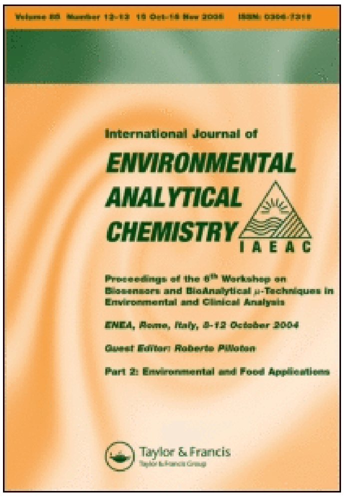

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Continuous ultrasound-assisted extraction of cadmium from vegetable samples with on-line preconcentration coupled to a flow injection-flame atomic spectrometric system

M. Carmen Yebra[a]; Sheila Cancela[a]; Amada Moreno-Cid[a]
[a] Department of Analytical Chemistry, Nutrition and Bromatology, Chemistry Faculty, University of Santiago de Compostela, 15782-santiago de composa, Spain

To cite this Article Yebra, M. Carmen , Cancela, Sheila and Moreno-Cid, Amada(2005) 'Continuous ultrasound-assisted extraction of cadmium from vegetable samples with on-line preconcentration coupled to a flow injection-flame atomic spectrometric system', International Journal of Environmental Analytical Chemistry, 85: 4, 305 — 313

To link to this Article: DOI: 10.1080/03067310412331334826

URL: http://dx.doi.org/10.1080/03067310412331334826

Intern. J. Environ. Anal. Chem.
Vol. 85, No. 4–5, 10 April–20 April 2005, 305–313

Continuous ultrasound-assisted extraction of cadmium from vegetable samples with on-line preconcentration coupled to a flow injection-flame atomic spectrometric system

M. CARMEN YEBRA*, SHEILA CANCELA
and AMADA MORENO-CID

Department of Analytical Chemistry, Nutrition and Bromatology, Chemistry Faculty,
University of Santiago de Compostela, 15782-Santiago de Compostela, Spain

(Received 21 April 2004; in final form 5 November 2004)

A fully on-line flow injection method is described for the quantitative acid extraction of cadmium, preconcentration of this trace metal using a minicolumn containing a chelating resin and its determination by flame atomic absorption spectrometry (FAAS). The on-line acid extraction step was carried out using a continuous ultrasound-assisted extraction system (CUES). The acid extract was preconcentrated on a minicolumn packed with a chelating resin (CHELITE P, with aminomethylphosphoric acid groups), cadmium elution was carried out with hydrochloric acid, and this trace metal was continuously monitored by FAAS. A Plackett–Burman experimental design was used for optimization of the analytical procedure. The method allowed a total sampling frequency of 16 samples per hour, with a relative standard deviation for the complete procedure of 2.1; the detection and quantification limits were 0.014 µg/g and 0.067 µg/g for 60 mg of sample, respectively. The accuracy was verified using a standard reference material (NCS DC 73351 tea leaves). The analytical procedure was applied to the determination of trace amounts of cadmium in real vegetable and fruit samples.

Keywords: Ultrasound-assisted acid extraction; Minicolumn preconcentration; Cadmium; Vegetable samples; Flame atomic absorption spectrometry; Flow injection analysis

1. Introduction

In view of the increase in vegetable consumption in recent years and the concurrent changes in atmospheric pollution levels, the determination of the cadmium content in the most consumed vegetables is of considerable concern from the human health standpoint. The ingestion of high levels of cadmium in foods and drinks can result rapidly in

---

*Corresponding author. Fax: +34-981595012. E-mail: qncayebi@usc.es

International Journal of Environmental Analytical Chemistry
ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310412331334826

feelings of nausea, vomiting, abdominal cramp and headaches. Long-term ingestion of cadmium causes serious renal damage, as well as bone disease leading to brittleness and even collapse of the skeleton [1]. The soils in which plants grow contain metals from a number of sources, principally the rocks from which the soil was formed, as well as fertilizers, waste and other materials added by man.

