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# Analysis of High Solid Content in Biological Samples by Flame Atomic Absorption Spectrometry

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## 1. Introduction

The analysis of organic samples by flame atomic absorption spectrometry (FAAS) involves the difficulties of the digestion step. This fact was partially overcome by the use of the microwave assisted digestion technique (Skip, 1998). The digestion of the samples has the analytical advantage of an appropriated presentation for the analysis by different techniques. Nevertheless it has some disadvantages as could be analyte losses, risk of contamination, higher cost, longer analysis time and obligatory dilution of analyte, in some cases to undetectable levels (Bugallo *et al*, 2007; Marcó and Hernández, 2004). It is desirable the development of methods those avoid the sample digestion step. The technique of flow injection atomic absorption spectrometry is well suited for this purpose (Trojanowicz, 2000), in the determination of elemental levels in slurry samples and high solid content samples. It allows to the analysis of solid samples in a simple manner. (Arroyo *et al*, 2002; Koleva and Ivanova, 2008).

The analysis of slurry samples gives the advantage of a liquid while allowing the introduction of a solid. The slurry method is reported for the analysis of prior dried samples (Januzzi *et al*, 1997; Da Silva *et al*, 2006; Mokgalaka *et al*, 2008), precalcined (Andrade *et al*, 2008). The analysis by FAAS of solid samples or high solid content samples, as could be crude clinical samples is not frequently found in the literature. It is reported the use of slurries from crude tissues combining the FAAS technique with nebulization with a Babington type nebulizer for the introduction of high solids content samples (Mohamed and Fry, 1981; Fry and Denton, 1977).

Brandao *et al*, 2011 reported a simple and fast procedure for the sequential multi-element determination of Ca and Mg in dairy products employing slurry sampling and high resolution-continuum source flame atomic absorption spectrometry (HR-CS FAAS). The main experimental conditions optimized were 2.0 mol L<sup>-1</sup> hydrochloric acid, sonication time of 20 min and sample mass of 1.0 g for a slurry volume of 25 mL. The elements were determined using aqueous standards for the external calibration with limits of quantification of 0.038 and 0.016 mg g<sup>-1</sup>, respectively. The precision expressed as relative standard deviation varied from 2.7 to 2.9% for a yogurt sample containing Ca and Mg concentrations of 1.40 and 0.13 mg g<sup>-1</sup>, respectively.

Erik G.P. da Silva *et al*, 2008 evaluated the slurry sampling flame atomic absorption spectrometric method for the determination of copper, manganese and iron in oysters (*Crassostrea rhizophora*), clams (*Anomalocardia brasiliiana*) and mussels (*Mytella guyanensis*; *Perna perna*). They optimized the variables nature and concentration of the acid solution for slurry preparation, sonication time and sample mass. The optimized conditions were 80 mg of sample grounded in a cryogenic mill, dilution using 1.0 mol L<sup>-1</sup> nitric /hydrochloric acid solution, sonication time of 30 min and a slurry volume of 10 mL. The calibration curves were prepared matching the acid concentration. This method allowed the determination of copper, manganese and iron by FAAS, with detection limits of 0.17, 0.09 and 0.46 µg g<sup>-1</sup>, respectively. The precision, expressed as relative standard deviation (RSD), was 3.0%, 2.9% and 3.8% (n= 10), for concentrations of copper, manganese and iron of 17, 22 and 719 µg g<sup>-1</sup>, respectively. The accuracy of the method was confirmed by analysis of the certified oyster tissue (NIST 1566b). The results showed no significant differences using the proposed method respect to those obtained after complete digestion and determination by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

The ultrasonic extraction is an interesting aid to the slurry sampling but its use is not widely spread as the digestion procedures (Taylor *et al*, 2002; Taylor *et al*, 2006). An ultrasound-assisted solid-liquid extraction procedure by using diluted mixed acid solution was developed by Manutsewee, *et al*, 2007 for determination of cadmium, copper and zinc in fish and mussel samples. They evaluated the effects of several parameters such as nitric acid concentration, hydrochloric acid concentration, hydrogen peroxide concentration, leaching solution volume, temperature and sonication time. After the optimization of these parameters the elements cadmium and copper were determined by graphite furnace atomic absorption spectrometry, and zinc was determined by flame atomic absorption spectrometry. The results were compared to those obtained by microwave-assisted digestion. The recoveries (%) of metal amount obtained by leaching technique to the amount obtained by digestion technique for cadmium, copper and zinc ranged from 92% to 114% for fish and from 88% to 103% for mussel samples. The accuracy of the developed method was verified with the dogfish muscle certified reference material (DORM-2). The Recoveries were in the order of 80.9 ± 0.3 and 87.2 ± 0.6%.

