Anal Bioanal Chem (2002) 373 : 153–158
DOI 10.1007/s00216-002-1298-7

ORIGINAL PAPER

M. D. Mingorance

Focused microwave-assisted digestion of vegetal materials for the determination of essential mineral nutrients

Received: 23 November 2001 / Revised: 26 February 2002 / Accepted: 23 March 2002 / Published online: 27 April 2002

Abstract An open focused microwave-assisted digestion procedure has been developed to decompose and dissolve vegetal matrices for subsequent macro- and micronutrients analysis. The parameters of the microwave oven were evaluated using an experimental design. Sulfuric acid (5 mL) and hydrogen peroxide (3 mL) were found to be suitable for quantitative determination of Ca, Cu, Fe, K, Mg, Mn, N, P, and Zn in 0.100–0.500 g of vegetal sample. The precision was better than 6% for all elements at different concentrations. Results for reference and laboratory control materials are in agreement with certified and indicative values. In addition, the sample digest could be used for ICP-OES of all the elements mentioned. The proposed microwave-assisted digestion procedure offers the ability to determine the most important essential plant nutrients in one unique solution by means of analytical techniques usually found in most laboratories.

Keywords Vegetal material · Focused microwave-assisted digestion · Spectroscopy analysis · Mineral elements · ICP

Introduction

Plants constitute important components of ecosystems as they provide the transport pathway from the abiotic to biotic environment. Uptake of metals by plants reflects their bioavailability in soils and affects plant yield and crop quality to the extent that animal or human health may be jeopardized. A previous digestion of the sample [1, 2, 3] must assure the sufficient destruction of the matrix to permit the total analysis of the element of interest. The preparation of the sample, a time-saving and highly risky step that contributes to the uncertainty of the final results, causes analyte losses and leads to sample contamination [4]. Many microwave-assisted digestions have been reported depending on the kind of sample and the element to be determined [5, 6, 7, 8]. The microwave digestion most frequently described for mineral elements in plants uses nitric acid as oxidant [9, 10, 11], but if nitrogen is determined, samples have to be digested with sulfuric acid [12, 13]. In recent years, the laboratory of the Estación Experimental del Zaidín has been engaged in various nutritional and environmental studies where the determinations of N, P, Ca, Mg, K, Fe, Cu, Mn, and Zn in numerous vegetal samples were requested. This prompted us to carry out a focused open microwave digestion procedure to determine macro- and micronutrients in the same plant matrix solution by using various analytical techniques: N and P by colorimetry, K by flame photometry and Ca, Cu, Fe, Mg, Mn, and Zn by atomic absorption spectrometry. The operating conditions of the digestion were optimized. The results obtained by the proposed method were compared with those obtained by a conventional heating method and with certified or indicative values. As an additional secondary aim the elements were also determined by ICP-OES. Thereafter, the method will be applied to routine analysis.

Experimental

Reagents and samples

All chemicals were of analytical reagent-grade. Working standard solutions of all analytes (Merck Tritisol, Merck, Germany) were prepared in 1.8 M H_2SO_4. Certified reference materials (CRMs) were purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD): citrus leaves SRM 1572, tomato leaves SRM 1573, and pine needles SRM 1575 and pepperbush SRM 1 from NIES (Ibaraki, Japan). Laboratory control materials came from the Comité Inter-Instituts d'Études des Techniques Analytiques (CII): leaves of apple fruit, codia, cotton, eucalyptus, luzerne, carnation, orange, palm, peach, apple golden, and vineyard.

Microwave digestion (MW)

The ground and dried material (0.250 g) was digested (Maxidigest MX 4350, Prolabo, Paris, France) with H_2SO_4 (5 mL) at 50%

M.D. Mingorance (✉)
Department of Earth Science and Environmental Chemistry,
Estación Experimental del Zaidín (CSIC), Prof. Albareda 1,
18008 Granada, Spain
e-mail: mdmingor@eez.csic.es

power for 4 min. The heating was then stopped, and H_2O_2 (3 mL) was added dropwise over 2 min. The solution was again heated (at 80%) for 4 min and at 90% for 5 min. After the samples had cooled, the digest was transferred into 50 mL flasks and the solutions made up with demineralized water. Blank solutions were prepared in the same way as the samples.

