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Talanta 66 (2005) 691–695
Talanta
www.elsevier.com/locate/talanta

# Fast on-line ultrasound-assisted extraction coupled to a flow injection-atomic absorption spectrometric system for zinc determination in meat samples

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Received 14 July 2004; received in revised form 29 October 2004; accepted 1 December 2004
Available online 5 January 2005

**Abstract**

A continuous ultrasound-assisted extraction system connected to a flow injection manifold has been used for the on-line determination of zinc in meat samples by flame atomic absorption spectrometry. An experimental design was used for the optimisation of the continuous manifold. This flow injection methodology allowed a sampling frequency of ca. 80 samples per hour with a relative standard deviation for the whole procedure of 0.3% (for a sample containing 163.6 $\mu g\,g^{-1}$ Zn). The detection limit was 0.6 $\mu g\,g^{-1}$ for a sample amount of 5 mg. Accurate results were obtained by measuring certified reference materials (BCR-186 (pig kidney) and BCR-184 (bovine muscle)). The analytical procedure was applied to different real meat samples with satisfactory results.


*Keywords:* Continuous ultrasound-assisted extraction system; Flame atomic absorption spectrometry; Zinc determination; Meat samples

## 1. Introduction

Zinc is an essential trace metal for human beings. Disorders of zinc metabolism are usually due to a deficiency rather than a surplus of this metal [1]. Hence, the determination of zinc in meat samples is an important aspect of food analysis. Nevertheless, meat samples are solid complex matrices that involve an arduous task when sample preparation is accomplished. Nowadays, the solid sample preparation is one of the target goals of present analytical chemistry. This analytical step tends to be labour-intensive and incompatible with the highly developed and automated instrumentation and data treatment that are used in the second and third stages of the analytical process, respectively. Most solid sample pretreatments procedures are still performed manually, which makes them slow, complex, and labour-intensive, being the source of analytical errors and contamination problems [2]. In this sense, several strategies have been proposed to achieve easier, faster and automated solid sample preparation, which include fusion [3,4], heating block digestion [5,6], microwave assisted digestion [7,8], acid or alkaline leaching [9,10] and slurry sampling [11–13].

Flow injection (FI) techniques have demonstrated to be a very useful tool for solid sample pretreatment automation. These methodologies have a simple foundation, simple and comfortable handling, great capacity to achieve accurate and precise results, and very high sample throughput [14]. In this way, the incorporation of a microwave oven into a FI manifold offers many advantages over its off-line counterpart: it enables completely automated operations, reduces extensive time delay between sample delivery and analysis, easy digestion of complex matrices, a safety increase for the analyst and a decrease in the losses of volatile analytes [15–20]. However, these advantages are accompanied by some drawbacks such as matrix interferences (the sample matrix is introduced into the detector) and the need of incorporating a cooling area into the FI system due to the high temperature achieved into the

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doi:10.1016/j.talanta.2004.12.019

oven. Ultrasound-assisted extraction can also be coupled to a FI manifold. Using this energy the direct leaching of a solid sample can be accomplished by using a closed loop including a sample container. This sample container is immersed in a thermostated water bath, and ultrasonic irradiation and heating are used for accelerating the extraction of analyte(s) from the sample. FI-ultrasound-assisted extraction methodology presents several advantages over its off-line counterpart, like as a shorter sonication time (reduced by factors of 6–12) and the centrifugation step to separate the liquid phase (10–20 min) is avoided. Therefore, the simplification of the analytical process is considerable. These advantages were experimented when our investigation team proposed a continuous ultrasound-assisted extraction system (CUES) incorporated to an on-line flow injection manifold for the determination of trace metals in seafood samples [21–24]. The CUES combines the benefits of ultrasound-assisted extraction and those achieved by FI systems. In this paper the CUES is coupled to an on-line flow injection manifold and this FI system was optimised for zinc determination in complex solid matrices such as meat samples.