The analysis of solid foodstuffs such as fruits and vegetables usually requires rather complicated and time-consuming sample preparation procedures. Both losses of test elements and sample contamination may occur during this step, which deteriorates the reliability of the analysis. Moreover, sample preparation substantially increases the duration and cost of the analysis. Wet digestion is the preferred sample preparation procedure for solid foodstuffs. The Analytical Methods Subcommittee recommends the use of a wet digestion with sulphuric acid and hydrogen peroxide [2]. Thus, a number of methods for cadmium determination in vegetables involving a wet digestion were proposed. The long time involved (overnight at 50°C and 2 h at boiling temperature [3,4]) and the use of concentrated acids (nitric acid [3,4], nitric and perchloric acids [5,6]) are the principal disadvantages of these procedures. Despite the use of the microwave digestion, which is fast and effective, the digestion operation entails the utilization of concentrated acids, hydrogen peroxide or their mixtures ($HNO_{3(c)}$–H_2O_2 [7,8] and $HNO_{3(c)}$, $HNO_{3(c)}$–$HClO_{4(c)}$ or $HNO_{3(c)}$–H_2O_2 [8]), which generate carcinogenic nitrous vapours as a consequence of the organic matrix destruction, so a safety control has to be taken into account. If dry ashing is used, the temperature must be kept below 500°C to prevent volatilization losses. This procedure takes a long time (18 h [9] and 28 h [10]).

Ultrasonic radiation is a powerful aid in the acceleration of various steps of the analytical process. This energy is of great help in the pretreatment of solid samples. It also obviates total sample matrix introduction into the nebulizer of the spectrometer, which occurs with solid sampling, slurry sampling and digestion procedures.

The content of cadmium in vegetables has been determined by several techniques, including flame atomic absorption spectrometry (FAAS) [6,10–13], electrothermal atomic absorption spectrometry (ETAAS) [3–5,8,14–16], inductively coupled plasma (ICP) [8] and inductively coupled plasma/mass spectrometry (ICP-MS) [7]. As it is simple and inexpensive, FAAS is a widely used technique for the determination of metallic elements. FAAS, using an air–acetylene flame, has been shown to yield accurate and sensitive results in cadmium determination. Nevertheless, the concentration of metallic ions in samples as fruits and vegetables is often lower than the detection limits attainable with the most sensitive detectors. Therefore, in order to determine trace levels of metals (such as lead, cadmium, chromium and mercury) using FAAS, it is necessary to enrich them first by coprecipitation, extraction or preconcentration on a chelating or ion-exchange resin [6, 12, 13].

In this paper, we describe a simple and continuous ultrasound-assisted extraction system (CUES) connected to an on-line flow injection manifold for acid leaching of cadmium, on-line preconcentration of this trace metal from the acid extract using a chelating resin (CHELITE P, with aminomethylphosphoric acid groups) and its determination by flow injection-flame atomic absorption spectrometry (FI-FAAS). This procedure combines the benefits of ultrasound-assisted extraction procedures and the high sensitivity achieved by the preconcentration processes with the advantages of an FI system. The method was applied to vegetable and fruit samples for the determination of cadmium.

2. Experimental

2.1. *Instrumentation*

A Perkin Elmer Model 5000 atomic absorption spectrometer (Perkin Elmer, Shelton, CT-USA) furnished with a cadmium hollow cathode lamp was used. The instrument was set at 228.8 nm. The spectrometer output was connected to a Perkin Elmer Model 50 Servograph Recorder with a range of 5 mV. The FI system includes two Gilson Minipuls 3 peristaltic pumps (Gilson, France) fitted with Viton tubes, an ultrasonic bath (Selecta, Barcelona), six Rheodyne injection or switching valves (USA), Models 5041 and 5301, a glass minicolumn (100 mm × 3 mm i.d., bed volume 700 μL) (Omnifit, UK). The ends of the minicolumn were plugged with filter paper (Whatman 541) and a Viton minicolumn (120 mm × 1 mm i.d.) packed with 50 mg of chelating resin for the preconcentration step. The ends of the minicolumn were plugged with glass wool.

2.2. *Reagents*

Ultrapure water of 18.2 MΩcm resistivity, obtained from a Milli-Q water purification system (Millipore, Bedford, MA) was used for the preparation of the reagents and standards. Hydrochloric acid, nitric acid (Scharlau Chemie, Barcelona, Spain) and 1000 μg/mL cadmium standard (Merck, Germany) were reagent grade. Chelating resin with aminomethylphosphoric acid groups, Chelite P (Serva Electrophoresis GmbH) and a particle size of 0.3–0.8 mm was used. The certified reference material used was NCS DC 73351 tea leaves (China National Analysis Center for Iron and Steel, Beijing, China).

