Microchemical Journal 134 (2017) 27–34

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Fast and simple flow-batch extraction procedure for screening of macro and micronutrients in dried plant leaves by ICP OES

Thiago L. Marques *, Joaquim A. Nóbrega

Group for Applied Instrumental Analysis, Department of Chemistry, Federal University of São Carlos, 13565-905 São Carlos, SP, Brazil

ARTICLE INFO

Article history:
Received 13 March 2017
Received in revised form 1 May 2017
Accepted 3 May 2017
Available online 04 May 2017

Keywords:
Flow-batch system
Extraction
Plant tissues
On-line determination
Multielement analysis
ICP OES

ABSTRACT

Acid extraction is an alternative procedure for sample preparation for determination of macro and micronutrients in plant tissues. This approach presents as advantages its speed, simplicity, low cost and use of diluted acid solutions in mild conditions (temperature and pressure). However, most of the extraction procedures are performed in batch mode, which makes them more laborious, time-consuming and susceptible to errors when compared to procedures in flow and flow-batch mode. In this work, the performance of a flow-batch extraction system for on-line determination of macro and micronutrients in dried plant leaves by ICP OES was evaluated. The proposed flow-batch system is simple for operation, inexpensive, allowed on-line determination of macro and micronutrients in dried plant leaves by ICP OES and could be easily applied in any routine analysis laboratory. The best efficiency extraction was achieved using 50 mg of dried spinach leaves, 10 mL of 8% V V^{-1} HCl and 4 min of extraction time. Despite applying 4 min of extraction time, it is important to highlight that some elements, such as Cu, K, Mg, Mn and Na, can be extracted in as short as 30 s extraction times. The flow-batch system presented good accuracy and most elements determined in CRMs and samples are in good agreement with certified values and concentrations determined after microwave-assisted acid digestion, respectively.

1. Introduction

Determination of macro and micronutrients in leaf tissues is important in agriculture management since the deficiency of these elements may affect plant growth, development, and yield of crops [1]. Dry-ashing [2–4], acid digestion with conductive heating [2,5], and microwave-assisted acid digestion [6,7] are sample preparation methods usually applied. Despite particularities, all these methods are performed under high temperatures and sometimes under medium and high pressure when using closed vessels, moreover, dilute or concentrated acids are applied, which requires special care and use of dedicated apparatus for carrying out each procedure [6].

Dilute acid extraction is a mild sample preparation approach which can be applied to determine macro and micronutrients in plant tissues. Basson & Böhmer [2] and Miyazawa et al. [8] demonstrated quantitative extraction and determination of Ca, Cu, K, Mg, Mn, and Zn in leaf tissues by flame atomic absorption spectrometry (FAAS) and flame photometry and Hunt [5] determined Ca, K and Mg in plant samples by FAAS. However, Fe and P were not extracted under the evaluated conditions because they are strongly linked to sample matrix [2,8].

Procedures based on ultrasonic assisted extraction were also investigated. Nascentes et al. [9] demonstrated the quantitative extraction of Ca, Mg, Mn and Zn from leaf tissues using an ultrasonic bath followed by determination using FAAS, however Fe determination was not feasible in most samples. Padilha et al. [10] successfully determined Cu, Mn and Se ultrasonically extracted from vegetable tissues using a Ti probe. Based on these studies it may be inferred that dilute acid extraction is a good approach for metals determinations in plant tissues, despite not being possible to determine elements such as Fe and P. These elements require more drastic methods, such as acid digestion or dry-ashing, to be released [8,11].

In general, conventional or ultrasonic-assisted acid extractions are performed in multiple steps and are prone to errors during sample preparation. Additionally, these procedures are time consuming and laborious despite the typically short extraction times (*ca.* 10 min). However, these disadvantages can be overcome using flow extraction systems as reported in the literature for plant tissues for organic and inorganic analysis by chromatographic [12–14] and spectroscopic [15,16] techniques. Other advantage of flow systems for sample preparation is the possibility to perform on-line measurements of target compounds or elements [12,16].