## 2. Experimental

### 2.1. Instrumentation

The overall manifold, as can be seen in Fig. 1, comprised two parts: (a) the continuous ultrasound extraction system (CUES), which accomplishes the sample lixiviation and (b) FI-FAAS continuous monitoring of zinc. The CUES is composed of a Gilson Minipuls-3 peristaltic pump fitted with PTFE tubes (P1), an ultrasonic bath (UB) (Selecta), a glass minicolumn (M) (50 mm × 3 mm i.d., bed volume 350 μl) (Omnifit) used as sample container (the ends of the minicolumn were plugged with filter paper) and two Reodyne (models 5041 and 5020) low pressure valves (SV1 and SV2). The on-line zinc monitoring was performed by a manifold that comprises a Gilson Minipuls-3 peristaltic pump (P2) fitted with PTFE tubes and two Reodyne (model 5020 and 5301) injection or switching valves (IV and SV3, respectively). PTFE tubes (0.8 mm i.d.) were employed to connect the FI manifold to a Perkin-Elmer 5000 atomic absorption spectrometer with a deuterium background correction (FAAS). This analytical instrument was furnished with a zinc hollow-cathode lamp and it was set at 213.9 nm. A standard air/acetylene flame was used. The spectrometer output was connected to a Perkin-Elmer 50 servograph recorder with a range of 5 mV. The signals measured were the heights of the absorbance peaks. Numerical analysis of experimental designs were performed by means of the STATGRAPHICS V.4.1 statistical package (Manugistic, Inc., Rockville, MD, USA, 1997) [25].

### 2.2. Material, reagents and solutions

Ultrapure water of 18.2 MΩ cm resistivity obtained from a Milli-Q water purification system (Millipore) was used for the preparation of samples, reagents and standards. The glassware used was cleaned in 4 M $HNO_3$ for 4 days and rinsed with ultrapure water before use. Hydrochloric acid, nitric acid and zinc standard were of reagent grade (Merck). The zinc stock solution was prepared by dilution of the zinc standard, which contains 1000 mg $l^{-1}$ of zinc. Thus, a solution containing 10 μg $ml^{-1}$ of zinc was obtained. Working solutions containing zinc in the range 0–1 mg $l^{-1}$ were prepared by a suitable dilution of the stock solution.

### 2.3. Reference materials

Two kinds of samples were used to validate the method proposed: BCR-186 (pig kidney) with certified concentration of 128 ± 3 μg $g^{-1}$ Zn and BCR-184 (bovine muscle) with certified concentration of 166 ± 3 μg $g^{-1}$ Zn (Community Bureau of Reference, Belgium).

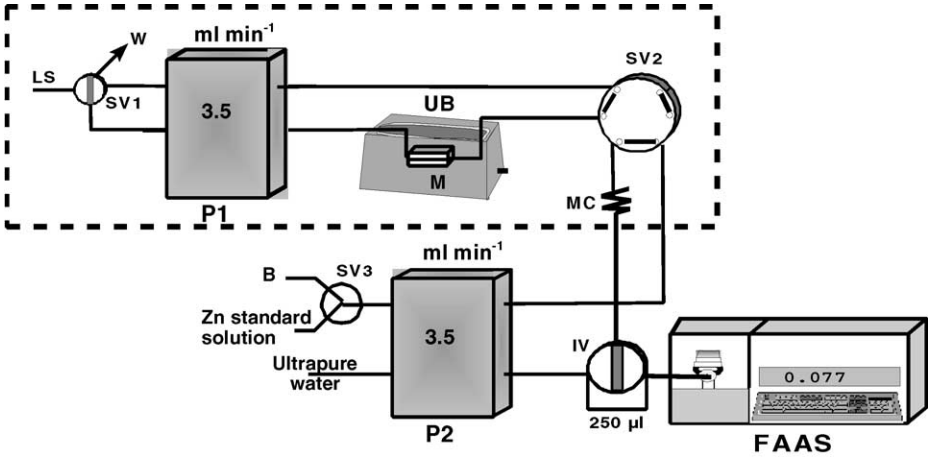


Fig. 1. Schematic diagram of the CUES and optimum working conditions for the on-line Zn determination: (I) on-line acid leaching step; (II) on-line Zn monitoring. P1 and P2, peristaltic pumps; UB, ultrasonic bath; LS, leaching solution; M, minicolumn; IV, injection valve; SV1, SV2 and SV3, switching valves; MC, mixing coil; W, waste; B, blank; FAAS, flame atomic absorption spectrometer.

### 2.4. Sample preparation

Meat samples were purchased in local markets. These samples were triturated, homogenised, freeze-dried, and then kept in clean, dry containers. After sieving, fractions with particle size under 100 μm were taken.