Bugallo *et al*, 2007 compared the method of slurry sampling to the microwave assisted digestion for the determination of calcium, copper, iron, magnesium and zinc in fish tissue samples by flame atomic absorption spectrometry. They found that in comparison to microwave-assisted digestion, the analysis of slurries is simple, requires short time and overcome the difficulty of the total sample dissolution before analysis. It is necessary the addition of acid for some analites as iron, to enhance the recovery. For Ca and Cu the quantification must be performed using standard addition. Both methods were accurate and the standard deviations obtained using slurry sampling method and microwave-assisted digestion were not significantly different and the mean relative standard deviation of the slurry sampling method for different concentration levels was below 12%.

In this chapter will be discussed the analysis by FAAS of two kinds of biological samples: brain and onion bulb tissues. The preparation procedures were simplified in order to perform the analysis of the crude samples with the aid of ultrasonic acid extraction for brain slurries and crude onion leachates. The introduction using a Flow Injection System avoids transport effects. The results by FAAS and flow injection atomic absorption spectrometry (FIAAS) were evaluated using the independent technique of TXRF.

### **1.1 Determination of Zn and Cu in crude human brain slurry samples by flow injection flame atomic absorption spectrometry**

The determination of metals in brain samples is required for the study of brain physiology, biochemistry and neurochemistry, or clinical purposes to find correlations between metal levels and some pathologies (Schizophrenia, Wilson disease, Alzheimer, etc.) (Andrási *et al*, 1995; Religa *et al*, 2006). In connection to neurochemistry, it is necessary to determine the elements Zn and Cu for the evaluation of neurotransmission processes. (Horning *et al*, 2000).

The brain matrix is mainly formed by high molecular weight carboxylic acids or greases of long chains, a complex organic matrix. The digestion of such a matrix prior to the analysis is reported in the literature, for different techniques (Andrade *et al*, 2008; Taylor *et al*, 2002; Taylor *et al*, 2006; Lech and Lachowicz, 2009). The main objective of this work was the determination of Cu and Zn in crude brain slurry samples by the method of flow injection atomic absorption spectrometry and the development of precise, accurate, efficient and cheap method of determination of metals in human brain samples. The results were compared to those obtained after microwave aided digestion of the samples. The independent technique of total reflection X-ray fluorescence (TXRF) was used for accuracy evaluation.

Crude brain dissected samples were homogenized with a high speed homogenator to obtain slurries. These slurry samples were properly diluted in 5% V/V HNO<sub>3</sub> to aid the analyte extraction to the aqueous phase, to carry out the determination of copper and zinc by flame atomic absorption spectrometry, following a procedure reported by Marco *et al*, 2003.

### **1.2 Determination of calcium, potassium, manganese, iron, copper and zinc levels in representative samples of two onion cultivars by flame atomic absorption spectrometry using ultrasonic assisted leaching**

The onion is one of the most important cultivars in the world. The determination of major, minor and trace element levels is an important tool for the enhancement of production efficiency in the field of agriculture, provenance, and contamination risk evaluation (Ariyama *et al*, 2007; Abdullahi *et al*, 2008; Abdullahi *et al*, 2009). There is a necessity for new analysis methods and simple sample preparation procedures. The chemical characterization of the cultivar samples, becomes important due to the fact that chemical composition is closely related to the quality of the products. (Akan *et al*, 2010; Hashmi *et al*, 2007). Alvarez *et al*, 2003, reported a preparation procedure for the elemental characterization, involving the acid extraction of the analytes from crude samples by means of an ultrasonic bath, avoiding the required digestion of samples in vegetable tissue analysis. The technique of total reflection X-ray fluorescence (TXRF) was successfully applied for the simultaneous determination of the elements Ca, K, Mn, Fe, Cu and Zn. The procedure was compared with the wet ash and dry ash procedures for all the elements using multivariate analysis and the Scheffe test. The technique of flame atomic absorption spectrometry (FAAS) was employed for comparison purposes and accuracy evaluation of the proposed analysis method. A good agreement between the two techniques was found when using the dry ash and ultrasound leaching procedures. The levels of each element found for representative samples of two onion cultivars (Yellow Granex PRR 502 and 438 Granex) were also compared by the same method.

