

Trace Metal Analysis in Plant Tissue by Inductively Coupled Plasma-Atomic Emission Spectrometry With Slurry Sample Introduction

Nereida Carrión*, Alberto Fernández, Eliás J. Eljuri, Miguel Murillo,
and Mario Franceschetto

Centro de Química Analítica
Facultad de Ciencias Universidad Central de Venezuela
Apartado 47102, Caracas 1041-A, Venezuela

INTRODUCTION

The very high temperatures reached in plasmas make these sources an efficient tool for evaporating and atomizing solid materials. However, drawbacks inherent to this technique such as differences in sample transport efficiency, analyte volatility and atomization, and matrix dependency are still problems to be solved. On the other hand, quantification has usually been carried out by comparison with known reference materials and so it appears that the use of internal standards would be essential for controlling some of the above variables.

In recent years, solid sample introduction by suspension of finely powdered material has become increasingly common using inductively coupled plasma-atomic emission spectrometry (ICP-AES). This method requires no special devices since the sample can be introduced into the plasma with a high solid nebulizer device (1). Several workers (1-8) demonstrated that the atomization efficiency in ICP-AES rapidly increases with particle size reduction. Saba et al. (2) studied the factors influencing the transport of metallic particles in lubricating oils to emission and absorption sources. They observed that the efficiency of nebulization decreases with increasing particle size. Ebdon et al. (3,4) and McCurdy et al. (5) used slurry nebulization with ICP-AES for the analysis of coal. It was found (3) that among such parameters as slurry concentration and sample pumping rate, particle size was the

ogical samples and found that the analyte behavior is dependent on particle granulometry as well as mineralogical and chemical composition. Laird et al. (9) reported that the matrix interferences might introduce substantial bias in results using slurry nebulization, especially in those samples containing particle sizes larger than 2 μm . Min and Xi-En (10) determined trace elements in ultrafine ZrO_2 powders (D. <0.1 μm), dispersed in an H_2O -glycerine medium. They found it necessary to introduce an empirical coefficient to correct the results. Fagioli et al. (11) successfully carried out the analysis of carbonaceous slurries of biological materials.

Furthermore, use of acidic dissolution, obtained from wet ashing and fusion procedures, may be a particular problem in ICP-MS. To overcome these interferences, slurry nebulization was applied to the analysis of catalysts (12) and coals (13). Good results were obtained for all elements determined, except for Al.

In this work, the introduction of finely powdered leaf samples into the plasma by nebulization of aqueous suspensions, using a V-groove nebulizer, was investigated. The influence of sieving (particle size), the use of acids as dispersants and slurry concentration, have been investigated. Pine needle samples (*Pinus caribaea*) and spinach (*Spinacia oleracea*) leaves were analyzed using the proposed method. The results were compared with those obtained with a standard dissolution method. The accuracy was assessed by analyzing the NIST SRM 1572 Citrus Leaves and SRM

ABSTRACT

A simple and fast method for the determination of manganese, magnesium, and calcium in leaf tissue samples by inductively coupled plasma-atomic emission spectrometry with slurry sample introduction is described. Samples were sieved through 74 μm mesh and suspended in 0.01 M nitric acid solution. Aqueous standard solutions were used for calibration purposes and complete recovery was obtained. The results for the present method are reported for two certified plant materials, SRM 1572 Citrus Leaves and SRM 1573 Tomato Leaves. Good agreement was obtained. The results were compared with a standard procedure. The precision varied from 0.5 to 2.0% with no internal standard.

one to have the highest effect on atomization efficiency. Different recoveries for Cu, Fe, Mn, Ni, and V have been found (4) when particle sizes of less than 30 μm were analyzed. Total recovery for sulfur determination was obtained (5) with a small particle size (diameter average of 6 μm) when slurry of coal samples was analyzed by this technique. Ebdon and Collier (6,7) found that particle size has a critical effect on transport efficiency. Improvements in atomization efficiency were observed (6) for a particle size of less than 4-5 μm . Acceptable results for the analysis of whole kaolin were reported (7) by using internal standard, aqueous calibration, and aqueous ammonia as a dispersant. Halicz and Brenner (5) introduced slurries of mineral-

*Corresponding author.

