room temperature in a dust free environment for pre-digestion and then, 1.0 mL of (30%) H_2O_2 was added. Quartz tubes were fully closed using Teflon and aluminum parts, Bakelite screw caps, and placed in the heating block. The heating program described in Table 2 was run. The intermediate temperature was set to 170 Cº. A hold time of 10 min at 170 Cº led to nitric acid generation and reduced overpressure, but kept the pressure high enough to increase the boiling point of the acid mixture during this step, improving digestion. The final solutions were transferred to polypropylene flasks and diluted up to 25 mL with water.

2.6. Microwave digestion procedure

In order to compare the results obtained with the CHDS, sugarcane leaf samples were digested in triplicate according to the following procedure: 200 mg of ground material were accurately weight in microwave PFA vessels and then 3.0 mL of concentrated (69%) HNO_3 were added. Pre-digestion was performed at room temperature for 15 min. Then, 1.0 mL of (30%) H_2O_2 and 2.0 mL of water were added. The following heating program was applied: (1) 20 ºC (room) to 200 ºC with a ramp of 20 min; (2) 15 min at 200 ºC; (3) 30 min cooling (from 200 ºC to 60 ºC). The final solutions were transferred to polypropylene flasks and diluted up to 25 mL with water.

3. Results and discussion

The accuracy of the CHDS with quartz digestion tubes was evaluated by using three certified reference materials. The results obtained for Al, B, Ca, Cu, Fe, K, Mg, Mn, P, S and Zn are presented in Table 3 and Table 4. For all 11 elements determined by ICP OES, the relative standard deviation (RSD) of three replicates was usually below 5%.

The method was then applied to ten sugarcane leaf samples. For comparative purposes, the determination was also performed in the samples after microwave digestion by ICP OES. The results for Al, B, Ca, Cu, Fe, K, Mg, Mn, P, S and Zn determinations, described in Table 3 and Table 4, were in fair agreement between two systems. The limit of quantification (LOQ) was calculated as the concentration corresponding to 10-fold the standard deviation of 10 independent sample blanks divided by the slope of the analytical curve.[12] According to the results presented in Table 5, the LOQ of each element was similar using both digestion systems. Boron, which was present in low concentrations (from 2.4 to 4.5 mg kg^{-1}) in sugarcane leaf samples, could be determined simultaneously by ICP OES because digestions were performed in quartz tubes, resulting in lower blank values and adequate LOQ for this element.

The dual view ICP optical emission spectrometer (iCAP 6500 Duo) allowed the selection of the plasma view (axial or radial), avoiding any additional dilution for measuring analytical lines of the elements in high concentrations (macronutrients). The axial plasma view was used for measuring the analytical lines of Al, B, Fe, Cu, Mn and Zn, and the radial plasma view was used for measuring the analytical lines of Ca, Mg, K, P and S.

The CHDS with quartz digestion tubes has a potential as a cheaper alternative to the MW digestion. The proposed quartz tube requires small volumes of the acid mixture (2 mL of HNO_3 and 1 mL H_2O_2), and there is no specification for a minimum volume of reagents. Nevertheless, MW digestion vessels require a minimum volume of the acid mixture (typically 6 - 8 mL). The CHDS and the MW output powers used in this study are 400 W and 1400 W, respectively. Considering the heating times for CHDS and MW digestion systems employed in this work are 40 and 35 min, respectively, the MW system consumes three times more energy than the CHDS.

In order to increase the sample throughput, the number of positions of the CHDS can be increased considerably by extending the length of the aluminum heating block. This strategy is difficult for MW digestion systems because the number of vessels is limited by the output power. The higher the number of vessels, the higher the power setting.

According to results obtained, it is possible to digest plant materials with the CHDS without pressure and temperature control. This is attained by performing a pre-digestion, limiting the sample mass to 200 mg, using adequate amount of hydrogen peroxide in the acid mixture to remove NO_2 from the gaseous phase[9] and optimizing a heating program with target temperatures, ramps and hold times. In the CHDS, the heat transfer is limited by the temperature of the aluminum heating block. In MW digestion systems, the heat transfer must be strictly controlled by a temperature sensor and when programming a power profile, the programmed power must be adapted to the number of vessels used. The lower the number of vessels, the lower the power.

In the CHDS, it is possible to apply the same heating program regardless the number of vessels. In MW digestion systems it is not possible to use less than 4 vessels, regardless of the type of rotor. Also, for uniform heating, the vessels must be loaded symmetrically into the rotor according to the respective rotor type. If a different number of vessels has to be used, the vacant positions must be filled with reagents. In recently developed MW digestion systems, it is possible to program a temperature profile. However, the power limit must be adapted to the number of vessels and using an internal temperature sensor is mandatory.