Conventional wet digestion (CD)

The dried and ground material (0.250 g) was digested with H_2SO_4 and H_2O_2 in a heating-block system according to the Analytical Methods Committee [14]. Blank solutions were prepared in the same way as the samples.

Determination of mineral elements

All elements were determined in the digest under the standard conditions given for the analytical instruments.

N [15] and P [16] were determined by colorimetry using automatic air-segmented continuous flow analysis (Bran+Luebbe TRACCS 2000, Bran+Luebbe, GmbH, Norderstedt, Germany). Potassium was analyzed by flame photometry (Jenway Ltd. PFP7, Essex, UK). Determination of Ca, Cu, Fe, Mg, Mn, and Zn was made by atomic absorption spectrometry (Perkin-Elmer 5000, Connecticut, USA) using air-acetylene. Also, nitrogen was determined by titration [17]. Finally, all elements except N were determined by emission atomic spectrometry ICP (Thermo Jarrell Ash IRIS Advantage, Franklin, MD, USA), with a cyclone axial spray chamber; nebulizator Ar flow, 0.64 L min^{-1}; generate RF-power, 1150 W; RF, 40.68 MHz; analytical wavelength (nm), 213.618 (P), 315.887 (Ca), 285.213 (Mg), 766.190 (K), 259.940 (Fe), 324.754 (Cu), 257.610 (Mn), and 213.856 (Zn).

Accuracy

To check the accuracy, linear regression was used for comparing the results obtained from the microwave digest (*Y*) with the indicative or certified values (*X*). The parameters of the regression line indicate the presence of systematic errors: an intercept value significantly different from zero (constant error) and a slope value different from one (proportional error). The confidence limit was 95%.

Results and discussion

Optimization of the microwave digestion method

After preliminary studies, five variables were considered (Table 1) to select the digestion factors which influenced the microwave system and a 2^{5-2} fractional factorial experiment (Table 2, runs 1–8 and 17–19) was designed. From the inference test (Table 3), it may be concluded that Ca recoveries are not affected by any of the factors. However, P1, P3, T3, and H_2O_2 volume are the most significant factors for N, P, K, and Mg recoveries. A 2^4 factorial (Table 2, runs 1–19) was then performed to evaluate the factors as well as the interactions. Results from an ANOVA test showed no significant effects ($p>0.05$) on Mg, K, and P recoveries, whereas P1 T3, P3, and P1*T3 have a significant influence ($p<0.05$) on N recovery.

The optimum values for each variable considered were obtained by a central composite face (Table 2, runs marked). The results, fitted to a second order model ($R^2=0.971$, $Q^2=0.734$), giving the following equation model after deletion of the insignificant terms:

$$\begin{aligned}\%\text{N recovery} = {} & 96.4 + 3.34\ \text{P1} + 2.35\ \text{P3} + 10.7 \times \text{T3} \\ & - 0.015\ \text{P1} * \text{P1} - 0.011\ \text{P3} * \text{P3} \\ & - 0.382\ \text{T3} * \text{T3} - 0.0168\ \text{P1} * \text{P3} \\ & - 0.113\ \text{P1} * \text{T3}\end{aligned}$$

The estimated contour response plot (Fig. 1) showed that recovery of N increases when the three factors increase. Taking into account that the maximum values of T3 led to overheating the digestion vessels; final selected conditions are given in Table 1.

Precision

The precision of the proposed method (Table 4) was lower than 6% in all cases depending of the element being analyzed and the vegetal sample, without any observable relationship. On the other hand, the MW method exhibits a precision similar or better than of the CD method (Table 5).