1573 Tomato Leaves certified reference materials.

EXPERIMENTAL

Apparatus

This study was performed using a high power argon ICP connected to a computer-controlled emission spectrometer. The instrumental parameters and their operating conditions are summarized in Table I. The measurement system consisted of a single channel monochromator-photomultiplier tube assembly, coupled to a computer data acquisition system. The PMT (Hamamatsu E955) signal was amplified by means of a high speed current amplifier, developed in our laboratory. All data were acquired and processed with a Hewlett Packard Model HP-86B microcomputer. The coupling between the photomultiplier and the microcomputer was accomplished by a 12-bit A/D converter with a BCD (HP) output for interfacing with the microcomputer. A variable external oscillator was used for clocking the unit. The software for the monochromator was written in BASIC. The sample slurry was introduced into the ICP by means of a V-groove nebulizer with an argon inlet of 70 microns. This nebulizer has been shown (14) to perform well for the introduction of liquids with high percentages of dissolved and suspended solids. The uptake rate was controlled by means of a peristaltic pump. Nebulizer clogging was avoided by using suitable uptake and rinsing cycles.

A vibrational-type Spex Model 5000 mixing mill with a porcelain cylinder and spheres was used to grind the samples. Homogenization of the slurries was carried out using a blackstone Model ST-2D ultrasonic device.

Reagents and Materials

All reagents were of analytical reagent grade (Merck).

Standard solutions were prepared from 1000 µg/mL stock solutions (BDH) of each element.

Deionized water (Milli-Q grade) was used to prepare samples and standards.

Pine needles and spinach leaves were analyzed in this work.

Biological standard reference material NIST (National Institute of Standards and Technology): SRM 1572 Citrus Leaves and SRM 1573 Tomato Leaves were used to test the accuracy of the proposed method.

Slurry Preparation

The sample treatment for preparation of the slurries was the same as that previously reported (16). The leaf samples were washed repeatedly with double-distilled water and oven-dried at 80°C for six hours. They were then ground in a mixing mill for 15 minutes. For particle size studies, the plant tissue samples were put into a series

of sieves and the different size fractions isolated by manual agitation. The particle size ranges were 154-103, 103-74, 74-44, and 44-27 µm. Particles smaller than 27 µm were not obtained. Slurry solutions (0.2% m/V) were prepared in water for each fraction and used to assess the effect of particle size on emission signal.

Particle size fractions of less than 74 µm were used to prepare the slurries for quantitative analysis. Slurries were prepared by weighting an appropriate amount (50-150 mg) of sieved sample and suspending it in 25 mL of 0.01 M nitric acid solution. Slurries were stirred by ultrasonic agitation before measurements.

Wet Acid Digestion of Sample

Leaf samples were dissolved using the procedure reported previously (16). Approximately 0.2 g of sample was weighed and digested with a mixture of 3 mL HNO₃-H₂SO₄ (2:1). Then, H₂O₂ (1 mL) was

TABLE I
Instrumental Parameters

Spectrometer	Spex 1.26 cm (Model 1269) with holographic grating, 3600 lines/mm, 0.2 nm/mm
RF generator	Durr Model 8100, maximum output 5 kW operating at 64 MHz
Forward power	1.2 kW Plasma torch Demountable assembly with Mermet-Trassy configuration (18)
Nebulizer	V-groove (14)
Spray chamber	Scott-type
Sample delivery	Peristaltic pump (Sorvall, Type 49061); forced feeding 1.5 mL/min
Microcomputer	Hewlett Packard Model HP-86B
Nebulizer flow rate	0.6 mL/min
Coolant flow rate	15 L/min
Observation height	15 mm above load coil
Analytical lines	Mn(II) 259.37 nm Mg(II) 279.53 nm Ca(II) 393.37 nm

added. This mixture was heated at 80°C for 2-3 hours and diluted to a final volume of 100 mL. The accuracy of the dissolution method was verified by analyzing SRM 1572 and SRM 1573 using atomic absorption spectrometry (AAS) as described earlier (16-18).

