JAAS Journal of Analytical Atomic Spectrometry

Flow-through Microwave Digestion System for the Determination of Aluminium in Shellfish by Electrothermal Atomic Absorption Spectrometry

MARCO A. Z. ARRUDA, MERCEDES GALLEGO AND MIGUEL VALCÁRCEL*

Department of Analytical Chemistry, Faculty of Sciences, University of Córdoba, E-14004 Córdoba, Spain

A flow-injection system incorporating a microwave oven was constructed for the digestion of food samples for the subsequent determination of aluminium by electrothermal atomic absorption spectrometry. A volume of 100 µl of sample slurry was simultaneously injected with 200 µl of 3 mol l^{-1} HNO_3 and transferred into a PTFE reactor (coiled around an Erlenmeyer flask filled with water) located inside a microwave oven. The digested sample and flushing solution were collected in an autosampler cup. The sample was diluted 20-fold within the manifold. The performance of the procedure was evaluated by determining aluminium in an oyster tissue reference material. The recovery of aluminium was about 90%; however, the results for replicate analyses showed no significant differences. The proposed method was applied to the determination of aluminium in shellfish.

Keywords: *Aluminium determination; shellfish analyses; electrothermal atomic absorption spectrometry; continuous microwave digestion*

The dramatic recent advances in analytical instrumentation have not been accompanied by significant changes in sample preparation methods. Trace analyses usually require acid digestion, which is labour-intensive and time-consuming (about 70–95% of the time devoted to the over-all analytical process); in addition, manual digestions are prone to material losses and/or contamination, subject to potential health hazards and occasionally expensive (particularly when ultrapure acids are needed). Not surprisingly, several workers have developed specific procedures for opening acid digestion beakers[1] and closing Teflon digestion containers.[2] Microwave digestion, as a heat source, is an effective alternative to conventional procedures as it reduces digestion time and problems associated with analyte losses and atmospheric contamination. As a result, the first application of this procedure[3] was soon followed by a host of uses involving a variety of sample types.[4–8] Notwithstanding these advantages, the manual volumetric transfer, addition of reagents (*viz.*, chemical modifiers) and dilution involved are still error-prone and the source of contamination and material losses. Many of these problems can be overcome or minimized by incorporating the microwave digestion device into a flow-injection (FI) system.

Continuous-flow systems including an on-line microwave digestor have been used in combination with a flame atomic absorption[9–11] or inductively coupled plasma atomic emission[12,13] spectrometer; however, only two such applications using electrothermal atomic absorption spectrometry (ETAAS) have so far been reported.[14,15] The simplest configuration of this type is that proposed by Burguera *et al.*;[9] the sample assayed was liquid (whole blood) and mineralization in the microwave oven only required dilute acids (100 µl of 0.3 mol l^{-1} HCl plus 0.4 mol l^{-1} HNO_3) and took only 1 min. Solid samples such as slurries call for more sophisticated flow systems because samples must be digested with highly concentrated acids, which rapidly destroy organic matrices in microwave ovens through an excessive gas pressure. The mineralization conditions used should allow for adequate de-gassing of the mineralized sample. For this purpose, various devices including several injection valves, back-flush filters, back-pressure regulators and antifreeze cooling baths have been tested in flow systems;[10,12,13] other configurations include a liquid–gas phase separator or an ice chamber.[14,15] Digesting the sample also takes some time, using a long coil (20 m)[10] or retaining the sample (2–5 min)[12,13] inside the microwave oven. A closed-flow system has also been used for on-line digestion in 2–4 min; the use of a cooled sample cell permits de-gassing of the samples after microwave irradiation, with good results.[11]

The aim of the present work was to develop a simple, fast procedure for mineralizing solid food samples as slurries. The flow system uses a small reactor located in a household microwave oven in which the slurry samples and acid solution are digested for a few minutes. The performance of the method was evaluated by determining aluminium in shellfish, which typically contains abundant organic matter, using ETAAS detection.