Herrera & Luque de Castro [15] developed a microwave-assisted flow extraction system for Cd and Pb determination in leaf tissues by graphite furnace atomic absorption spectrometry (GFAAS). Extractions were carried out using 500 mg of sample packed inside a PTFE microcolumn which was immersed in a 30 mL water bath put inside a microwave oven cavity. The microcolumn was placed in a 2 mL sample

* Corresponding author.
E-mail address: thiagolm_chemistry@hotmail.com (T.L. Marques).

http://dx.doi.org/10.1016/j.microc.2017.05.002
0026-265X/

loop which was held in load position during extraction with 1% V V^{-1} HNO_3. The extraction solution was carried out in reversible flow direction for 20 s at 0.8 mL min^{-1} and the system was constantly irradiated at 300 W for 10 and 15 min during Cd and Pb extraction, respectively. Final extracts were diluted to 4 mL before off-line measurements by GFAAS [15].

The main disadvantage of these previously mentioned flow extraction systems is the use of a microcolumn, which must be repeatedly packed for each sample analysis [13–16]. Moreover, sample is kept static while extraction solution flows through it, so analyte concentrations are different in each extract fraction. Then, the whole extracted portion should be homogenized before measuring or completely driven to detection system to obtain accurate results [12]. Flow-batch system can overcome this problem since it avoids sample dispersion. Additionally, it increases analytical signals which become similar to those obtained in batch mode [17]. This approach allows to combine the advantages of flow, batch and multicommutation strategies and also the coupling with conventional analytical instruments [18,19]. In these systems some parameters as washing time, flow-rates, tube diameters and lengths are not so critical when compared to conventional flow systems [19]. However, there is no flow-batch extraction system applied to multielement determination in plant tissues. In this sense, the aim of the work here described was to develop and to evaluate a new flow-batch extraction system to determine macro and micronutrients in leaf tissues by inductively coupled plasma optical emission spectrometry (ICP OES). The flow-batch extraction system was developed based on minimum costs, easy assembling and operation, and applicability for routine analysis.

2. Experimental

2.1. Instrumentation and apparatus

Determination of analytes in extracts were performed using dual view ICP OES (iCap 6000 Dual view, Thermo Scientific, Cambridge, England). Extracts were introduced using a concentric nebulizer and cyclonic chamber when performing off-line measurements. On the other hand, a V-groove nebulizer and a double pass cyclonic chamber were used for on-line measurements. The other operational parameters applied for macro and micronutrients determination in extracts by ICP OES are summarized in Table 1. The particle size distribution was measured using static laser light scattering instrument (Fritsch Analysette 22 MicroTec plus, FRITSCH, Idar-Oberstein, Germany).

An ultrasound bath (AquaWave 9374, Barnstead Lab-Line, Elma Ultrasonic, Singen, Germany), an aquarium air pump (A 230, Big Air Super Pump, China) and a magnetic stirrer (752A, Fisatom, São Paulo, Brazil) were tested for slurry homogenization during extraction. A peristaltic pump (IPC Model 78001-12, Ismatec, Glattburg, Switzerland) with eight channels was applied for propulsion of solutions. A Perspex commutator (2-3-2) and PTFE tubes (0.80 mm) were used for solutions management. Metallic rod, claws and clamps were used to assemble the flow-batch extraction system.

Table 1
Operational parameters applied for multielement determinations by ICP OES.