5. Conclusion

The CHDS with quartz tubes is simple, easy-to-operate, low cost (acquisition, operation and maintenance), effective and robust. Nevertheless, MW digestion systems are considerably more expensive and require higher skilled analysts in relation to the CHDS. The CHDS is definitely an interesting and feasible alternative for laboratories dedicated to large scale routine analysis of leaf samples.

Acknowledgements

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) for the financial support (Grant 2012/02891-7). The authors are also grateful to the Conselho Nacional de Desenvolvimento Tecnológico (CNPq) (Grant 303255/2013-7). We also thank the technical support provided by Thermo Scientific and Analitica.

References

(1) M. A. Rendon-Sagardi, C. Sanchez-Ramirez, G. Cortes-Robles, G. Alor-Hernandez and M.G. Cedillo-Campos, *Appl. Energy*, 2014, http://dx.doi.org/10.1016/j.apenergy.2014.01.023.

(2) M. Ewing and S. Msangi, *Environ. Sci. Policy*, 2009, **12**, 520.

(3) L. C. Freitas and S. Kaneko, *Energy Policy*, 2011, **39**, 2289.

(4) A dimensão do setor Sucroenergético: mapeamento e quantificação da safra 2013/14. M. F. Neves and V. G. Trombin, Ribeirão Preto: Markestrat, Fundace, FEA-RP/USP 2014. <http://www.unica.com.br/download.php?idSecao=17&id=25818107>. Retrieved 24 June 2014.

(5) L. C. Nunes, J. W. B. Braga, L. C. Trevizan, P. F. de Souza, G. G. A. de Carvalho, D. Santos, R. J. Poppi and F. J. Krug, *J. Anal. At. Spectrom.*, 2010, **25**, 1453.

(6) S. R. Oliveira, J. A. Gomes Neto, J. A. Nóbrega and B. T. Jones, *Spectrochim. Acta Part B*, 2010, **65**, 316.

(7) R. M. Prado and G. Caione, *Plant Analysis, Soil Fertility*, Dr. Roland Issaka (Ed.), ISBN: 978-953-51-0873-3, InTech, 2012. DOI: 10.5772/53388. Available from: http://www.intechopen.com/books/soil-fertility/plant-analysis.

(8) S. Husted, D. P. Persson, K. H. Laursen, T. H. Hansen, P. Pedas, M. Schiller, J. N. Hegelund and J. K. Schjoerring, *J. Anal. At. Spectrom.* 2011, **26**, 52.

(9) K. Miranda, E. R. Pereira-Filho, and J. A. Gomes Neto, *J. Anal. At. Spectrom.,* 2014, DOI: 10.1039/C3JA50369K.

(10) G.L. Moore, *Introduction to Inductively Coupled Plasma Atomic Emission Spectrometry*, Elsevier, Amsterdam, 1989.

(11) R. A. Reis Jr. and P. H. Monnerat, *J. Plant Nutr.*, 2002, **25**, 2831.

(12) A. Gustavo González and M. Ángeles Herrador, *Trends Anal. Chem.*, 2007, **26**, 227.

(13) E. M. M. Flores, J. S. Barin, M. F. Mesko and G. Knapp, *Spectrochim. Acta Part B*, 2007, **62**, 1051.

Figure Captions

Figure 1. Quartz tube used for digestion in the CHDS with (1)Teflon hollow rod, (2) aluminum hollow rod with embedded Teflon ring, (3) aluminum screw cap, (4) Teflon cover with FFKM o´ring and (5) Bakelite screw cap (a) before and (b) after closing.

Figure 2. Details of the quartz digestion tube clogging system.

Figure 3. Teflon capsule to (a) insert the quartz tube and (b) release the vapors after the completion of the heating and cooling processes.

Table 1. Istrumental operating conditions of ICP OES.

Parameter	Operating condition	Observation view
RF generator frequency (MHz)	40	-
RF applied power (kW)	1.15	-
Purge gas	Argon	-
Plasma gas flow rate (L min^{-1})	12 L min^{-1}	-
Auxiliary gas flow rate (L min^{-1})	0.5 L min^{-1}	-
Nebulizer gas flow (L min^{-1})	0.7 L min^{-1}	-
Analytes	line (nm)	-
Al (I)	396.152	Axial
B (I)	249.773	Axial
Ca (I)	422.673	Radial
Cu (I)	324.754	Axial
Fe (II)	238.204	Axial
K (I)	766.485	Radial
Mg (I)	285.211	Radial
Mn (II)	257.610	Axial
P (I)	213.618	Radial
S (I)	181.972	Radial
Zn (I)	213.857	Axial

(I) = atomic ; (II) = ionic.