EXPERIMENTAL

Apparatus

A Perkin-Elmer (Überlingen, Germany) 1100-B atomic absorption spectrometer with deuterium arc background correction equipped with an HGA-700 graphite furnace and an AS-70 autosampler was used. The aluminium hollow cathode lamp was operated at 25 mA (wavelength, 309.3 nm; spectral bandpass, 0.7 nm). Pyrolytic graphite coated graphite tubes (Perkin-Elmer part No. B-013-5653) and L'vov platforms (Perkin-Elmer part No. B-012-1091) were used. The graphite furnace programme is shown in Table 1. Argon was used as the inert gas in all experiments. Integrated absorbance (peak area) readings, printed on an Epson (Wembley, UK) FX-850 printer, were used to evaluate the results. The FI system consisted of

Table 1 Recommended furnace programme*

Step	Temperature/°C	Ramp time/s	Hold time/s	Read
Dry 1	100	10	35	
Dry 2	200	15	15	
Pyrolysis 1	800	10	5	
Pyrolysis 2	1700	15	15	
Atomize	2500	0	4	–1
Clean	2650	1	1	

* Purge gas, argon; flow rate, 300 ml min^{-1} (flow stopped during atomization step); injected volume, 20 µl sample + 10 µl modifier.

* To whom correspondence should be addressed.

a Gilson (Villiers-Le-Bel, France) Minipuls-2 peristaltic pump fitted with poly(vinyl chloride) tubes, a laboratory-made three-piece injector commutator[16] with built-in T-shaped Perspex connectors and PTFE transmission lines of 0.8 mm id and a household microwave oven (AEG Micromat Duo, Madrid, Spain) equipped with a magnetron of 2450 MHz with a nominal maximum power of 800 W as marketed. The coil reactor was introduced through the vent holes of the microwave oven in order to avoid drilling of the walls.[11]

A household electric mixer (Moulinex R-32, Madrid, Spain) was used to grind samples to a particle size below 200 μm, and an ultrasonic bath (Bandelin-Tk 52, Berlin, Germany) was employed to homogenize the slurry samples prior to injection into the flow system.

Chemicals

All reagents were of analytical-reagent grade (Merck, Darmstadt, Germany) and high-purity water (Milli-Q Water System, Millipore, Madrid, Spain) was used throughout. A 1000 mg l^{-1} aluminium stock solution was prepared by dissolving 1.000 g of aluminium wire in 20 ml of concentrated H_2SO_4 plus 50 ml of concentrated HNO_3, and diluting to 1 l with water. Working standard solutions containing 100–1000 μg l^{-1} of aluminium were prepared from the stock by serial dilution with 0.2% v/v HNO_3 prior to use. These standard solutions were diluted 20-fold inside the FI manifold. The chemical modifier, 0.01 mol l^{-1} $Mg(NO_3)_2$, was dissolved in water.

Material and Cleaning

Reagents were stored in polyethylene containers (glass vessels were ruled out in order to minimize aluminium release and adsorption). All vessels were cleaned by soaking in 10% v/v HNO_3 for 48 h, rinsing five times with Milli-Q water and filling with ultrapure water until use.[17]

Sample Preparation

Fresh shellfish samples were frozen for 2 d, after which they were readily shelled without thawing. The shelled samples were allowed to thaw at room temperature on filter-paper and then ground using the mixer. The resulting slurry was lyophilized by freeze-drying at 6 Pa for 72 h, after which an accurately weighed amount of about 100 mg was mixed with 25 ml of 0.2% v/v HNO_3. The slurry thus formed was shaken in an ultrasonic bath for 10 min in order to homogenize solid particles in the solution prior to introduction into the FI system.

Standard Reference Material (SRM) 1566a Oyster Tissue (National Institute of Standards and Technology, NIST) was employed throughout to check the recovery, accuracy and precision of the proposed method. The SRM was dried at a pressure of 3 Pa for 20 h, following the supplier's recommendations.

Procedure

The proposed FI system for the on-line digestion of samples is depicted in Fig. 1. In the position shown in the figure, both loops, holding 100 μl of standard (containing 100–1000 μg l^{-1} of aluminium) or sample slurry and 200 μl of digesting solution (3 mol l^{-1} HNO_3), were inserted into the carrier stream (0.2% HNO_3) at a flow rate of 0.25 ml min^{-1} and driven to the merging point (X); the mixed solution was then led to the PTFE digestion coil (200 cm long, 0.8 mm id) located inside the microwave oven. The coil was wrapped around an Erlenmeyer flask filled with water. About 1.5 min after sample injection, when the sample plug was inside the digestion coil,