2.3. *Sample preparation and procedure*

Fruits and some vegetables (carrot, potato and courgette) were peeled. Vegetable and fruits were washed with ultrapure water, cut in small pieces and dried for 5 days at 50°C. Then, the samples were triturated and pulverized in a porcelain mortar, and after sieving, fractions with a particle size less than 30 μm were taken.

The continuous cadmium determination system is shown in figure 1. Vegetable and fruit samples (50–60 mg) were directly weighed into the glass minicolumn. Then, the minicolumn was connected to the CUES. First, the CUES circuit (2 mL) was loaded with the acid leaching solution (3 M nitric acid). Once the CUES circuit was closed, the leaching solution circulates through the minicolumn under ultrasonic energy action at a flow rate of 3.5 mL/min for 2 min. The direction of the flow was changed each 10 s to prevent sample accumulation at the minicolumn end. Then, the switching valve (SV2) was switched to its opposite position, and the acid extract was homogenized in the mixing coil. After this, the acid extract channel converged with a buffer solution stream (16 M ammonium acetate) to obtain a pH value > 3. The mixture was homogenized in a second mixing coil and then passed through the preconcentration minicolumn at a flow rate of 2 mL/min. The cadmium was retained quantitatively as its chelate and then eluted by injection of 92 μL of 3 M hydrochloric acid into a water carrier stream and swept to the detector where cadmium was continuously monitored.

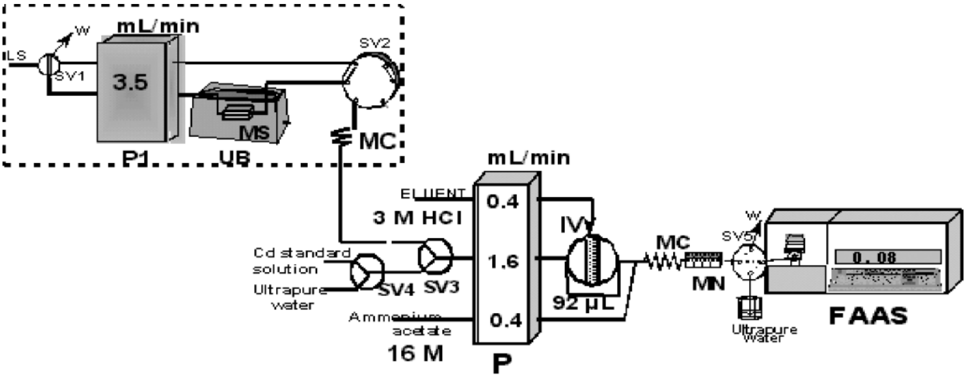

Figure 1. Flow-injection manifold for the whole procedure (CUES and preconcentration devices) for cadmium determination in vegetable and fruit samples. P1 and P2, peristaltic pumps; LS, leaching solution; W, waste; UB, ultrasonic bath; MS, minicolumn containing the sample; IV, injection valve; SV1, SV2, SV3, SV4 and SV5, switching valves; MC1 and MC2, mixing coils; MN, minicolumn containing the chelating resin (Chelite P) and FAAS, flame atomic absorption spectrometer.

Standard solutions containing 0.000–0.035 µg/mL of Cd in the same acid medium as the leaching solution were introduced into the flow system. Blank determinations were performed using the acid leaching solution, and in all instances the absorbance was 0.000.

3. Results and discussion

3.1. *Optimization of the cadmium preconcentration step*

Plackett–Burman designs were used principally when the number of variables influencing the analytical system was very large, because the number of experimental runs can be reduced (as the designs would provide knowledge about the system tendencies [17]). In this paper, a Plackett–Burman 2ˆ6 * 3/16 factorial type III resolution design with one centrepoint was selected, allowing 6 degrees of freedom, which involves 13 non-randomized runs. To optimize the preconcentration step, cadmium was measured on-line. Six experimental variables were optimized: HCl concentration (eluent solution), elution flow rate, sample pH to obtain a quantitative retention of Cd on the chelating resin (Chelite P), sample flow rate, eluent volume and minicolumn diameter. The lower and upper levels for each studied variable are listed in table 1. These values were chosen from experiences developed in previous experiments. The results of the design, expressed as percentage recovery are also shown in table 2. These results can be visualized by a standardized ($P = 95.0$ %) main effects Pareto chart (figure 2). It can be seen that the cadmium preconcentration/elution process appeared to be affected by one statistically significant factor: the sample pH. This variable is affected by a positive estimated effect. This means that when the pH value was increased for the levels tested, cadmium preconcentration/elution processes were favoured. This variable was studied at pH 2–9 to obtain the pH range where Cd retention was quantitative. At pH 9, partial precipitation of Cd occurs as $Cd(OH)_2$, so the recovery obtained was 46.2%. At pH 3–8, a quantitative recovery was achieved ($>95\%$), and when the pH value was <3, Cd retention was not complete

Table 1. Factor levels studied in the Plackett–Burman design (2ˆ6 * 3/16) for the Cd preconcentration-elution process.