Table 2. Heating program of the CHDS.

Step	Time (min)	Temperature (ºC)
1	10	20 (room) to 120
2	10	170
3	10	170 to 220
4	10	220
Cooling	30	220 to 60

Table 3. Results (mean ± standard deviation) for macronutrients (mg kg^{-1}) in 3 CRMs digested (n = 3) by the CHDS and in 10 sugarcane leaf samples (S1 to S10) digested (n = 3) by the CHDS and by closed-vessel microwave digestion system (MW).

		Element				
		Ca	K	Mg	P	S
CRM						
Apple Leaves	Certified	15260 ± 150	16100 ± 200	2710 ± 80	1590 ± 110	(1800)
	Found	14298 ± 237	15008 ± 301	2625 ± 48	1518 ± 27	1867 ± 84
	Recovery (%)	94	93	97	95	104
Pine Needles	Certified	2500 ± 100	4170 ± 70	(1060 ± 170)	1070 ± 80	NC
	Found	2364 ± 53	4019 ± 38	1090 ± 14	1035 ± 10	798 ± 41
	Recovery (%)	95	96	103	97	-
White Cabbage	Certified	(7768 ± 655)	NC	(1362 ± 127)	(3307 ± 241)	NC
	Found	7785 ± 33	27693 ± 204	1269 ± 7	3441 ± 24	7242 ± 111
	Recovery (%)	100	-	93	104	-
Sample						
S1	MW	5408 ± 101	12209 ± 176	1612 ± 33	1511 ± 54	1514 ± 89
	CHDS	4968 ± 103	12312 ± 51	1632 ± 8	1537 ± 17	1616 ± 25
S2	MW	3810 ± 81	11175 ± 218	1616 ± 34	1488 ± 48	1388 ± 104
	CHDS	3593 ± 38	11849 ± 165	1707 ± 23	1620 ± 29	1337 ± 62
S3	MW	3845 ± 24	11099 ± 83	1170 ± 7	1536 ± 20	1118 ± 110
	CHDS	3587 ± 44	11959 ± 132	1188 ± 11	1645 ± 24	1268 ± 31
S4	MW	4473 ± 153	10897 ± 419	1454 ± 45	1528 ± 72	1363 ± 120
	CHDS	4196 ± 53	11267 ± 216	1491 ± 25	1579 ± 49	1443 ± 120
S5	MW	3371 ± 170	12264 ± 510	1107 ± 48	1531 ± 112	1406 ± 90
	CHDS	3359 ± 63	13457 ± 230	1192 ± 18	1739 ± 17	1447 ± 31
S6	MW	5380 ± 95	11103 ± 192	2539 ± 39	1651 ± 44	1578 ± 63
	CHDS	5268 ± 66	12012 ± 286	2826 ± 33	1827 ± 32	1800 ± 60
S7	MW	5744 ± 154	12615 ± 302	1662 ± 45	1649 ± 69	1975 ± 167
	CHDS	5523 ± 24	12797 ± 146	1787 ± 2	1785 ± 20	1989 ± 45
S8	MW	3667 ± 180	8982 ± 452	1653 ± 66	1113 ± 85	1287 ± 161
	CHDS	3677 ± 73	9738 ± 246	1848 ± 37	1272 ± 43	1311 ± 43
S9	MW	2768 ± 36	12222 ± 151	921 ± 8	1278 ± 13	1205 ± 6
	CHDS	2746 ± 78	12828 ± 284	990 ± 30	1424 ± 30	1253 ± 86
S10	MW	4121 ± 127	8558 ± 252	953 ± 26	1368 ± 76	1260 ± 73
	CHDS	4075 ± 37	9269 ± 70	1038 ± 7	1551 ± 8	1346 ± 16

Values in brackets are not certified.

Table 4. Results (mean ± standard deviation) for micronutrients (mg kg^{-1}) in 3 CRMs digested (n = 3) by the CHDS and in 10 sugarcane leaf samples digested (n = 3) by the CHDS and by closed-vessel microwave digestion system (MW).