Accuracy

The accuracy of analytical results was tested to ensure that it produces results which make it suitable for the intended purpose. Four certified reference samples were digested and the results were compared with the certified values using

Table 1 Experimental domain and final factors values

Factor	Step	Symbol	Low level (–)	High level (+)	Optimum values
Microwave power (%W)	1	P1	20	60	50
Heating time (min)	1	T1	3	7	4
Microwave power (%W)	3	P3	40	80	70
Heating time (min)	3	T3	1	7	4
Volume of H_2O_2 (mL)	2	V	3	6	3
Microwave power (%W)	4		fixed		90
Heating time (min)	4		fixed		5
Mass of sample (g)			fixed		0.250
Volume of H_2SO_4 (mL)			fixed		5

Table 2 Design matrix and experimental recovery

Run	P1	T1	P3	T3	V	Recoveries (%)				
						N	P	Ca	K	Mg
1[a]	–	–	+	–	–	61.8	61.7	93.5	90.0	94.5
2[a]	+	–	–	–	–	86.4	96.3	90.3	83.3	92.7
3[a]	–	+	–	–	–	34.6	93.8	96.8	70.0	98.2
4[a]	+	+	–	+	–	100	88.9	87.1	93.3	89.1
5	–	–	+	+	+	92.4	67.9	90.3	90.0	94.5
6	+	–	+	–	+	80.3	93.8	90.3	70.0	92.7
7	–	+	+	–	+	71.5	92.6	83.9	96.7	90.9
8	+	+	+	+	+	100	86.4	90.3	93.3	89.1
9[a]	+	+	+	–	–	93.9	100	93.5	100	87.3
10[a]	–	–	–	+	–	68.1	93.8	93.5	90.0	94.5
11[a]	–	+	+	+	–	97.7	96.3	100	83.3	94.5
12[a]	+	–	+	+	–	95.5	97.5	80.6	76.7	94.5
13	–	–	–	–	+	1.00	90.1	100	76.7	89.1
14	+	+	–	–	+	91.7	95.1	83.9	83.3	90.9
15	–	+	–	+	+	69.5	86.4	80.6	76.7	89.1
16	+	+	–	+	+	95.5	85.2	96.8	76.7	94.5
17[a]	0	0	0	0	0	97.0	98.8	90.3	90.0	92.7
18[a]	0	0	0	0	0	89.4	97.5	83.9	86.7	94.5
19[a]	0	0	0	0	0	93.9	92.6	87.1	83.3	89.1
20[a]	–	0	0	0	0	75.5				
21[a]	+	0	0	0	0	97.9				
22[a]	0	0	–	0	0	81.1				
23[a]	0	0	+	0	0	97.9				
25[a]	0	0	0	–	0	86.7				
26[a]	0	0	0	+	0	95.1				

[a]Central composite design.
P1 microwave power of step 1, *T1* heating of step 1, *P3* microwave power of step 3, *T3* heating of step 3, *V* volume of hydrogen peroxide.

Table 3 Importance of the regression coefficients (VIP) corresponding to 2^{5-2} designs for each element

Factor	VIP (cum)[a]	N	P	K	Ca	Mg
T3	1.25	10.69	1.98	–1.55	–0.788	–1.72
P1	1.19	10.43	–4.47	3.68	0.0643	–0.715
V	0.982	2.89	–1.78	1.44	–0.745	0.449
P3	0.982	2.89	–1.78	1.44	–0.745	0.449
T1	0.314	0.419	0.860	–0.702	–0.366	–0.279

[a]Importance of the regression coefficients for all elements together.

the *Z*-scores (Fig. 2) which give a estimation of deviations. The values for Ca, P, K, and Mg can be considered acceptable or questionable (*Z*-score<3), but unsatisfactory for Ca in pepperbush and pine samples (*Z*-score>3).