### 2.5. Procedure for zinc determination in meat samples

The continuous zinc determination system is shown in Fig. 1. Meat samples 5 mg, are directly weighted into a glass minicolumn, and in order to avoid sample losses into the CUES circuit, its ends are plugged with filter paper (Whatman 541). Then, the minicolumn is connected to the CUES. First, 1 ml of leaching solution (LS; 0.75 M nitric acid or 0.75 M hydrochloric acid) is aspirated and directed into the CUES circuit. Then, the SV1 is switched to its other position to close the CUES circuit. The leaching solution is circulated at 3.5 ml $min^{-1}$ through the minicolumn during 0.5 min under the action of ultrasound energy. Elapsed this time, the SV2 is switched to its other position and the acid extract circulates towards the mixing coil (MC; total length 2 m) in order to be homogenised. A volume of 250 μl of this acid extract is injected by means of a IV into an ultrapure water carrier stream that transport it to the detector for on-line Zn monitoring. While the sample measurement is accomplished, the CUES circuit is washed with ultrapure water in order to avoid carry-over and a new minicolumn is inserted in the leaching circuit for the next analysis. Standard solutions containing between 0 and 1 mg $l^{-1}$ of zinc, in the same acid medium as leaching solution, are injected into an ultrapure water carrier stream by mean of IV.

The samples were also analysed by a conventional off-line sample digestion method using a concentrated acid: an amount of 0.5 g of sample was mixed with nitric acid in a glass beaker and heated on a hot-plate until complete sample dissolution. Once cool, the solution was transferred into a 100 ml calibrated flask and made up to volume with ultrapure water with subsequent zinc determination by FAAS.

## 3. Results and discussion

### 3.1. Optimisation of the variables involving the CUES

The optimisation of the whole proposed procedure was focussed to the continuous ultrasound-assisted extraction step. The detection step was optimised taken into account the optimum values founded for the CUES. Therefore, the variables studied to start with the optimisation process are those involving the CUES (nitric acid concentration, hydrochloric acid concentration, sonication time, leaching temperature, flow-rate of the CUES and leaching solution volume). In order to study the behaviour of these variables, a factorial Plackett-Burman $2^6 \times 3/16$ type III resolution design involving 12 runs plus one center point was built. This factorial design has been applied on 5 mg of a reference material (BCR-184) with a zinc content of $166 \pm 3$ μg $g^{-1}$. To optimise the CUES, zinc was measured on-line in the leachate by FAAS with a flow system similar to that depicted in Fig. 1. Table 1 shows the lower and upper levels for each studied variable. These values were chosen from available data and experiences developed in previous experiments. The variable response was % Zn extraction efficiency. The numerical analysis of the results produced the standardized main effects Pareto Chart (Fig. 2). The conclusions of this screening study are that concentration of nitric and hydrochloric acids are influential factors because these factors overtake the limit of statistical significance (95%). Both significative factors have a positive influence, this means that for the levels tested, an increase of these factors would provide a better efficiency in the zinc extraction. Sonication time and leaching volume are affected by a positive estimated effect, but they are not statistically influential factors for the levels tested. With regard to the leaching temperature and flow-rate of the CUES are also not statistically influential factors, but in this case they are affected by a negative estimated effect.

Table 1
Factor levels in the Plackett-Burman factorial design and their optimum values

| Factor | Key | Low | High | Optimum |
|---|---|---|---|---|
| $HNO_3$ concentration (M) | A | 0 | 3 | 0.75 or 0[a] |
| HCl concentration (M) | B | 0 | 3 | 0 or 0.75[a] |
| Sonication time (min) | C | 0.5 | 5 | 0.5 |
| Leaching temperature (°C) | D | 20 | 70 | 20 |
| Flow-rate of the CUES (ml $min^{-1}$) | E | 3.5 | 6 | 3.5 |
| Leaching solution volume (ml) | F | 1 | 3 | 1 |

[a] The optimum leaching solution is $[HNO_3] = 0.75$ M or [HCl] = 0.75 M.