Variable	Key	Low (−)	High (+)	Optimum
Sample pH	A	1	7	3–8
Sample flow rate (mL/min)	B	0.5	4.0	2.0
Eluent concentration (HCl, M)	C	0.1	3.0	3.0
Eluent volume (μL)	D	70.4	190	92.0
Eluent flow rate (mL/min)	E	3.0	5.0	3.0
Minicolumn diameter (mm)	F	1.0	2.0	1.0

Table 2. Design matrix and response values in the Plackett–Burman design (2ˆ6 * 3/16) for the preconcentration and continuous ultrasonic acid extraction of Cd.

Run No.	[a]A [b]G	B H	C I	D J	E K	F L	% Cd recovery[c]	% Cd recovery[d]
1	+	−	+	−	−	−	22.1	105.0
2	+	+	−	+	−	−	39.4	31.0
3	−	+	+	−	+	−	0	49.5
4	+	−	+	+	−	+	90.1	102.0
5	+	+	−	+	+	−	9.2	25.5
6	+	+	+	−	+	+	13.9	95.0
7	0	0	0	0	0	0	26.8	40.5
8	−	+	+	+	−	+	0	74.5
9	−	−	+	+	+	−	0	55.0
10	−	−	−	+	+	+	0	17.0
11	+	−	−	−	+	+	6	45.0
12	−	+	−	−	−	+	0	59.7
13	−	−	−	−	−	−	0	38.0

[a] Experimental factors for the preconcentration step.
[b] Experimental factors for the continuous acid extraction step.
[c] Percentage Cd recovery in each experiment of the preconcentration optimization.
[d] Percentage Cd recovery in each experiment of the acid extraction optimization.

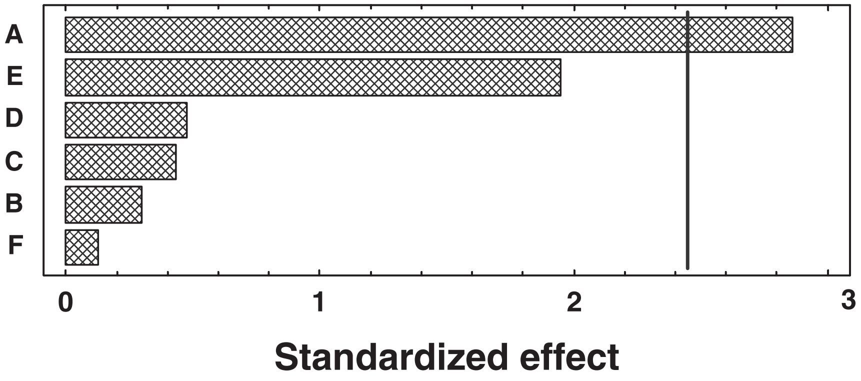

Figure 2. Standardized ($P = 95\%$) main effects Pareto chart for the Plackett–Burman design (2ˆ6 * 3/16) for on-line preconcentration of cadmium in vegetable and fruit samples.

(recovery $<95\%$). All other parameters were not statistically influential factors. As the Plackett–Burman design only provides the tendencies to the optimum values of the variables, these factors were fine-tuned outside the framework of the design. The aims of this study were to decrease the eluent volume and increase the sample flow rate.

In order to achieve a greater concentration factor, eluent volumes of 70, 92 and 190 µL were studied. An eluent volume of 70 µL was not enough to obtain a quantitative Cd recovery (65%), but a volume of 92 µL provides a complete elution of cadmium from the minicolumn, so that 92 µL was selected as the optimum eluent volume. To obtain the greatest sampling frequency, a possible increase in the sample flow rate was studied. This variable was tested at 0.5–3.0 mL/min. A flow rate of 3.0 mL/min provides a 56% recovery, but a flow rate of 2.0 mL/min attains a quantitative recovery (>95%), so this value was selected as the optimum for cadmium retention. To simplify the analysis, an eluent flow rate of 3.5 mL/min, 1 mm minicolumn diameter, and 3 M HCl were selected as optimum values for these variables. The optimum conditions for cadmium preconcentration-elution are listed in table 1.