To complete the accuracy study, four laboratory control vegetal samples from CII were digested by the MW digestion method, and the results obtained were compared with those from CD digestion (Table 5). Few significant differences ($p<0.05$) are observed between the digestions without any observable pattern. The method should be validated over a different range of analyte concentrations to check the possible systematic errors. Eleven CII samples were chosen at a wider concentration range (Table 6). Regression parameters of the line for all the elements indicate the presence of proportional bias in some cases with errors lower than 10% with respect to the indicate values, which is considered acceptable in a routine elemental analysis.

Effect of vegetal mass on the digestion efficiency

Masses of two CII samples in the range 0.1–1.0 g were tested and we found that a mass higher than 0.500 g produced dark solutions. From an ANOVA of the results (Fig. 3), it can be seen that there are no significant differences

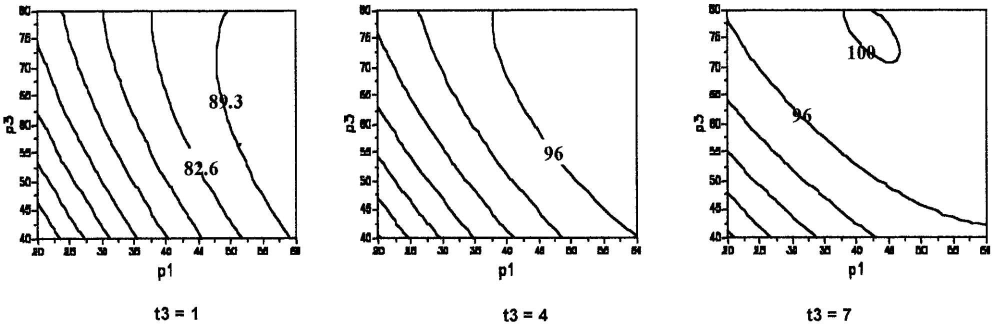

Fig. 1 Contour response plots for N recoveries versus microwave power for step 1 (P1) and 3 (P3) at different heating times in step 3 (T3)

(peach, p=0.120; carnation, p=0.736). A mass of 0.100 g gave the higher standard deviation in both cases because of low homogeneity in the analytical sampling step.

Table 4 Precision of the microwave-assisted digestion expressed as %RSD (n=4). Mean (%) (w/w)

		N	P	Ca	Mg	K
Palm	Mean	2.67	0.17	0.85	0.28	1.02
	Within-run	0.54	0.79	2.41	0.91	2.38
	Between-run	1.21	1.12	2.84	5.02	3.85
	Overall	1.33	1.37	3.72	5.10	4.53
Orange	Mean	2.28	0.15	5.16	0.32	1.82
	Within-run	0.63	2.55	2.34	1.57	3.61
	Between-run	1.46	2.35	1.93	4.53	3.02
	Overall	1.59	3.47	3.03	4.79	4.71
Peach	Mean	4.44	0.28	2.32	0.48	2.53
	Within-run	3.54	3.18	3.49	3.65	3.25
	Between-run	3.07	3.53	2.61	2.09	4.10
	Overall	4.69	4.75	4.36	4.21	5.23
Carnation	Mean	1.58	0.61	1.96	0.34	5.51
	Within-run	1.70	2.57	1.58	4.44	3.15
	Between-run	2.43	3.29	5.67	4.69	2.55
	Overall	2.97	4.17	5.89	6.46	4.05

Overall precision as $(s_w^2+s_b^2)^{1/2}$ where s_w is within-run precision and s_b is between-run precision.

Applicability to other elements

To increase the scope of the described method Fe, Cu, Mn, and Zn were determined (Table 7). The results show that Fe, Cu, and Mn can be determined without bias, but Zn shows both errors (5.3%). These are not important enough to introduce considerable errors and may have no chemical significance for diagnostic results.