In this work a sample preparation procedure for onion bulb elemental characterization by FAAS is proposed, involving the acid extraction of the analytes by means of an ultrasonic

bath. The procedure was compared with the wet ash and dry ash procedures for all the elements using multivariate analysis and the Scheffé Test. The accuracy was also verified with the TXRF technique. The onion samples were grounded and homogenized with deionized water (1:1) in a domestic homogenator. In a second step, nitric acid was added at different concentrations for the optimization of acid levels. The samples were placed for 30 minutes in the ultrasonic bath. It was found an optimal concentration of 5% V/V of nitric acid and 10% of wet sample mass. Then samples were filtrated. The filtrates were also analyzed by flow injection flame atomic absorption spectrometry (FI-AAS) for all the elements, using deionized water as carrier, an optimized injection volume of 150  $\mu$ L and an optimized flow of 3.5 ml/min. The results obtained were compared to those obtained using the methods of wet ash and dry ash sample digestion and flame atomic absorption analysis after the humidity correction (dry base). For two kind of cultivars (Yellow Granex PRR 502 and 438 Granex). it was found that the dry ash method was statistical equal to the method of ultrasonic extraction-FIAAS. No significant differences were found between the results obtained by FAAS and TXRF. The precision was always below 5% of relative standard deviation in all the cases. It was concluded that the proposed method is the most reliable in the basis of its simplicity, shorter analysis time and minor use of reagents and glassware.

## 2. Experimental

### 2.1 Analysis of brain samples

#### 2.1.1 Samples

Brain samples from healthy, male individuals, who suffered accidental and/or instantaneous death were taken at Morgue of the Central Hospital of Barquisimeto, Edo. Lara, Venezuela. Brains were dissected, not more than 24 hours after death, and kept at -50 °C until sample preparation.

#### 2.1.2 Sample treatment

The brain sections, such as cerebellum, hypothalamus, frontal cortex and encephalic trunk, were weighed and homogenised with deionized water with a high speed homogeniser at 23000 rpm (Ultra-Turrax P25 Janke and Kumkel, IKA registered mark-LABORTECHNIK). Homogenates with a 50-60% w/V (wet weight) of brain tissue were obtained and kept at -20 °C until analysis. Some samples were lyophilized after homogenization at -50 °C in a digital LABCONCO lyophiliser, LYPH-LOCK.

The digestion of the homogenates and lyophilized samples for comparison purposes was carried out in a Domestic microwave oven using closed teflon vessels, in two steps: 15 minutes at medium power and 10 minutes at maximum power. An ultrasonic bath Cole Palmer was employed for slurry treatment and for homogenization. The whole sample treatment is detailed by Marcó and Hernández, 2004.

#### 2.1.3 Slurries

Slurries were prepared taking aliquots of the 50-60% w/V crude brain homogenates with volumetric pipettes and transferring to calibrated flasks, following strictly the next procedure:

1. An appropriate aliquot of homogenate is taken with the glass volumetric pipette, depending on the desired concentration of the slurry .



2. The homogenate portion is then transferred with the pipette to the volumetric flask. In this step, the pipette is rinsed with deionised water helping the remaining homogenate in the inner wall of the pipette falling to the volumetric flask.
3. Deionized water is added to the sample into the flask to fill approximately half of its volumetric capacity and flask was slightly agitated for few seconds to form an homogeneous slurry.
4. In the case of acid addition, the appropriate amount of the nitric acid is added to the flask, depending of the desired acid concentration.
5. The calibrated flask with the slurry is finally filled to the labelled volume, with deionised water.
6. The sample slurries in the flask are treated for 10 minutes in ultrasonic bath at 25 °C.

It is important to remark that this procedure must be followed in all the cases, in order to get stable, and homogeneous slurry. When the step order is not followed, it is frequently to observe the instantaneous denaturalization of the homogenate and the formation of particles of non desirable size.

Additional reagents for assurance of the slurry stability, such as Viscalex, Triton among others were not necessary. Problems with foaming were not found.

#### 2.1.4 Digested samples

In a similar way as described in steps 1 and 2 of the slurry preparation procedure, a sample aliquot of 5 ml of homogenate was transferred to the teflon vessels, instead of the volumetric flasks. Then 5 ml of ultrapure concentrated nitric acid and drops of hydrogen peroxide were added to the teflon vessels. Vessels were closed for digestion in the microwave oven.