RESULTS AND DISCUSSION

Influence of Particle Size on Atomization

Our previous report (16) on AAS determination of trace elements in plant tissues revealed that the stability of the slurry depended on its particle size. For these materials, sieved particle sizes greater than 160 μm were not used because of the agglomeration produced by the particles. Therefore, slurries of sieved samples with particle sizes in the ranges of 154-103, 103-74, 74-44, and 44-27 μm were prepared (2 mg/mL) and measured to assess the influence of particle size on the emission signal.

The results for Mg, Mn, and Ca for the pine needle and spinach leaf samples are shown in Figure 1. It can be seen that the analyte signal generally increases with decreasing particle size. The magnitude of this effect depends on the element. Signal variation of Ca and Mg is higher than that shown by Mn. It is also observed in Figure 1 that for the sieved fraction of less than 74 μm , a maximum signal is obtained for both samples. This seems to indicate that slurries of leaf tissue with a particle size of less than 74 μm contain a high concentration of fine particles that are small enough to be efficiently transported through the sample introduction system to the plasma.

Unfortunately, we did not have a measurement system for the determination of particle size distribution. However, it has been reported (19) that for bituminous coals, ground with a swing mill and sieved

with a sieve opening of 44 μm , a median particle diameter average of 11 μm was measured.

Particles less than 74 μm were chosen to prepare the slurries throughout this work. They produced emission signals whose intensities were similar to those signals produced by the particles of less than 44 μm .

Effect of Acid Concentration on Slurry Atomization

It has been reported (16-18) that the presence of acids improved the atomization efficiency of slurries. In

order to determine the optimum acid concentration, the intensity of the emission signal for Mg, Ca, and Mn was observed by suspending 0.2% (m/V) of the sample at different concentrations of nitric acid. The acid concentration varied from 10^{-6} to 10^{-1} M (pH 6 to 1). The results of this study are shown in Figure 2. It can be seen that the emission intensity signal generally increases with the acid concentration, reaching a maximum around pH 2 for spinach leaf samples and around pH 3 for pine needle samples. No emission signal was

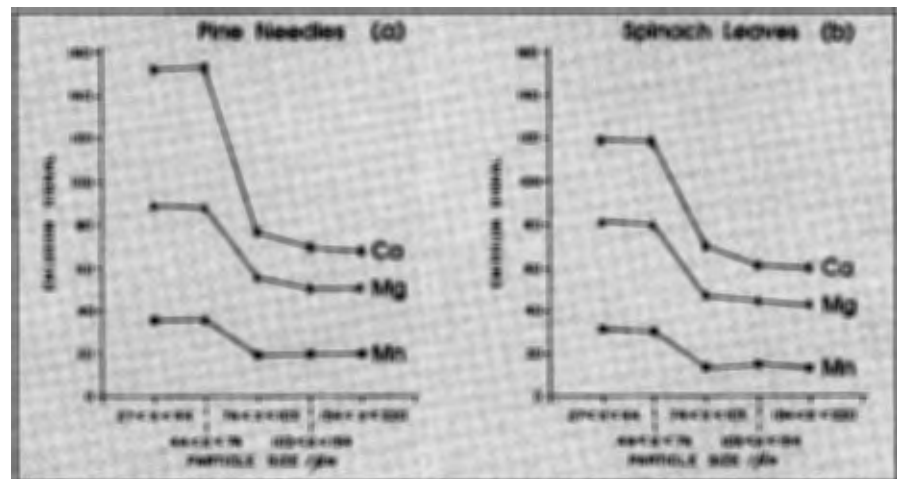


Fig. 1. (a and b). Emission signal for Mn, Mg, and Ca as a function of particle size for pine needles and spinach leaves.