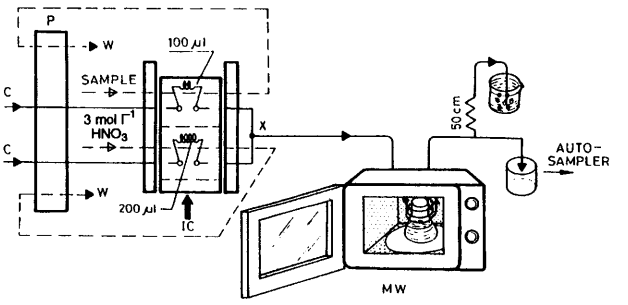

Fig. 1 FI system for the wet digestion of shellfish slurry samples: C, carrier solution; P, peristaltic pump; IC, injector commutator; W, waste; MW, microwave oven; r, restrictor. Flow rate of carrier (0.2% HNO_3), 0.25 ml min^{-1}

the microwave oven was switched on at 800 W for 2 min; the sample was then collected in an autosampler cup for 2 min until the latter was completely filled (total volume, 2 ml). The sample was thus diluted 20-fold. A 50 cm long × 2 mm id restrictor inserted in the FI system prior to the point where digested sample was collected removed any gas produced during the digestion step. The restrictor was immersed in a beaker filled with water in order to reduce atmospheric contamination. The over-all time required for digestion and dilution of the sample after injection was about 5 min. The chemical modifier was 0.01 mol l^{-1} $Mg(NO_3)_2$ and was placed in another autosampler cup. Blanks of 0.2% HNO_3 were also inserted into the FI system and digested similarly to the sample. Each measurement was made in triplicate.

RESULTS AND DISCUSSION

Optimization of the Digestion Conditions

In a preliminary test, direct introduction of a slurry of the NIST SRM 1566a Oyster Tissue resulted in a recovery of only 40%; hence such a direct insertion procedure was ruled out.

The digestion conditions or degree of dissolution in the microwave oven were governed by three variables, namely, acid concentration, digestion time and microwave power. Hence, the interaction of the acid solution and microwave energy with the slurry sample influences the destruction of organic matter, as demonstrated by several workers.[4–6] Dilute acids absorb microwave power more strongly than do concentrated acids; the difference is attributed to the larger fraction of water present in more dilute acids.[18] On the other hand, high acid concentrations expedite the attack of sample matrices in microwave ovens, but tend to produce excessive gas pressure. Because equilibrium is never reached in flow systems, completely avoiding uncontrolled high pressures within the manifold is difficult.[15] In addition, sample digestion should be achieved with the lowest possible acid mixture concentration and shortest digestion time in order to minimize potential hazards.

Food mineralization can be accomplished with various acids including HNO_3, HCl, H_2SO_4 and $HClO_4$. We rejected H_2SO_4 because it attacks PTFE at high temperatures;[14] $HClO_4$ was also ruled out because it is potentially explosive in contact with organic materials. The most frequently used acids for digesting foods in microwave ovens are HCl and HNO_3.[13,15] Hydrochloric acid must also be discarded for the determination of aluminium because it interferes in the vapour phase.[19,20] Nitric acid was therefore selected for attacking the matrices used in this work.

The NIST SRM 1566a Oyster Tissue (certified aluminium content, 202.5 ± 12.5 μg g^{-1}) and the flow system depicted in Fig. 1 were used to optimize the proposed method. The

digestion coil, 200 cm × 0.8 mm id, was wrapped around an Erlenmeyer flask filled with water (to prevent the magnetron from operating under dry conditions). The effect of the HNO_3 concentration was studied over the range 0.03–0.3 mol l^{-1} by injecting 200 µl of the acid simultaneously with 100 µl of sample slurry. The microwave power was set at 425 W; the oven was switched on for about 1.5 min once the sample plug was located inside the digestion coil in all instances. If the oven was kept on for a longer time, the sample/acid plug was largely segmented by the gases produced and the sample dispersed beyond the bounds of the digestion coil; hence it left the microwave. This problem could not be avoided by using a longer digestion coil, as this entailed circulating the carrier solution (0.2% HNO_3) for a longer time in order to sweep the whole sample and flush the manifold, which resulted in a final collected sample volume exceeding the capacity of the autosampler cups. The problem is usually solved by including several injection valves and back-pressure regulators. In order to avoid complicating the assembly, it was decided to switch the oven on once the sample was in the digestion coil. The influence of the acid concentration was studied by retaining the sample in the oven for different periods (0, 2, 4 and 6 min) in order to optimize the digestion time. Halting the flow for more than 2 min had no significant effect at a power of 425 W. However, the recovery of aluminium was only about 70%. Next, the oven power was varied over the range 170–800 W and the HNO_3 concentration between 0.03 and 3 mol l^{-1} (the sample flow was not halted, however, because the sample/acid plug overflowed the digestion coil inside the oven above 600 W). Because these two variables (acid concentration and oven power) were the most influential on the recovery of aluminium from the SRM, their effect was plotted graphically as a response surface (Fig. 2). As can be seen, the effect of both variables was closely related. The optimum HNO_3 concentration was above 2 mol l^{-1} and the optimum oven power was above 600 W; an HNO_3 concentration of 3 mol l^{-1} and a power of 800 W, with no stopping of the sample inside the microwave oven, were selected for further experiments. Under these conditions, the recovery of aluminium from the SRM was about 90%.