About 0.35 g of Lyophilised brain samples were weighed and digested in closed teflon vessels with 5 ml. of concentrated nitric acid and drops of hydrogen peroxide using the same microwave assisted digestion followed with the brain homogenates.

#### 2.1.5 Standards and reagents

Aqueous calibration standards of Zinc and Copper were prepared by serial dilution of the stock solution (1000  $\mu\text{g mL}^{-1}$ ), Titrisol, Merk. Zinc determination was carried out using always aqueous calibration curves. Copper determination was carried out using the aqueous calibration curve and also the standard addition method. Suprapur, 65% v/v  $\text{HNO}_3$  (MERCK, Germany) and 30% v/v  $\text{H}_2\text{O}_2$  (Riedel de Haen, Germany) were employed for leaching and digestion purposes. Nitric acid (Riedel de Haen, Germany) was used for cleaning quartz reflectors for the TXRF analysis. Distilled, deionized water (16 MVcm ) was employed for rinsing and dilution purposes and also as FIAAS carrier.

#### 2.1.6 FAAS analysis

Measurements were performed in a 2100 Perkin Elmer flame atomic absorption spectrometer. FIAAS set up without peristaltic pump, using the nebulizer aspiration flow for sample and carrier propulsion as shown in figure 1 was used to avoid the clogging of the system. This system was made of Teflon pieces from a chromatographic column kit. In one position of the valves the loop is charged while the carrier is passing direct to the nebulizer. In the other position of the valves, the sample in the loop is aspirated and the carrier immediately passes through the loop to the nebulizer. As the system has the possibility of two loops, when one of the loops is being charged the carrier is passing through the other

loop and viceversa. The carrier container is an extraction funnel fixed 30 cm above the entry of nebulizer. The volume of the sample loop was approximately 100 microliters. The length of the tube from injection port to nebuliser was 35 cm and the internal diameter 0.8 mm.