Instrument parameter	Operational conditions
RF applied power (W)	1150
Argon external flow rate (L min^{-1})	12
Argon intermediate flow rate (L min^{-1})	0.50
Argon nebulizer flow rate (L min^{-1})	0.70
Integration time (s)	5
Replicates	3
Sample uptake delay (s)	30
Stabilization time (s)	5
Sample uptake rate (mL min^{-1})	1.0
Elements and wavelengths (nm)	
Axial view: Ba(II) 455.403, Cu(I) 327.396, Fe(II) 238.204, Mn(II) 259.373, Sc(II) 424.683, Sr(II) 421.552 and Zn(I) 213.8564	
Radial view: C(I) 193.091, Ca(II) 317.933, K(I) 766.490, Mg(I) 285.213, Na(I) 588.995 and Sc(II) 424.683	

2.2. Reagents and samples

Extraction solutions were prepared with sub-boiling acids (HNO_3 and HCl) and deionized water (18 MΩ cm, Milli-Q Direct 8, Millipore, Billerica, MA, USA). Multielement calibration curve were prepared from 1000 mg L^{-1} Ba, Ca, Cu, Fe, K, Mg, Na, Sc, Sr and Zn monoelement certified standard solutions (Fluka Analytical, Sigma-Aldrich, Buchs, Switzerland) and 10,000 mg L^{-1} Ca and K stock solutions prepared from analytical grade salt of $CaCO_3$ (Mallinckrodt Chemicals St. Louis, USA) and KCl (Merck, Darmstadt, Germany).

The samples investigated were dried spinach, orange and tomato leaves. Dried spinach leaves were selected to optimize extraction conditions. These samples were collected from local farmers in São Carlos, SP, Brazil and prepared as described before [20]. Accuracy was checked using certified reference materials (CRM) produced by National Institute of Science and Technology (Gaithersburg, MD, USA): spinach leaves (NIST 1570a), apple leaves (NIST 1515), tomato leaves (NIST 1573a), and peach leaves (NIST 1547).

2.3. Flow extraction system

Three shaking techniques were evaluated for performing on-line extractions: mechanical shaking by stir bar and magnetic stirrer, suspension bubbling by aquarium air pump with a glass tube inserted into the extraction vessel, and ultrasonic radiation by ultrasound bath. In these experiments 10 mL of 8% V V^{-1} HCl were added to polyethylene (PE) tubes containing 50 mg of dried spinach leaves which presented a small mean particle size (*ca.* 35 μm) with high homogeneity. These characteristics are similar to those presented in CRMs which allows to work with small quantities of sample without compromising the accuracy and precision of the extraction procedure [21]. Silvestre & Nomura showed that it is possible to use lower sample masses (0.3 mg) than those recommended by CRMs producers (100–500 mg), since the CRMs are finely powdered materials [21]. Thus, it was possible to perform quantitative analyses using low sample masses of dried plant leaves as proposed here. Experiments were performed in 50 mL PE tubes for mechanical shaking and 15 mL PE tubes for suspension bubbling and sonication. These tube volumes were selected for improving sample homogenization. Suspensions were continuously stirred for 5 min at room temperature. After extraction, suspensions were filtrated using paper filter and extracts were stored until measurements by ICP OES. Paper filters were only used in this experiment, since they may be seemed as potential source of sample contamination. Procedural blanks were prepared by washing paper filters with 10 mL of 8% V V^{-1} HCl.

Later on, different types of reactors and filters were studied to perform flow-batch extraction with on-line detection by ICP OES. They were made with easily purchased materials which are available in any routine analysis laboratory, such as: 50 mL Falcon® tubes, Tygon® tubes, rubber o-rings, 100 μL pipet tips, cotton fabric, glass wool and super glue. Reactors and respective filters evaluated in the flow-batch extraction system are presented in Fig. 1.

Reactor 1 was made with a Falcon® tube with its bottom cut off. Thus, sample could be added through the open base, while cap was used to adapt a filter. Filter 1 and Filter 2 were made with thin and thick cotton fabric, respectively. These reactors and filters were named as R1F1 and R1F2.

Reactor 2 was made with a Falcon® tube with upper side cut off to facilitate sample introduction. Three connections were made in this tube where the superior and intermediate were used for introducing the extracting solution and for filtration of the extract, respectively.