Optimization of the Flow System

The FI system was optimized using the univariate method. The slurry of the SRM was injected simultaneously with 3 mol l^{-1} HNO_3 into the carrier solution (0.2% HNO_3 at 0.25 ml min^{-1}); the mixture was then passed through the microwave oven (2 min at 800 W), which was switched on once the sample plug was located inside it (after 1.5 min). The mineralized sample and washing solution (carrier, 0.2% HNO_3) were collected in an autosampler cup. The delivery of an effluent volume of 2 ml was checked gravimetrically by using a standard of 500 µg l^{-1} that was introduced into the digestion system. The relative standard deviation (s_r) thus obtained was about 3% ($n=5$). The peak area was measured using aluminium standards in 0.2% HNO_3, also treated in the microwave oven.

Injected volumes of food sample slurry used for on-line microwave digestion range from 100 µl[11] to 20 ml.[12] Obviously, the injected volume influences the digestion efficiency and hence the accuracy and precision of the results. However, the volume could not be raised at will because large acid and sample loops increased the pressure inside the FI system and resulted in undesirable gas evolution. The effect of the injected volume of sample slurry was therefore studied by using a constant volume of 200 µl of 3 mol l^{-1} HNO_3 and varying the sample volume between 50 and 200 µl. For this purpose, four slurries of NIST SRM 1566a Oyster Tissue were prepared by accurately weighing an appropriate mass of 200, 100, 65 or 50 mg of solid and mixing it with 25 ml of 0.2% HNO_3 to obtain a 0.8, 0.4, 0.26 or 0.2% m/v slurry, respectively. The selection of the above slurry proportions was dictated by the need for the amount of aluminium in the injected volume to remain constant at 50 (0.8%), 100 (0.4%), 150 (0.26%) and 200 µl (0.2%), respectively. The recoveries ($n=5$) of aluminium thus achieved were similar in all instances, namely: 90.7 ($s_r=5.7\%$), 91.4 ($s_r=2.0\%$), 91.0 ($s_r=2.3\%$) and 91.8 ($s_r=2.5\%$) for an injected volume of 50, 100, 150 and 200 µl, respectively. An injected volume of 100 µl was therefore selected in order to ensure that the slurry concentration, 0.4%, was not too low; in addition, the sample plug, 100 µl, was intercalated between that of the acid, 200 µl, thereby favouring digestion. The dimensions of the digestion coil were not optimized because, as stated above, no stopping of the sample/acid plug inside the microwave oven was needed at the maximum power of the oven. Hence, a digestion coil of 200 cm × 0.8 mm id was selected because longer coils increased the dispersion of the sample plug and required larger volumes of washing solution, which resulted in higher sample dilution.

Preliminary experiments intended to determine the potential influence of the flow rates of the carrier (0.2% HNO_3), slurry and acid solutions on the digestion process revealed that low flow rates (*viz.*, 0.3 ml min^{-1}) provide better recoveries as a result of the sample/acid plug being slowly transferred into the digestion coil, thereby increasing the dissolution efficiency in the microwave oven. The carrier flow rate chosen was 0.25 ml min^{-1}, so the sample/acid mixture was circulated through the system at a flow rate of 0.5 ml min^{-1}.