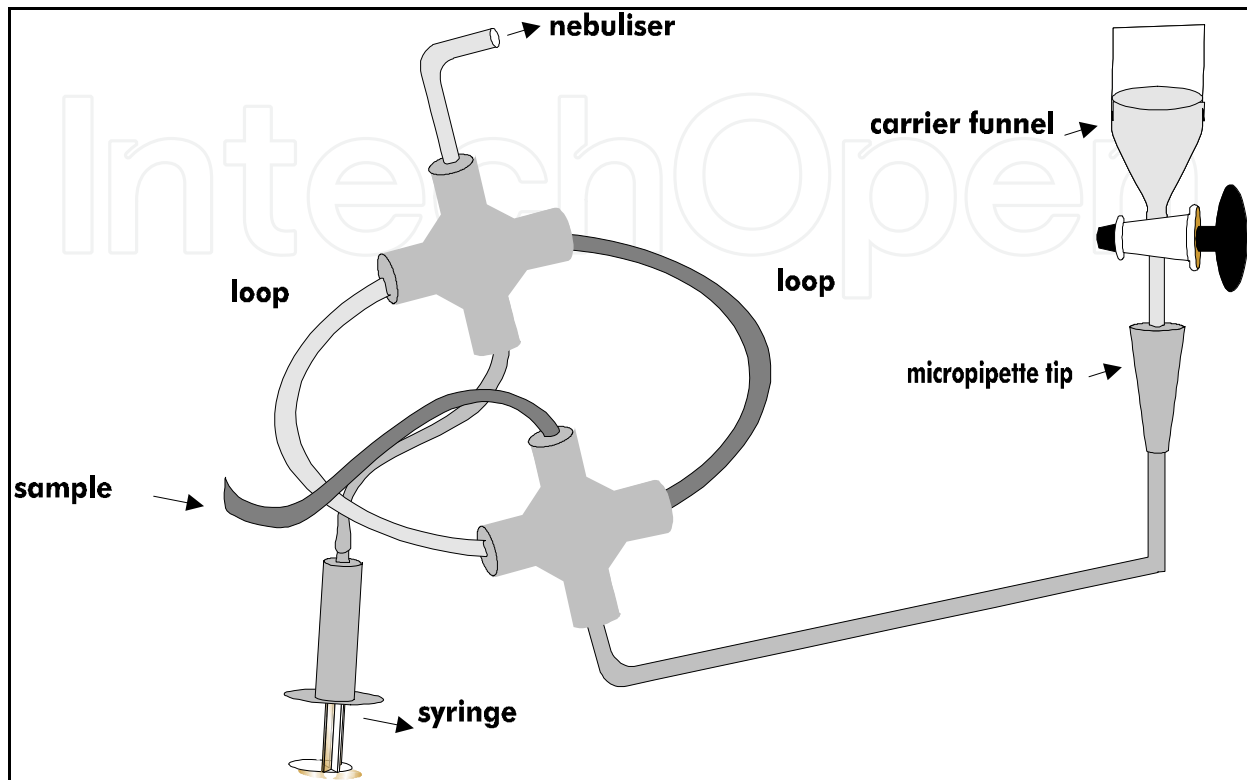


Fig. 1. Sample introduction system

#### TXRF measurements

A CANBERRA Energy Dispersive X-Ray Spectrometer, with Si-Li detector and set up for TXRF, Excitation with Mo tube, 17.4 Kev line was used for X-Ray analysis. TXRF set up with TLAP crystal as monochromator. Details of the TXRF measurements are given in the works of Marcó *et al*, 1999 and Marcó and Hernández, 2004.

## 2.2 Analysis of onion samples. (According to Álvarez *et al*, 2003).

### 2.2.1 Reagents and standards

Titrisol, 1000 standard solutions (MERCK, Germany) were employed for preparation of calibration curves. Suprapur, 65% v/v HNO<sub>3</sub> (MERCK, Germany) and 30% v/v H<sub>2</sub>O<sub>2</sub> (Riedel de Haen, Germany) were employed for leaching and digestion purposes. Nitric acid (Riedel de Haen, Germany) was used for cleaning quartz reflectors for the TXRF analysis. Distilled, deionized water (16 MVcm) was employed for rinsing and dilution purposes and also as FIAAS carrier.

The standards for FAAS and FIAAS analysis were prepared by serial dilution of titrisol standards, according to the linear range of each of the analyzed elements, as reported by the manufacturer.

For the TXRF analysis aqueous, multielement (K, Ca, V, Mn, Cu, Se and Sr) standards were prepared by mixing and dilution of the corresponding stock solutions with distilled, de-

ionized water to yield final concentrations of 5, 10 and 20 mgL<sup>-1</sup>. The element Co was used as internal standard and added to standards and samples. The further quantification is well explained by Alvarez *et al*, 2003.

### 2.2.2 Sampling procedure

Two kinds of onion cultivars were collected, Yellow Granex PRR of Sumblex and Texas Grano 438, Asgrow, at the main market of Barquisimeto following the next procedure: ten bags of 60 Kg from each cultivar were random selected. Then from each bag were taken 10 onions. A total amount of 100 onions for each cultivar were collected.

#### Sample preparation

The onion samples were grounded and homogenized with deionized water (1:1) in a domestic homogenizator in a previous step (30 random selected onions for each preparation procedure from each cultivar). An amount of 5 g of wet weight or 10 g of the homogenate was digested by a wet ash procedure and prepared by ultrasonic leaching, as explained bellow. In similar way an amount of dry onion corresponding to 5 g of wet weight (calculated on the basis of the dry matter content) was used for the dry ash procedure. The humidity correction and the determination of dry masses were performed separately by drying in oven at a fixed temperature of 60 °C. The values of dry matter percentage were 8% for Yellow Granex and 9% for 438 Granex cultivar. The results are expressed in dry basis. In all the cases four independent replicas were prepared.

### 2.2.3 Ultrasonic extraction

An ultrasonic bath, Cole Palmer (USA) with temperature control was used. Temperature was fixed to 70 °C. Time of sonication was 30 min. Ten grams of the homogenate (5 g of wet sample) were placed in flasks and mixed with different nitric acid concentrations (0, 5, 10 and 15% v/v) for the optimization of acid levels. The samples were placed for 30 min in the ultrasonic bath with a fixed temperature of 70 °C. Then samples were filtrated with Whatman filters by gravity and the supernatants were quantitatively transferred to 50 mL volumetric flasks.

### 2.2.4 Wet ash

The wet digestion was performed weighing 5 g of the wet sample and adding 15mL of concentrated HNO<sub>3</sub> and drops of H<sub>2</sub>O<sub>2</sub>. The digestion was performed in a hot plate. After the digestion procedure the sample were aphorized to a final volume of 50 mL.