Since the Plackett-Burman design only provides the tendencies to the optimum of the variables, these factors were fine tuning outside the framework of the design. Although the concentrated acids (leaching solution) are statistically influential factors, the goal to achieve was decreased by these concentrations because high acid concentrations can produce serious damages in the spectrometer nebuliser of FAAS. In

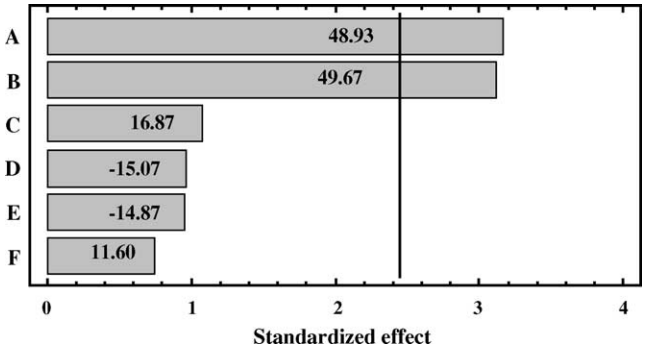


Fig. 2. Standardized Pareto chart for the Plackett-Burman design ($2^6 \times 3/16$) for the continuous ultrasonic acid extraction of zinc from meat samples. The vertical line indicates the statistical significance ($P = 95\%$) bound for the effects.

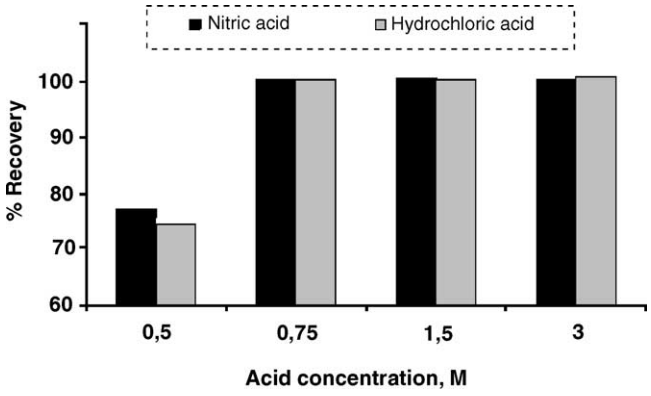


Fig. 3. Optimisation outside the framework of the design of the acids concentration as leaching solution.

this sense, several experiments were carried out to study the optimum acids concentration (Fig. 3). These experiments showed that a nitric acid concentration of 0.75 M (without hydrochloric acid) or hydrochloric acid concentration 0.75 M (without nitric acid) as leaching solutions were enough to obtain a quantitative extraction efficiency for Zn (100.2 and 100.1%, respectively). Nevertheless, a lower acid concentration (0.5 M) produced a no quantitative extraction efficiency (77.4 and 74.7%, respectively). With regard to sonication time and leaching solution volume, with the aim of achieve higher sampling frequency and more sensitive results, was developed a study (outside the framework of the design) to diminish the values of these variables. In this study, it was observed that 0.5 min of sonication time and 1 ml of leaching solution provided quantitative recoveries (100.6 and 100.3%, respectively). In the case of the no significative factors leaching temperature and flow-rate of the CUES, they were not fine tuning because the optimum values proposed by the design (20 °C and 3.5 ml $min^{-1}$) simplifies the manifold, because a temperature control was not necessary. Given these findings, we decided to work with the operational conditions of the CUES given in Table 1 under the optimum values heading.

Another variable that can affect the acid extraction process is the sample particle size. This variable was studied by using the optimum conditions of the CUES. Thus, particle sizes smaller than 30 μm and between 30–100 μm were tested. The results obtained indicated that this variable does not affect the extraction process, which can be explained as result of the high energy supplied by the ultrasounds (frequency of 40 kHz), which increases the contact between the sample and the leaching solution. The variable sample amount was not studied due to the high zinc concentration founded in the samples. Sample amounts higher than 5 mg provided absorbance signals that overtake the lineal range for this metal in the FAAS detector.

Other flow parameters involving zinc determination were also optimised. The mixing coil length was fixed to 200 cm (equivalent to 1 ml of leachate), because shorter lengths would not achieve the total homogenisation of the extract and a longer lengths would increase too much the time of analysis, diminishing in this way the sampling frequency. The carrier flow-rate was studied between 3 and 6 ml $min^{-1}$ and the injected volume of the acid extract between 100 and 400 μl. The aspiration flow-rate of the nebuliser was adjusted to be the same as the flow-rate of the carrier solution. Although the highest aspiration flow-rate provides better sensitivity, at the same time a higher dispersion takes place because the carrier flow-rate is increasing. Therefore, a carrier flow-rate of 3.5 ml $min^{-1}$ (dispersion coefficient equal to 1.1) was chosen as a compromise to obtain the minimum dispersion in the flow system. With regard to the injected volume it was verified that, as it is logical, when the volume was increased the sensitivity was increased. Therefore, a volume of 250 μl was chosen, which, in addition, allows making at least two injections of each acid extract verifying its homogeneity.