Since quantitative extraction of Cd in vegetable and fruit samples needs an acid medium, and the optimum pH for Cd retention on the chelating resin is in the range 3–8, an ammonium acetate buffer solution was proposed to achieve the optimum pH for Cd retention. In order to carry out this pH increase on-line, a study of concentration, volume and flow rate of the ammonium acetate buffer solution was developed. Thus, a 16 M concentration, 0.5 mL and 0.4 mL/min were selected as a compromise for the ammonium acetate channel.

3.2. *Optimization of the continuous ultrasonic acid extraction of cadmium*

A Plackett–Burman 2ˆ6 * 3/16 factorial type III resolution design with one centrepoint was selected for optimization of the continuous ultrasonic acid extraction of cadmium. This design involved 13 non-randomized runs. This factorial design was applied to 50–60 mg of a cabbage sample with a cadmium content of 0.391 ± 0.003 µg/g (this Cd concentration was obtained using an off-line acid digestion procedure, $n = 11$). To optimize the CUES, cadmium was measured on-line by FAAS with a flow system that involves the preconcentracion step previously optimized. Six experimental variables were optimized: HNO_3 and HCl concentrations (leaching solution), sonication time, leaching temperature, flow rate of the CUES and leaching volume. Table 3 shows the lower and upper levels for each studied variable and the results of the experimental design. These results can be visualized by a standardized ($P = 95.0\%$) main effects Pareto chart (figure 3). It can be seen that the cadmium extraction from vegetable samples appeared to be affected by three statistically significant factors: sonication time, flow rate of the CUES and nitric acid concentration. Sonication time and nitric acid concentration were affected by a positive estimated effect. In order to increase the sampling frequency, a possible reduction in the sonication time was studied.

Table 3. Factor levels in the Plackett–Burman design (2ˆ6 * 3/16) for the continuous ultrasonic acid extraction of cadmium from vegetable samples.

Variable	Key	Low (−)	High (+)	Optimum
HNO_3 concentration (M)	G	0	3	3
HCl concentration (M)	H	0	3	0
Sonication time (min)	I	0.5	5	2
Leaching volume (mL)	J	2	5	2
Leaching temperature (°C)	K	20	70	20
Flow rate of the CUES (mL/min)	L	3.5	6.0	3.5

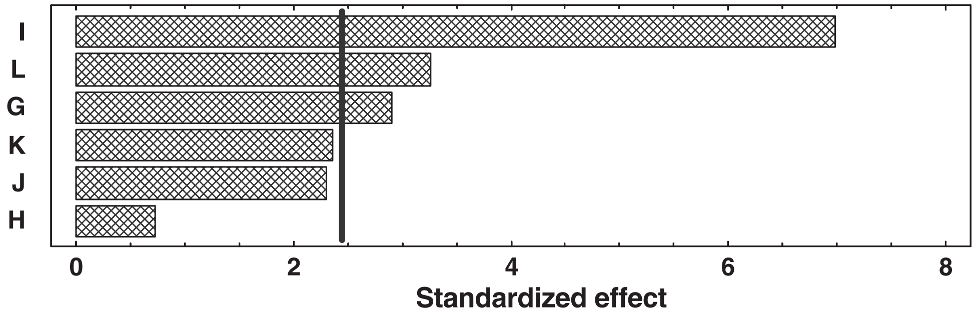

Figure 3. Standardized ($P = 95\%$) main effects Pareto chart for the Plackett–Burman design (2ˆ6 * 3/16) for continuous ultrasonic acid extraction of cadmium from vegetable and fruit samples.

Thus, the sonication time was studied between 1 and 2 min. The results obtained showed that a sonication time of 1.5 min was not enough to obtain a quantitative Cd extraction, but 2 min provided a 101.4% recovery. Nitric acid concentration, affected by a positive estimated effect, was fixed in 3 M. The flow rate of the CUES was affected by a negative estimated effect, so 3.5 mL/min was fixed as the optimum. To simplify the analysis, 0 M HCl, room temperature and 2 mL of leaching volume were selected, since these parameters were not statistically influential factors. The optimum conditions for continuous ultrasonic acid extraction of Cd are listed in table 3.