Determinations by ICP

The validity of the overall procedure was evaluated by analyzing all elements under study in CII samples by a different analytical technique, ICP-OES. In the case of N determination we used the titrimetry as an alternative technique to colorimetry. From the slope values in Table 8, it can be seen that recoveries are >90% except for P, Fe, and Zn which ranged between 75–80%. These low recoveries could be explained by a partial solubilization of the elements, inclusion on the silica-based material, or by matrix effects. Data suggested the last of these reasons because the results provided by the other analytical techniques used, such as flame AAS, did not show low recoveries. It is known that the ICP signal is susceptible to both the presence of major constituents [18] that may produce a

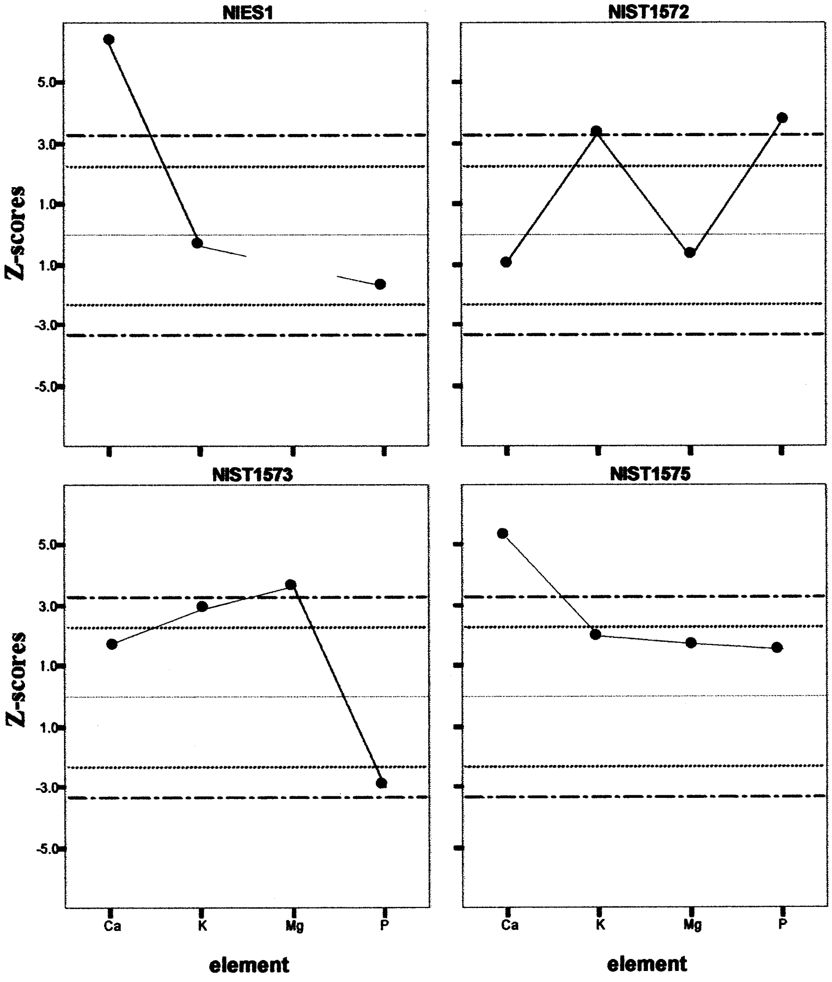

Fig. 2 Z-score values for comparison of the analytical data from MW digestion and certified values for certified reference materials. Mg content is not available for NIES 1 (*solid line* shows acceptable values and *broken line* shows questionable values)

Table 5 Comparison of results for mineral contents (n=4) from MW and CD digestions. Concentrations are % dry weight