		Element					
		Al	B	Cu	Fe	Mn	Zn
CRM							
Apple Leaves	Certified	286 ± 9	27 ± 2	5.64 ± 0.24	83 ± 5	54 ± 3	12.5 ± 0.3
	Found	250 ± 5	26.5 ± 0.3	4.91 ± 0.04	73 ± 2	48 ± 1	11.4 ± 0.3
	Recovery (%)	87	98	87	88	89	91
Pine Needles	Certified	580 ± 30	(9.6 ± 0.2)	2.8 ± 0.2	46 ± 2	(488 ± 12)	38 ± 2
	Found	539 ± 17	10 ± 1	2.65 ± 0.04	42 ± 1	456 ± 3	35 ± 1
	Recovery (%)	93	104	95	91	93	92
White Cabbage	Certified	NC	(27.7 ± 1.9)	2.89 ± 0.12	55.0 ± 2.5	13.3 ± 0.5	79.7 ± 2.7
	Found	4.1 ± 0.2	27.3 ± 0.5	2.6 ± 0.1	54 ± 1	12.2 ± 0.1	81.3 ± 0.5
	Recovery (%)	-	99	90	98	92	98
Sample							
S1	MW	209 ± 4	4.3 ± 0.4	4.2 ± 0.1	162 ± 5	40.0 ± 0.9	13.9 ± 0.3
	CHDS	200 ± 8	4.7 ± 0.6	3.9 ± 0.1	158 ± 4	40.5 ± 0.1	14.31 ± 0.03
S2	MW	221 ± 5	3.9 ± 0.1	4.2 ± 0.1	162 ± 4	34.2 ± 0.6	13.2 ± 0.1
	CHDS	225 ± 8	3.6 ± 0.3	3.9 ± 0.2	165 ± 4	33.9 ± 0.7	13.3 ± 0.3
S3	MW	164 ± 3	4.2 ± 0.2	4.2 ± 0.1	135 ± 2	25.5 ± 0.6	13.3 ± 0.2
	CHDS	154 ± 13	3.8 ± 0.1	3.8 ± 0.1	129 ± 8	25.8 ± 0.3	13.6 ± 0.3
S4	MW	180 ± 3	3.7 ± 0.2	3.4 ± 0.1	143 ± 2	57 ± 2	14.5 ± 0.6
	CHDS	162 ± 6	3.5 ± 0.1	3.3 ± 0.1	130 ± 6	53 ± 2	14.7 ± 0.3
S5	MW	126 ± 11	2.9 ± 0.2	3.1 ± 0.4	96 ± 7	30 ± 2	11 ± 1
	CHDS	123 ± 1	3.1 ± 0.2	2.9 ± 0.1	98 ± 2	30.8 ± 0.4	11.8 ± 0.3
S6	MW	188 ± 10	3.2 ± 0.2	3.8 ± 0.1	155 ± 8	42 ± 1	14.7 ± 0.5
	CHDS	178 ± 6	3.4 ± 0.3	3.7 ± 0.2	151 ± 5	42 ± 1	15.2 ± 0.1
S7	MW	304 ± 10	4.5 ± 0.2	4.6 ± 0.1	226 ± 10	43.9 ± 0.6	18.5 ± 0.3
	CHDS	288 ± 9	4.7 ± 0.3	4.1 ± 0.1	207 ± 7	41.4 ± 0.9	17.6 ± 0.4
S8	MW	174 ± 7	2.6 ± 0.3	3.2 ± 0.1	136 ± 7	44 ± 2	13.4 ± 0.7
	CHDS	173 ± 3	2.4 ± 0.2	3.0 ± 0.1	136 ± 3	43.3 ± 0.5	13.8 ± 0.3
S9	MW	214 ± 7	2.47 ± 0.07	3.8 ± 0.2	151 ± 7	83 ± 2	14.8 ± 0.2
	CHDS	212 ± 3	2.7 ± 0.2	3.6 ± 0.2	144 ± 2	78.1 ± 0.3	15.3 ± 0.3
S10	MW	168 ± 5	2.4 ± 0.1	3.9 ± 0.1	130 ± 4	38 ± 1	12.8 ± 0.3
	CHDS	152 ± 2	2.7 ± 0.3	3.6 ± 0.1	117 ± 5	36 ± 1	12.9 ± 0.3

Values in brackets are not certified.

Table 5. Limits of quantification (LOQ) obtained with the CHDS and with a closed-vessel MW digestion system.