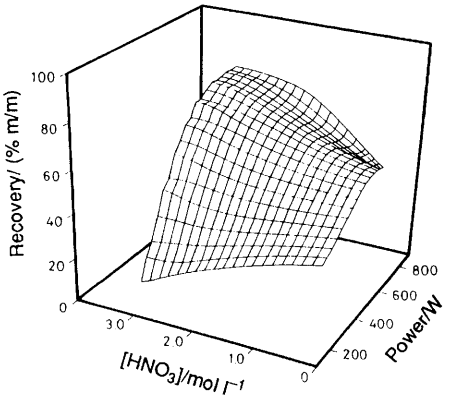

Fig. 2 Response surface for the recovery of aluminium in NIST SRM 1566a Oyster Tissue at variable digesting acid concentrations and microwave oven power

Analytical Figures of Merit

Under the previously established optimum conditions (Table 1), the linear portion of the calibration graph, obtained using the manifold depicted in Fig. 1, aqueous standard solutions and 0.01 mol l^{-1} $Mg(NO_3)_2$ as the chemical modifier ($r>0.997$; $n=7$), ranged from 100 to 1000 µg l^{-1} of aluminium (5.0–50.0 µg l^{-1} of aluminium after 20-fold dilution in the FI system). The background signal was very small (0.010–0.030 s) and was corrected for background noise. The detection limit, calculated according to IUPAC recommendations,[21] was 10 µg l^{-1} of aluminium. The characteristic mass, determined for a standard solution containing 400 µg l^{-1} of aluminium, was 200 pg per 0.0044 s for the diluted system. The precision was calculated by analysing NIST SRM 1566a Oyster Tissue in duplicate, both within-run [$s_r=4.3\%$ as repeatability ($n=12$)] and between-day [$s_r=14.4\%$ as reproducibility ($n=10$)].

The accuracy of the proposed method was validated by applying it to NIST SRM 1566a Oyster Tissue. For this

Table 2 Aluminium contents ($\mu g\ g^{-1}$)* in lyophilized shellfish samples as determined by FI-ETAAS ($n=5$)

Sample		Aluminium/$\mu g\ g^{-1}$
NIST SRM 1566a	Oyster Tissue†	201.8 ± 4.9
	Oysters	191.7 ± 3.2
	Cockles	140.2 ± 10.3
	Clams	242.8 ± 14.0
	Prawns	175.3 ± 8.8
	Mussels	117.5 ± 8.6

* Mean $\pm$ standard deviation.
† Certified value: $202.5 \pm 12.5\ \mu g\ g^{-1}$.

purpose, an accurately weighed amount of about 100 mg of the SRM was mixed with 25 ml of 0.2% v/v HNO_3. The average content found in five consecutive determinations on independent test portions of the SRM was $181.6 \pm 4.4\ \mu g\ g^{-1}$. The recovery of aluminium from the SRM was about 90%, because the matrix material was incompletely decomposed. Karanassios *et al.*[12] obtained recoveries of aluminium from botanical samples and bovine liver using microwave digestion that were below 100% because the element is intrinsically difficult to digest (recoveries for other elements including Ca, Cu, Fe, Mg, Pb and Zn in the same samples were about 100%). These workers showed that the recovery of aluminium by use of *aqua regia* increased with the digestion time beyond 16 min. They used a correction factor for this element that provided an acceptable degree of confidence. In order not to complicate the proposed experimental set-up or lengthen analyses unduly, we also used a correction factor for aluminium in the SRM because replicate determinations resulted in no significant differences; the corrected value, $201.8 \pm 4.9\ \mu g\ g^{-1}$, was consistent with the certified value.

Selectivity

The presence of major potential interfering ions usually included in NIST SRM 1566a Oyster Tissue was investigated. For this purpose, a standard containing $400\ \mu g\ l^{-1}$ of aluminium was employed. The ion concentrations assayed were $7\ mg\ ml^{-1}\ Na^+$ (nitrate), $3\ mg\ ml^{-1}\ Ca^{2+}$ (nitrate), $10\ mg\ ml^{-1}\ K^+$ (nitrate), $10\ mg\ ml^{-1}\ Cl^-$ (ammonium salt), $10\ mg\ ml^{-1}\ S$ ($CuSO_4$) and $8\ mg\ ml^{-1}\ P$ (KH_2PO_4). Magnesium was not studied because it was employed as the chemical modifier. If the presence of a given species resulted in a change by more than $\pm 10\%$ in the standard measurements, it was considered to interfere significantly. No interference was observed in any instance; however, the differences between the analytical responses (-9.6 to $+10.2\%$) were fairly small. Therefore, none of these ions interferes significantly with the determination of aluminium at concentrations above those typically present in shellfish.