Sample	Element	MW		CD		Difference (%)	Mean equiv[a] at α=0.05	sd equiv[a] at α=0.05
		mean	sd	mean	sd			
Palm	N	2.67	0.0058	2.8	0.0716	–5	no	MW< CD
	P	0.165	0.0013	0.163	0.0013		yes	yes
	Ca	0.855	0.0191	0.955	0.0387	–10	no	yes
	Mg	0.28	0.0025	0.301	0.0058	–7	no	yes
	K	1.01	0.024	0.94	0.032	7	no	yes
Orange	N	2.28	0.019	2.3	0.092		yes	MW< CD
	P	0.155	0.0039	0.155	0.0053		yes	yes
	Ca	5.16	0.12	5.51	0.065		yes	yes
	Mg	0.327	0.005	0.0323	0.005		yes	yes
	K	1.82	0.065	1.68	0.038	–8	no	yes
Peach	N	4.44	0.159	4.14	0.13		yes	yes
	P	0.284	0.0093	0.294	0.0062		yes	yes
	Ca	2.32	0.084	2.62	0.065	–11	no	yes
	Mg	0.484	0.019	0.487	0.0096		yes	yes
	K	2.53	0.081	2.4	0.065	5	no	yes
Carnation	N	1.58	0.022	1.51	0.056		yes	yes
	P	0.661	0.016	0.631	0.017		yes	yes
	Ca	1.96	0.11	1.93	0.017		yes	yes
	Mg	0.343	0.015	0.338	0.013		yes	yes
	K	5.51	0.17	5.32	0.23		yes	yes

[a] Means (t-test) and sd (F-test) equivalents at 95% confidence limit. *MW* microwave-assisted digestion, *CD* conventional digestion, *sd* standard deviation.

Table 6 Parameters of the regression lines (MW=a+b·IV) for CII samples digested by MW procedure (n=22)

	Intercept (se)	Slope (se)	R^2	Constant error[a]	Proportional error[a]
N	0.0645 (0.069)	0.962 (0.027)	0.985	no	no
P	–0.000716 (0.007)	0.952 (0.023)	0.988	no	no
Ca	–0.0347 (0.019)	1.056 (0.009)	0.999	no	yes
Mg	–0.0320 (0.011)	1.051 (0.037)	0.976	3.2	no
K	–0.0881 (0.029)	1.099 (0.014)	0.997	9.0	9.0
All elements	–0.0244 (0.013)	1.034 (0.07)	0.995	no	3.4

[a] Expressed as %. *MW* microwave-assisted digestion, *se* standard error, *IV* indicative values.

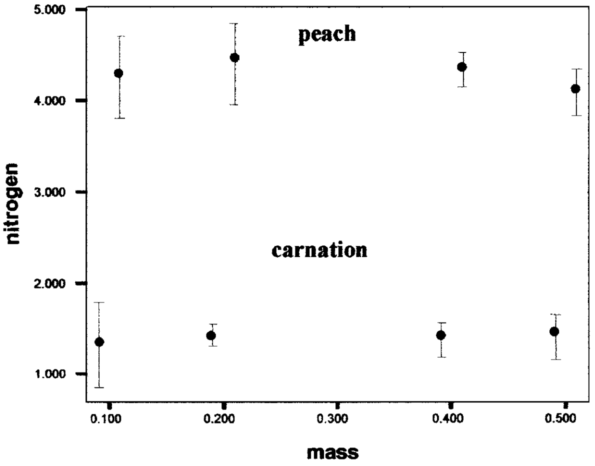

Fig. 3 Effect of sample mass for N in two different samples digested by MW procedure. The *bars* indicate the confidence interval of the mean (n=4) at 95%

signal depression, and the presence of sulfuric acid in the test solution that has a negative effect on the analyte signal [19, 20] by modifying the efficiency nebulization. Therefore, further work should be done to investigate calibration errors and the operating conditions of ICP.

Conclusions

The described digestion method effectively brings all the desired elements, including N, into solution in a relatively short time. Under optimized microwave conditions, the whole procedure including heating and cooling steps and sample preparation, took about 35 min. The advantages of working at atmospheric pressure and adding sequentially reagents render it an attractive alternative.

Although some element analysis, as for P, Fe, and Zn by ICP is not satisfactory, the joint analysis of nine elements is accomplished with good accuracy and precision using either affordable analytical techniques or ICP.