Element	LOQ (mg kg^{-1})	
	CHDS	MW
Al	2.67	2.20
B	2.20	2.33
Ca	2.87	2.40
Cu	0.63	0.70
Fe	1.33	1.47
K	1.33	1.10
Mg	0.50	0.47
Mn	0.23	0.27
P	10.0	8.47
S	1.07	1.17
Zn	0.67	0.47

Figure 1

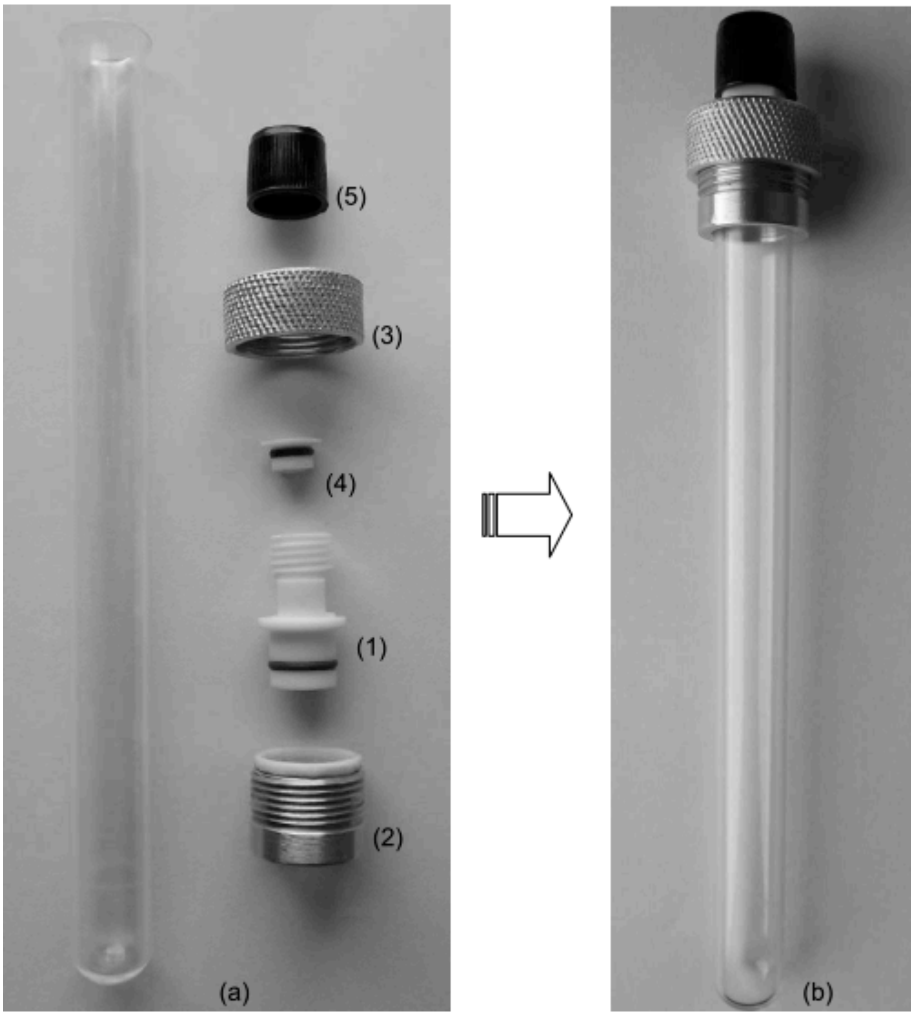


Figure 2

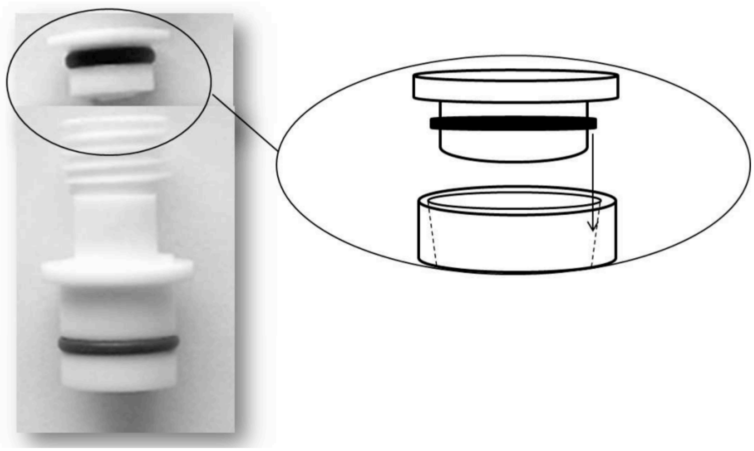


Figure 3