### 2.2.5 Dry ash

The dry digestion was performed weighing 0.4 g of dry sample (approx. 5 g of wet sample) and calcining at 700 °C for 2 h. Then, the ashes were dissolved with nitric acid and samples were quantitatively transferred to 50 mL volumetric flasks.

### 2.2.6 FAAS analysis

The samples were analyzed in a Perkin Elmer (USA) 3110 Atomic Absorption Spectrometer under conditions suggested by the manufacturer.

The FIAAS manifold was designed for low dispersion and two channels (carrier and sample), using a An ISMATEC peristaltic pump IPC model for sample and carrier



introduction controlled by a Temporizer GrabLab model 900 and a control valve Cole-Palmer, model 625E Bunker CT. See Figure 2.

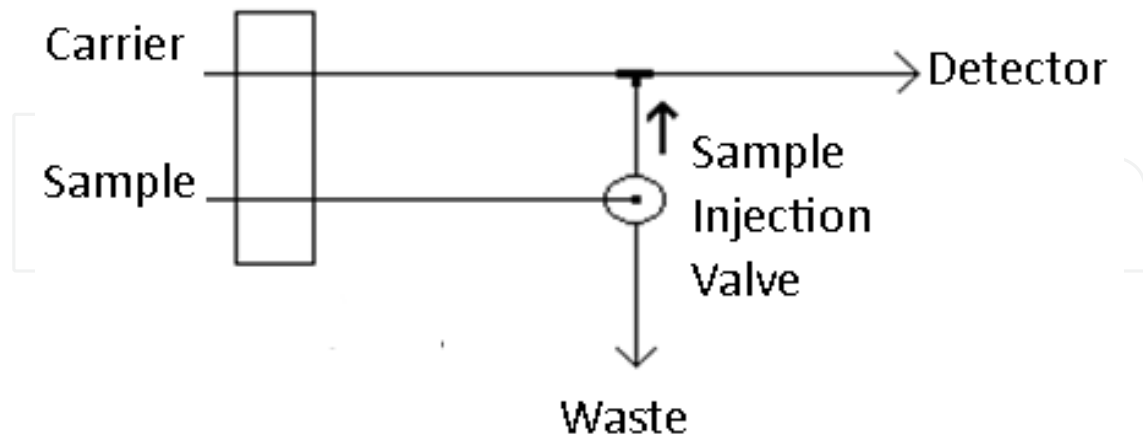


Fig. 2. FIAAS manifold

### 2.2.7 TXRF analysis

The TXRF analysis was carried out in a Canberra XRF Spectrometer with a modified TXRF module designed at the Atominstitut der Osterreichischen Universitaeten, Vienna. The excitation was performed with the  $K\alpha$  (17.4 keV) line of a molybdenum anode X-ray tube, operated at 40 kV and 20 mA, as detailed by Alvarez *et al*, 2003. A carbon-molybdenum multilayer crystal was used for monochromation of the incident beam and a Si(Li) detector (Resolution 180 eV at Mn line, 5.8 keV) was used for the detection of the fluorescence signal. The spectra were collected in a PC based multichannel analyzer (Canberra S100), with live collection time of 200 s. The spectral data analysis was conducted with the AXIL fitting program and QXAS package supplied by the International Atomic Energy Agency.

## 3. Results

### 3.1 Analysis of brain samples

The feasibility of the crude brain slurry direct introduction was tested at a first stage using different concentration (w/V) of brain tissue in water with and without acid. The introduction of the samples was performed by the use of a simple flow injection system described in the figure 1 due to frequent obstruction of the valve of the FIAAS manifold with peristaltic pump. Optimal slurry concentration was in the range of 2.3% w/V to 12.5% w/V for zinc determination while for copper the slurry concentration must be higher than 20% w/V for an appropriate detection and less than 24% w/V to avoid matrix interferences. The results obtained by the slurry method were compared to those obtained after sample digestion and also to the independent technique total reflection X-ray fluorescence. A good agreement between results confirmed the accuracy of the proposed sample preparation procedure. The mean precision for the zinc and copper determination was less than 5% for most of the samples.