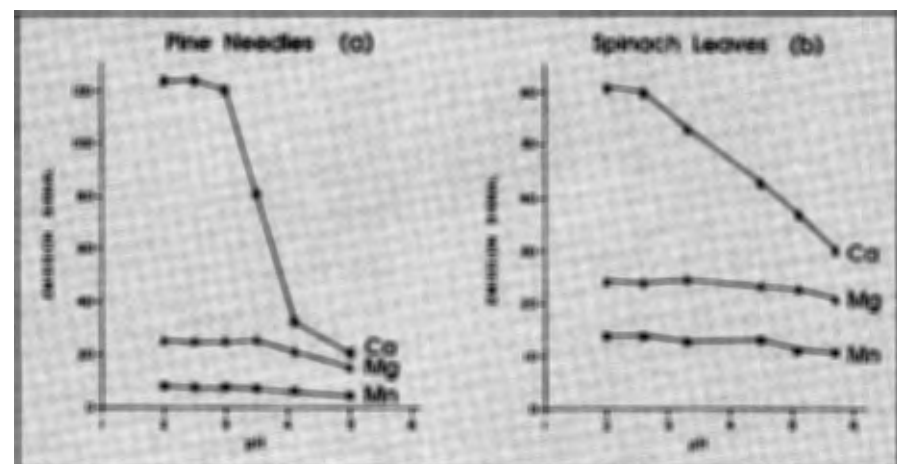


Fig. 2. Effect of acid addition on slurry atomization for pine needles and spinach leaves for Mn, Mg, and Ca.

observed when the supernatant was analyzed after centrifugation of the slurries. It can be said, therefore, that an extraction of the analytes is not taking place. Maximum acid dependency was observed for Ca.

Figure 3 shows that the emission intensity of all particle size fractions was increased after adding 0.01 M nitric acid. The highest enhancement of the signal was observed for the fractions of less than 74 μm . It can be said that the acid acts as a dispersant, neutralizing the charges, and also as a stabilizer, improving the efficiency of the slurry transport and atomization.

Effect of Acid on Solution Atomization

The efficiency of nebulization can be altered by changes in acid concentration from one solution to another. The depressive effect on line intensities with increasing acid strength has been observed (20). Yoshimura et al. (21) showed that aspiration of mineral acids alters the physical state of the plasma. They also observed that the excitation temperature decreased in the presence of mineral acids. In order to ascertain whether an interference effect from the acid (used during sample digestion) may be present, solutions containing 1 $\mu\text{g}/\text{mL}$ Mn, 5 $\mu\text{g}/\text{mL}$ Mg, and 10 $\mu\text{g}/\text{mL}$ Ca in an $\text{HNO}_3\text{-H}_2\text{SO}_4\text{-H}_2\text{O}_2$ (2:1:1) mixture were studied in the range of 1-5% (v/v). The results of this study show that Mn and Mg signal intensities were not significantly different in the acid concentration range studied. However, a depression of line intensity was obtained for Ca with increasing acid concentration. A minimum emission signal intensity was obtained at the acid concentration used during sample digestion (Figure 4). These results suggest that the acid effect could not be attributed to the increasing viscosity of the solution because the effect was not similar for all elements.

Figure 4 also shows the effect of the individual acids (HNO_3 and H_2SO_4) on the emission signal of Ca. The same acid concentrations were used. It was observed that the emission intensity decreased with in-

creasing H_2SO_4 concentration. However, a slight decrease was obtained with HNO_3 . These results are contradictory to those obtained by Yoshimura et al. (21) who found a similar depression for both acids.