Finally, two variables that can affect the acid extraction process were studied. These variables are the sample particle size and the amount of sample. By using the optimum conditions, particle sizes of 30–100 µm were tested. The results obtained showed that this variable does not affect the extraction process. As optimum results were obtained when a sample amount of 60 mg was used to optimize the CUES system, we presumed that smaller quantities would also be suitable. However, it is important to know the maximum amount of sample that is quantitatively leached in order to increase the sensitivity of the method. Therefore, the use of sample amounts higher than 60 mg were studied. The results obtained showed that amounts of sample greater than 60 mg produced pressure in the CUES, causing losses of sample. Thus, the maximum amount of sample that can be used is 60 mg.

4. Analytical figures of merit

The calibration graph was run ($n = 7$) under optimum chemical and flow conditions for the whole process, the equation was absorbance $= 7.8 \times 10^{-4} + 3.12\,X$ ($r = 0.999$), where X is cadmium concentration expressed as µg/mL. The linear range tacking into account 60 mg of sample and 92 µL of eluent volume was 0.067–3.243 µg/g.

The validation of the method was performed using 60 mg of a certified reference material, NCS DC 73351 tea leaves, with a Cd content of 0.057 ± 0.008 µg/g. Due to the low Cd concentration of reference material, two acid extractions were carried out, and the acid extracts were preconcentrated in the same preconcentration minicolumn. The Cd content obtained (mean ± SD, $n = 3$) was 0.059 ± 0.002 µg/g, which agrees with the certified value.

The precision of the preconcentration step was verified using 1 mL Cd standard solution containing 0.024 µg/mL ($n = 11$), and the precision of the whole process was checked using a cabbage sample containing 0.391 ± 0.003 μg/g Cd ($n = 11$). The results obtained, expressed as relative standard deviation were 1.9 and 2.1%, respectively. To evaluate the proposed procedure for possible sample matrix interferences, a standard addition method was carried out. In this way, the reference material (NCS DC 73351) was spiked with several cadmium standard solutions added into the leaching solution (0–0.03 μg/mL). In these conditions, an addition calibration graph was run ($n = 6$) under the optimal chemical and flow conditions for the whole process, the equation was absorbance $= 0.03 + 3.26X$ ($r = 0.999$), where X is Cd concentration expressed as μg/mL. This equation and the calibration graph have a similar slope, demonstrating that there are no matrix interferences for Cd determination.

The limit of detection (LOD) based on three times the standard deviation of the blank ($n = 30$) was found to be 0.014 μg/g. The quantification limit (LOQ) based on ten times the standard deviation of the blank ($n = 30$) was found to be 0.067 μg/g. The preconcentration factor of the method based on the rate between the direct calibration graph and FIA calibration graph slopes was found to be 20.5. The theoretic value of this factor was 21.7. The resin capacity based on maximum amount of cadmium that was able to retain the chelating resin was 7.1 μmol Cd/g resin. The sample throughput taking into account the whole process was about 16 samples/h.

5. Analysis of samples

The method was applied to determine Cd in several fruit and vegetable samples, some being crops from Galicia, Spain. The concentration of cadmium in these samples ranged from 0.118 to 0.640 μg/g. The results obtained with the proposed method were compared with those achieved by a conventional off-line sample digestion method with concentrated nitric acid, a preconcentration step using the chelating resin Chelite P and determination by FAAS. To compare the results obtained by both methods, the paired *t*-test was applied [18]. As shown in table 4, neither method

Table 4. Determination of cadmium in vegetable and fruit samples and paired *t*-test.

Sample	[Cd][a] (μg/g)	[Cd][b] (μg/g)	Recovery (%)
Cabbage	0.391 ± 0.003	0.396 ± 0.009	101.3
Lettuce	0.643 ± 0.005	0.640 ± 0.003	99.5
Turnip tops	0.169 ± 0.001	0.166 ± 0.004	98.2
Red cabbage	0.181 ± 0.006	0.178 ± 0.006	98.3
Silver beet	0.473 ± 0.005	0.479 ± 0.002	101.3
Potato	0.266 ± 0.002	0.256 ± 0.001	96.2
Courgette	0.398 ± 0.001	0.400 ± 0.007	100.5
Carrot	0.266 ± 0.007	0.268 ± 0.007	100.8
Tea	0.233 ± 0.002	0.233 ± 0.006	100.0
Lime tea	0.354 ± 0.006	0.355 ± 0.009	100.3
Camomila	0.231 ± 0.010	0.230 ± 0.010	99.6
Banana	0.119 ± 0.004	0.118 ± 0.004	99.2
Pear	0.147 ± 0.003	0.143 ± 0.003	97.3
Apple	0.258 ± 0.009	0.259 ± 0.008	100.4
Peach	0.153 ± 0.004	0.143 ± 0.001	93.5

[a] Off-line acid digestion, preconcentration with a chelating resin (Chelite P) and FAAS Cd determination.