Analysis of Real Samples

Five fresh shellfish samples were analysed, after lyophilization for water removal, by using the proposed FI system. The same amount of lyophilized shellfish slurry (100 mg in 25 ml of 0.2% HNO_3) was used in order to ensure that the analytical signal fell inside the linear working range of the instrument (after 20-fold dilution in the FI system). The results, expressed in $\mu g\ g^{-1}$ of aluminium, and their precision ($n=5$, standard deviation) are summarized in Table 2. As the recovery of aluminium from the SRM was about 90% and the samples analysed had similar matrices, the same correction factor was also used for the results given in Table 2. The results reveal that clams and oysters contain higher aluminium concentrations than do mussels.

CONCLUSIONS

The proposed method allows rapid preparation of shellfish slurry using on-line microwave digestion. However, the difficulty involved in digesting aluminium imposed a maximum recovery of about 90%, which entailed using a correction factor in order to determine the real amounts of metal in the samples. This is no hindrance as total digestion of the sample is unnecessary provided that reproducible recoveries are obtained; furthermore, a short sample preparation time is more important. The combined sample preparation and analysis time for one sample was about 20 min. Any problems associated with pressure changes during the digestion of food samples were avoided by collecting the digested sample in an open autosampler cup; hence, the FI system was not coupled on-line to the ETAAS instrument. The proposed method is simpler than other on-line digestion procedures for slurry samples (no gas diffusion cell, ice chamber, multiple valves or special devices are necessary); it also permits the sequential and economical treatment of different samples.

The Spanish CICYT is acknowledged for financial support (Grant PB94-0450). M.A.Z.A. is also grateful to the Brazilian Government (Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq, Brasília, Brazil) and the Spanish Government (Programa de Cooperación Científica con Iberoamérica, Madrid, Spain) for additional funding.

REFERENCES

1 Nadkarni, R. A., *Anal. Chem.*, 1984, **56**, 2233.
2 Fernando, L. A., Heavner, W. D., and Gabrielli, C. C., *Anal. Chem.*, 1986, **58**, 511.
3 Abu-Samra, A., Morris, J. S., and Koirtyohann, S. R., *Anal. Chem.*, 1975, **47**, 1475.
4 Kingston, H. M., and Jassie, L. B., *Anal. Chem.*, 1986, **58**, 2534.
5 Kimber, G. M., and Kokot, S., *Trends Anal. Chem.*, 1990, **9**, 203.
6 Kuss, H.-M., *Fresenius' J. Anal. Chem.*, 1992, **343**, 788.
7 Heltai, G., and Percsich, K., *Talanta*, 1994, **41**, 1067.
8 López, J. C., Reija, C., Montoro, R., Cervera, M. L., and de la Guardia, M., *J. Anal. At. Spectrom.*, 1994, **9**, 651.
9 Burguera, M., Burguera, J. L., and Alarcón, O. M., *Anal. Chim. Acta*, 1986, **179**, 351.
10 Haswell, S. J., and Barclay, D., *Analyst*, 1992, **117**, 117.
11 Carbonell, V., Morales-Rubio, A., Salvador, A., de la Gardia, M., Burguera, J. L., and Burguera, M., *J. Anal. At. Spectrom.*, 1992, **7**, 1085.
12 Karanassios, V., Li, F. H., Liu, B., and Salin, E. D., *J. Anal. At. Spectrom.*, 1991, **6**, 457.
13 Gluodenis, T. J., Jr., and Tyson, J. F., *J. Anal. At. Spectrom.*, 1993, **8**, 697.
14 Burguera, M., and Burguera, J. L., *Lab. Robot. Autom.*, 1993, **5**, 277.
15 Burguera, J. L., and Burguera, M., *J. Anal. At. Spectrom.*, 1993, **8**, 235.
16 Arruda, M. A. Z., Gallego, M., and Valcárcel, M., *Anal. Chem.*, 1993, **65**, 3331.
17 Wilhelm, M., and Onesorge, F. K., *J. Anal. Toxicol.*, 1990, **14**(4), 206.
18 Kingston, H. M., and Jassie, L. B. in *Introduction to Microwave Sample Preparation. Theory and Practice*, eds. Kingston, H. M., and Jassie, L. B., American Chemical Society, Washington, DC, 1988.
19 Bermejo-Barrera, P., Beceiro-González, E., and Bermejo-Barrera, A., *Microchem. J.*, 1992, **45**, 90.
20 Slavin, W., Carnrick, G. R., and Manning, D. C., *Anal. Chem.*, 1982, **54**, 621.
21 Analytical Methods Committee, *Analyst*, 1987, **112**, 199.

Paper 5/00859J
Received February 14, 1995
Accepted April 4, 1995