Table 7 Parameters of the regression lines (MW=a+b◡IV) for CII samples digested by MW procedure (*n*=22)

	Intercept (se)	Slope (se)	R^2	Constant error[a]	Proportional error[a]
Cu	–0.452 (0.984)	0.955 (0.096)	0.925	no	no
Fe	–15.618 (8.921)	0.986 (0.021)	0.996	no	no
Mn	–1.774 (1.433)	0.994 (0.006)	1.000	no	no
Zn	–2.564 (0.866)	1.053 (0.015)	0.998	2.5	5.3
All elements	–2.107 (2.127)	0.969 (0.006)	0.997	no	3.1

[a] Expressed as %. *MW* microwave-assisted digestion, *se* standard error, *IV* indicative values.

Table 8 Parameters of the regression lines (ICP=a+b◡IV) for CII samples by ICP after MW digestion (22 data)

	Intercept (se)	Slope (se)	Proportional error[b]	R^2
N[a]	0.0178 (0.047)	1.017 (0.019)	no	0.993
P	0.0738 (0.026)	0.740 (0.122)	26	0.821
Ca	0.0195 (0.051)	0.936 (0.023)	no	0.995
Mg	0.0170 (0.012)	0.900 (0.040)	no	0.983
K	–0.0618 (0.073)	0.901 (0.041)	no	0.985
Cu	0.659 (1.050)	0.899 (0.004)	no	1.000
Fe	61.507 (16.960)	0.768 (0.038)	13.2	0.981
Mn	–2.258 (3.954)	1.045 (0.014)	no	0.999
Zn	7.356 (2.350)	0.814 (0.051)	12.6	0.973
All elements	1.720 (1.675)	0.974 (0.010)	no	0.993

[a] Titrimetric measurement. [b] Expressed as %. *IV* indicative values, *se* standard error.

Method validation confirms that the determination of the elements after the MW procedure digestion is suitable for its intended use.

References

1. Pökiö R, Torvela H, Perämäki P, Knokkanen T, Rökkömäki R (2000) Analusis 28:850–854
2. Kataba-Pendias A, Pendias H (1972) Trace elements in soils and plants. CRC Press, Boca Raton
3. Hoenig M, Baeten H, Vanhentenrijk S, Vassileva E, Quevauviller Ph (1998) Anal Chim Acta 358:85–94
4. Hoenig H, de Kersabiec AM (1996) Spectrochim Acta 51B: 1297–1307
5. Kingston MH, Haswell SJ (1997) Microwave-enhanced chemistry: Fundamentals, sample preparation and applications. ACR Washington USA
6. Campos E, Barahona E, Mingorance MD (1998) Anal Chim Acta 369:235–243
7. Rodushkin I, Ruth T, Huhtasaari A (1999) Anal Chim Acta 378:191–200
8. Borkowska-Burnecka J (2000) Fresenius J Anal Chem 368: 633–637
9. Mingorance MD, Pérez-Vázquez ML, Lachica M (1993) J Anal Atom Spectrom 8:853–858
10. Sun DA, Waters JK, Mawhinnwy TP (1997) JAOAC 80:647–650
11. Polkowska-Montrenko H, Danko B, Dybczynski R, Amnnerlad AK, Bode P (2000) Anal Chim Acta 418:89–95
12. Suard CL, Feinberg MH, Ireland Ripert J, Maurel M (1993) Analusis 21:287–291
13. Chemat Z, Hadj-Boussaad DE, Chemat F (1998) Analusis 26:205–209
14. Analytical Methods Committee (1976) Analyst 101:62–66
15. Krom MD (1980) Analyst 105:305–316
16. Capitán F, Martinez F (1954) Ann Edaf XIII:767–790
17. Bouat A, Crouzet C (1965) Ann Agron 16:107–118
18. Thompson M, Walsh JN (1983) A handbook of inductively coupled plasma spectrometry. Balck & Son, Glasgow, UK
19. Hoenig M, Doceskalova M, Baeten HJ (1998) Anal Atom Spectrom 13:195–199
20. Thompson M, Ramsey M, Coles BJ (1982) Analyst 107:1330–1334