#### 3.1.1 Optimization of experimental conditions

Experimental conditions for the FAAS method were fixed following the routine recommended by the spectrometer manual. The parameters as slit, lamp current and gas flow were

automatically selected. For the FIAAS method, variations of the gas flow were performed. Gas flow was changed from 5 L/min to 8 L/min. A slight increment in sensitivity was observed when using 5L/min of gas flow, nevertheless the obstruction of the capillary tube was frequently observed under this condition due to the high solid content of the brain slurry samples. High gas flow was necessary to avoid the capillary tube obstruction.

*Sample volume:* three sample loop sizes were tested: 100, 150 and 200 microliters. The 100 microliters loop was selected as optimal volume, since no significant changes are observed in peak height respect to higher volumes, dispersion is low and risk of memory effect are minimized. The dispersion was 1.3 for Cu and Zn in the optimized set up.

*slurry concentration and stability:* Slurries with concentration w/V of 2.3%, 12.5% and 23 % were tested. Simultaneously, the effect of nitric acid 5% w/V was evaluated. The slurry 2.3% corresponded to the minimal concentration that allowed to the measurement of Zinc signal in the lower value of the working calibration range. The 12.5% is a concentration in the range recommended by Mohamed and Fry, 1981 for homogeneized tissues and 23.5% corresponds to the critical matrix due to the high solid content. The stability of the absorbance signal does not depend on the acid at concentration levels lower than 12.5%, evidencing that the analyte is mostly in the aqueous phase. It was deduced that for Zn a slurry concentration less than 12.5 % w/V and 5% HNO<sub>3</sub> V/V is adequate for the analysis. Concentrations higher than 13% are over the linear calibration range (0.1-1 µgml<sup>-1</sup>). In the case of copper, slurry concentration must be higher than 12.5% for an appropriate detection since linear range lies at higher concentration values, between 1-10 µgml<sup>-1</sup>. The critical higher concentration was 24%, due to matrix effects. See figure 3.