### 3.2. Analytical figures of merit

The calibration graph of the method was run ($n = 8$) under the optimal chemical and flow conditions for the whole process. The equation was: absorbance = $5.24 \times 10^{-4} + 0.21$ [Zn] ($r = 0.999$; [Zn] = 0–1 mg $l^{-1}$).

To evaluate the zinc determination for possible sample matrix interferences, a standard addition method was performed. In this way, a sample with a zinc content of $163.6 \pm 0.9$ μg $g^{-1}$ was spiked with several zinc standard solutions added to the leaching solution into the CUES (0–0.1 mg $l^{-1}$). In these conditions, an addition calibration graph was run ($n = 7$) under the optimal chemical and flow conditions for the whole process, the equation was absorbance = $1.72 \times 10^{-1} + 0.21$ [Zn] ($r = 0.999$), where [Zn] is zinc concentration, expressed as mg $l^{-1}$. This equation and the calibration graph present the same slope, demonstrating that Zn determination is free of matrix interferences. Therefore, one can make the calibration graph with Zn standard solutions to avoid the spiking process and so, speed up the analysis time.

The validation of the method was performed using 5 mg of two certified reference materials, BCR-186 (pig kidney) and BCR-184 (bovine muscle) with a Zn certified concentration of $128 \pm 3$ and $166 \pm 3$ μg $g^{-1}$, respectively. The Zn contents obtained (mean ± S.D., $n = 3$) were $128.2 \pm 0.6$ and $166.4 \pm 0.6$ μg $g^{-1}$ for BCR-186 and BCR-184, respectively, which agrees with the certified values.

The precision of the continuous analytical method obtained for real samples was checked using a sample containing $163.6 \pm 0.9$ μg $g^{-1}$ Zn (rabbit liver) and the result expressed as relative standard deviation was 0.3% ($n = 11$). The limit of detection (LOD) based on three times the standard deviation of the blank ($n = 30$) was found to be 0.6 μg $g^{-1}$ for 5 mg of sample. The sample throughput, taking into account the flow injection process, was ca. 80 samples per hour.

### 3.3. Analysis of real samples

The method was applied to the determination of zinc in several meat samples. In order to compare the results ob-

Table 2
Determination of zinc in meat samples and Paired *t*-test

| Samples | Zn ($\mu g\,g^{-1}$ dry mass) | | Difference | EF (%)[a] |
|---|---|---|---|---|
| | Off-line acid digestion and FAAS determination[b] | Present method[b] | | |
| Lamb kidney | 102.8 ± 0.9 | 103.0 ± 0.6 | −0.2 | 100.3 |
| Rabbit liver | 163.6 ± 0.9 | 164.2 ± 0.6 | −0.6 | 100.4 |
| Chicken muscle | 58.4 ± 1.79 | 58.8 ± 0.9 | −0.4 | 100.5 |
| Veal muscle | 156.6 ± 0.9 | 156.5 ± 0.6 | −0.1 | 99.9 |
| Pig muscle | 94.4 ± 1.3 | 94.1 ± 0.9 | 0.3 | 99.7 |
| Lamb muscle | 195.7 ± 0.9 | 195.7 ± 0.6 | 0 | 100.0 |
| Turkey muscle | 76.4 ± 1.3 | 76.9 ± 0.9 | −0.5 | 100.7 |

X (mean difference): −0.2; S.D.: 0.3; $n = 7$; experimental value of $t = 1.47$; critical value of $t$ ($P = 0.05$) = 2.45.

[a] Extraction efficiency (%) = [([Zn]continuous acid leaching)/([Zn] off-line acid digestion)] × 100.

[b] Mean ± standard deviation (S.D.) ($n = 3$).

tained by the proposed method, these samples were also pretreated by using a conventional off-line acid digestion, and their zinc concentrations were measured by FAAS. The results expressed as $\mu g\,g^{-1}$ Zn and their standard deviation ($n = 3$) obtained by these two methodologies are shown in the Table 2. To compare the results obtained by both methods, the Paired *t*-test ($P = 0.05$) [26] was applied and it concluded that both methods do not give significantly different values for the zinc concentration and thus, the agreement between the two methods is satisfactory.

## 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). A.M.C.B. acknowledges a grant from the Ministerio de Educación, Cultura y Deporte, AP 2001-0367.

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