[b] Cd concentration obtained by the present method. Experimental value of $t = 1.01$; Critical value of t ($n - 1 = 14$, $P = 0.05$) $= 2.15$.

gives significantly different values, so the agreement between the two methods is satisfactory.

6. Conclusions

The continuous ultrasound-assisted extraction system (CUES) combined with FI preconcentration is a rapid, precise and accurate sample pretreatment procedure and an effective approach for improving the sensitivity of the FAAS determination of cadmium in solid samples. The main goals obtained with the proposed method are a reduction in sample contamination as well as analyte losses (because less manipulation of the sample is required), a reduction in the amount of sample and reagent consumption (compared with off-line digestion procedures that involved 2–3 g [3], 2–10 g [6] and 0.5 g [8] of sample and upper volumes of concentrated acids), and a reduction in the sample preparation time (compared with digestion procedures: overnight at 50°C and 2 h at boiling point [3,4]) or dry ashing procedures (18 h [9] and 28 h [10]). These achievements imply an increase in sample throughput. A good match was obtained between off-line digestion and on-line ultrasound-assisted extraction, thus confirming the validity of the whole procedure. The proposed system is simple and obviates the use of expensive and sophisticated instruments.

Acknowledgements

The authors are grateful to the financial support provided by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Ministerio de Ciencia y Tecnología (Project no. CAL01-043).

References

[1] C. Reilly, *Metal Contamination of Food*, Elsevier, London (1991).
[2] Analytical Methods Subcommittee, Sample preparation, *Analyst*, **100**, 761 (1975).
[3] R. Tahvonen, J. Kumpulainen, *Fresenius J. Anal. Chem.*, **340**, 242 (1991).
[4] R. Tahvonen, J. Kumpulainen, *Food Addit. Contam.*, **12**, 263 (1995).
[5] R.W. Dabeka, A.D. McKenzie, *J. AOAC Int.*, **75**, 386 (1992).
[6] D. Zhang, C. Li, L. Yang, H. Sun, *J. Anal. At. Spectrom.*, **13**, 1155 (1998).
[7] L.A. De Pieri, W.T. Buckley, C.G. Kowalenko, *Can. J. Soil Sci.*, **77**, 51 (1997).
[8] M.J. McLaughlin, N.A. Maier, G.E. Rayment, L.A. Sparrow, G. Berg, A. McKay, P. Milham, R.H. Merry, M.K. Smart, *J. Environ. Qual.*, **26**, 1644 (1997).
[9] N.I. Ward, J.M. Savage, *Sci. Total Environ.*, **146/147**, 309 (1994).
[10] J. Gzyl, *Sci. Total Environ.*, **96**, 199 (1990).
[11] R. Srikanth, S. Reddy, Raja Papi, *Food Chem.*, **40**, 229 (1991).
[12] R. Barbera, R. Farre, M.J. Roig, *An. Bromatol.*, **42**, 345 (1990).
[13] M. Yaman, S. Guecer, *Analyst*, **120**, 101 (1995).
[14] Y.A. Vil'pan, I.L. Grinshtein, V.A. Kopeikin, S.I. Solov'eva, *J. Anal. Chem.*, **52**, 543 (1997).
[15] J. Stupar, F. Dolinsek, *Spectrochim. Acta B*, **51**, 665 (1996).
[16] I.L. Grinshtein, Y.A. Vilpan, A.V. Saraev, L.A. Vasilieva, *Spectrochim. Acta, B*, **56**, 261 (2001).
[17] S.N. Deming, S.L. Morgan, *Experimental Design: A Chemometric Approach*, Elsevier, Amsterdam (1993).
[18] J.C. Miller, J.N. Miller, *Statistics for Analytical Chemistry*, Ellis Horwood, Chichester, UK (1994).