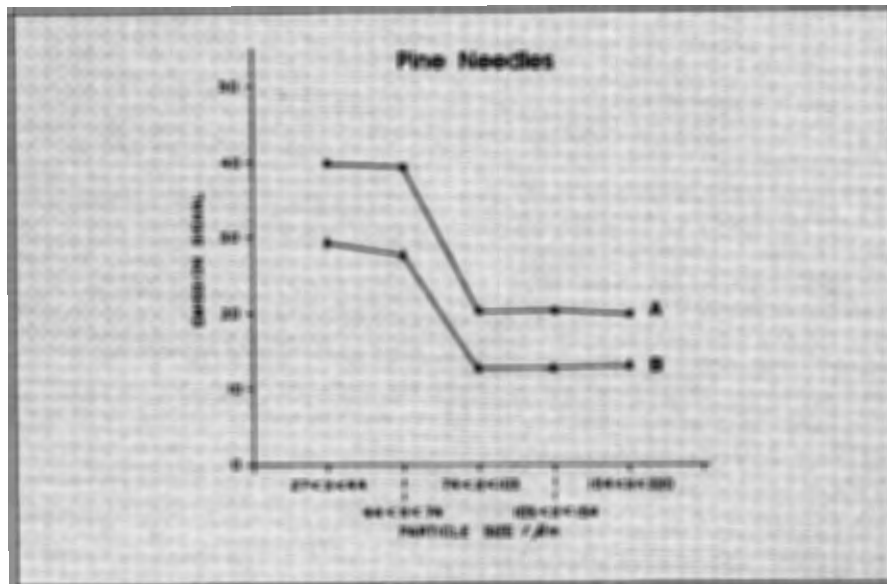


Fig. 3. Emission signal of Mn(II) at the 259.37-nm line as a function of particle size for pine needles (A) with acid and (B) without acid.

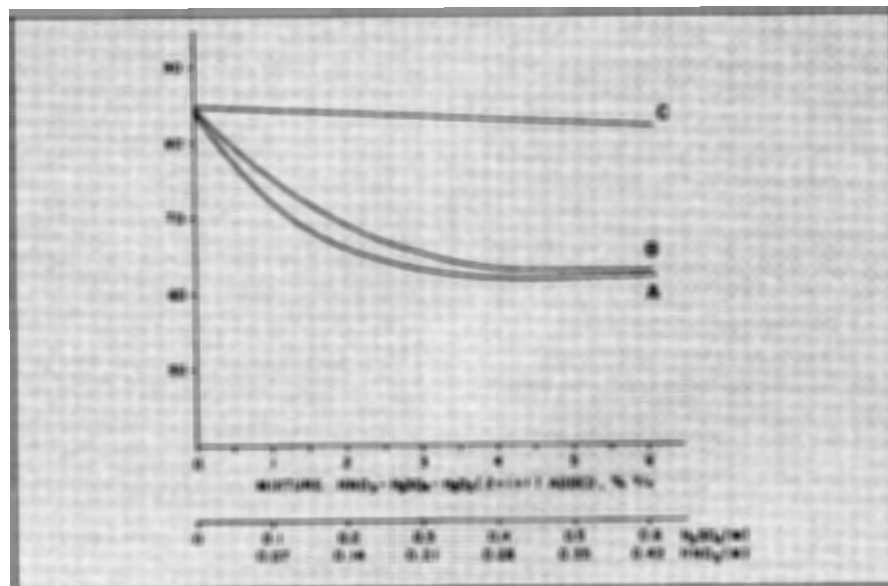


Fig. 4. Effect of acid addition on atomization of the solution for Ca in (A) acid mixture, (B) sulfuric acid, and (C) nitric acid.

Effect of Slurry Concentration

Slurries of the sieved material of less than $74\ \mu\text{m}$ can be prepared up to $10\ \text{mg}$ of solid/mL. Slurry concentrations greater than this lead to flocculation; thus, they must be kept in suspension by continuous stirring during nebulization. Optimization of the slurry concentration is shown in Figures 5 and 6. The plots show the effect of varying the concentration of the slurries on the Mg and Ca signals. A linear response is obtained for slurry concentrations in the range of 0.2–1.0% (m/V) for both elements. In comparison to the slopes obtained, the suspended and the digested samples lead to the same slope for Mg. A similar result was obtained for Mn. However, Ca in a dissolved sample shows a slope much lower than that obtained by suspension (Figure 6). This may be due to the interferences produced by the presence of the acidic matrix.

A further result derived from this study is that aqueous calibration standards can be used to quantify these elements in this type of suspended material. Also, it avoids use of certified reference materials, sometimes not commercially available and costly. Regarding Ca determination, the interference observed from acids (HNO_3 - H_2SO_4) can be avoided by using slurry atomization and calibration can be carried out using aqueous standards.

Comparison of Slurries and Standard Acid Digestion Procedures

A comparison study of both methods was carried out for the pine needle and spinach leaf samples. Table II gives comparative results for samples analyzed by slurry and by the analysis of solutions using ICP-AES. It can be seen that the precision obtained by the slurry method is similar and sometimes slightly better than that obtained by conventional acid digestion procedures. Good

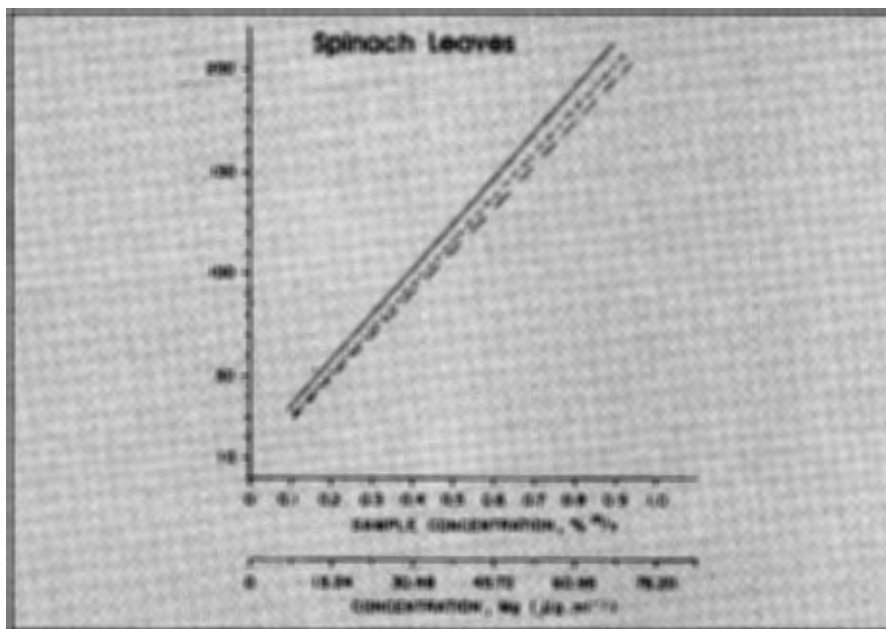


Fig. 5. Emission signal of the Mg(II) 279.553-nm line vs. sample concentration for spinach leaves.

- Slurry
- - - Dissolution (wet acid digestion)
- · · Aqueous standards

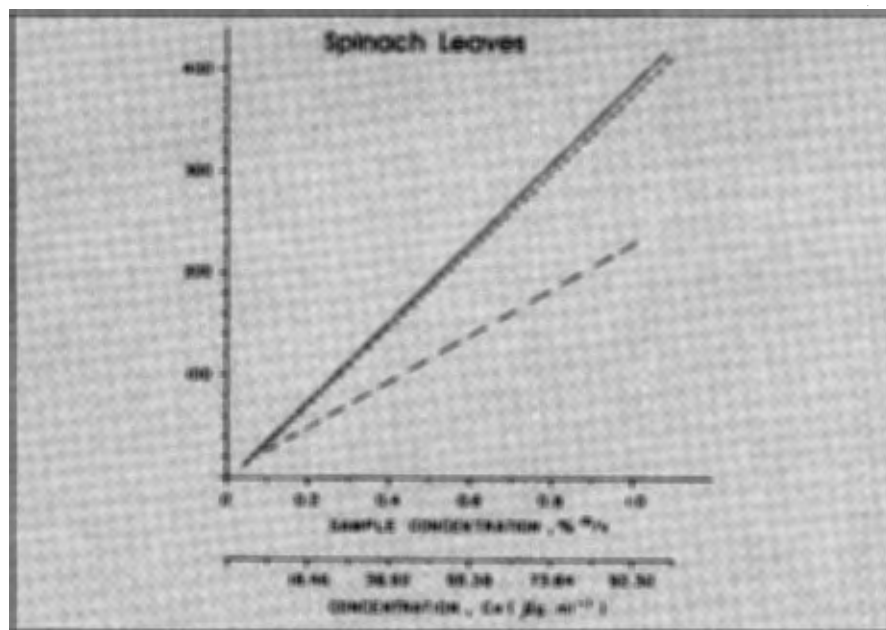


Fig. 6. Emission signal of the Ca(II) 393.366-nm line vs. sample concentration for spinach leaves.

- Slurry
- - - Dissolution (wet acid digestion)
- · · Aqueous standards

agreement between the two methods was obtained. The Student *t*-test and the *F*-test (22) were applied to the results. For a confidence level of 95%, the results showed that there was no statistically significant difference between the mean of both populations.

Analysis of SRM Materials by the Slurry Method

In order to assess the accuracy of the slurry method, two NIST certified materials were analyzed: SRM 1572 Citrus Leaves and SRM 1573 Tomato Leaves. Aqueous standard solutions were used for calibration purposes. The results are shown in Table III. Significance tests (22) indicated that no significant difference at the 95% confidence level was observed.

CONCLUSION

The results illustrate the feasibility of applying the slurry technique to the analysis of plant tissue samples and similar materials with ICP-AES.

Statistical evaluation of the results obtained by direct slurry analysis, calibrated against aqueous standards with no internal standard included, indicates that the method is capable of accurate and precise determinations and is comparable with those obtained by traditional wet ashing procedures.

Similarly to AAS, the precision is a more meaningful parameter when a typical slurry is analyzed. The precision of the method, indicated by % RSD, was less than 2%.

The results illustrate that with the method described, the matrix

TABLE II
Comparative Results – Solution ICP-AES vs Slurry ICP-AES

Sample	Mn		Mg		Ca	
	Concn (% m/m)	RSD (%)	Concn (% m/m)	RSD (%)	Concn (% m/m)	RSD (%)
Pine Needles						
Wet ashing	327	2	0.208	2	0.436	2
Slurry	316	2	0.200	0.5	0.435	1
Spinach Leaves						
Wet ashing	723	1	0.762	1	0.923	1
Slurry	710	1	0.773	1	0.934	0.5

Errors given for a confidence level of 95%.

TABLE III
Analysis of SRM Materials by Slurry Method and Calibration With Aqueous Standards

Element	NBS 1572 Citrus Leaves		NBS 1573 Tomato Leaves	
	Certified	Found	Certified	Found
Manganese (µg/g)	23±2	—	238±7	220±10
Magnesium (% m/m)	0.58±0.03	0.53±0.03	(0.7) ^a	0.53±0.02
Calcium (% m/m)	3.15±0.10	2.99±0.03	3.00±0.03	2.83±0.09

^a Uncertified value.

matching procedure can be avoided. Acid interference, such as found for Ca determination, can therefore be eliminated.

The acid added to the slurry as “dispersing agent” has an important role in the stabilization of the slurry.

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