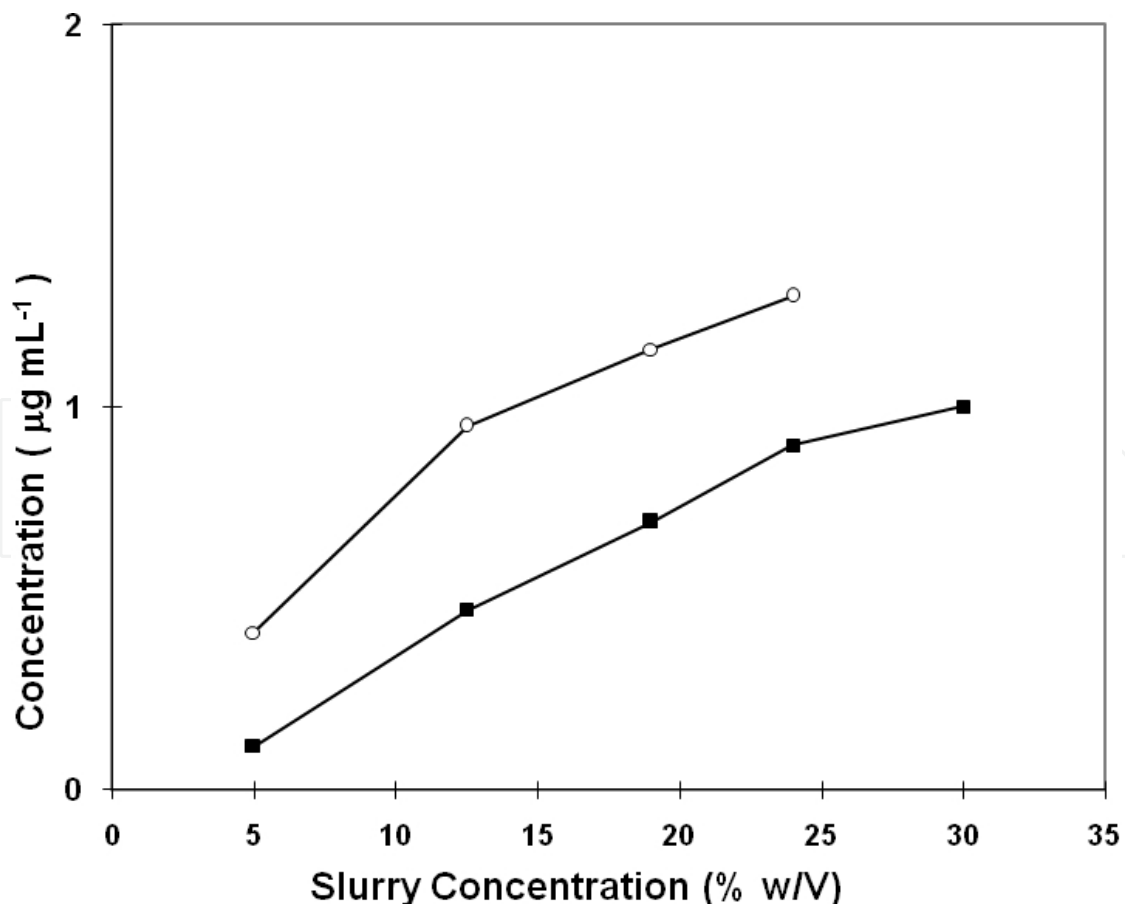


Fig. 3. Effect of the Slurry Concentration on Zn (■) and Cu(○) Signals.

*Effect of nitric acid:* the effect of nitric acid was evaluated at concentrations of 5% w/V, 10% w/V and 20% w/V. An increment of the absorbance signal is observed when the acid is added. The quality of the slurries in terms of particle size is improved. The clogging of the capillary was observed for samples without acid. Acid concentrations above 5% v/V do not affect significantly the absorbance signal. The acid blank was tested and its influence discarded on the measurement.

### 3.1.2 Determination of Zn

The determination of Zn can be carry out by slurry sampling at slurry concentration levels (%w/V) between 2.3 and 13% if nitric acid is added. Nevertheless, for concentration levels near the lower limit (2.3%) a high relative standard deviation is obtained. See table 1.

SAMPLE	CONCENTRATION	RSD (n=5)
Ce-2	8.8 (12%)	3%
	8.5 (4.8%)	4%
Te-2	7.7 (12%)	2%
Te-1	5.6 (4.8%)	3%
Cf-1	9.6 (4.8%)	2%
Ce-1	11.0 (4.8 %)	2%
Hp-1	10.9 (4.8%)	3%
Te-3	14.2 (2.3%)	9%
Hp-3	10.0 (2.7%)	10%
Cf-3	5.6 (4.7%)	5%

Table 1. Concentration ( $\mu\text{g g}^{-1}$ ) of Zn in Crude Brain Slurry Samples. The values in parenthesis indicate the slurry concentration. RDS: Relative Standard Deviation. Ce: Cerebellum; Te: Encephalic trunque; Hp: Hypothalamus; Cf: Frontal Cortex.

A good concordance between results obtained by slurry sampling and those obtained after microwave digestion of crude brain samples was observed, as shown in table 2. If the results for crude samples are compared to the corresponding liophylized and digested samples, the analyte concentration is in concordance to the dry weight correction (about 20% in brain samples ( Andrásí *et al*, 1995 ). The test of the correlation factor F at the 0.05 level of confidence was applied (  $F = 120.7$   $p = 0.002$  and critical F 10.1) demonstrating the agreement between results. See table 2.

The precision ranges between 2% of relative standard deviation (RSD) and 10%. This parameter is independent of the sample preparation procedure (slurry or digested) or the brain section. The precision depends on the analyte concentration. For slurry concentration above 4.8% w/V the value is under 5% of RSD. The precision when comparing independent replicates was similar.

The accuracy was evaluated by comparison to the technique of TXRF for the slurry and digested samples (see table 3). The t-Student at 95% of confidence level was applied ( $t= 0$  and  $p=1$ ).

Sample	Crude	Digested	Lyophilised and Digested
CE-2	8.8 (3%)	9.0 (2%)	
Et-1	5.6 (3%)	5.1 (4%)	5x5.4 27 (3%)
CE-1	11.0 (2%)	10.5 (5%)	5x13.2 66 (2%)
Hp-1	10.9 (3%)	9.9 (2%)	
Et-3	14.2 (9%)	12.9 (3%)	

Table 2. Comparative Results of Zn Concentration ( $\mu\text{gg}^{-1}$ ) in Crude Brain Slurries and Digested Brain Samples. The values in parenthesis correspond to relative standard deviation.

Sample	TXRF	FIAAS
Cf1	2.3 +/-0.2	
	**2.1 +/-0.2	2.3 *(3%)
Ce1	2.3 +/-0.2	2.6 (2%)
	**2.4 +/-0.2	**2.5 (5%)
Cf2	2.2 +/-0.3	2.1 (1%)
		**2.2 (2%)
Ce2	2.2 +/-0.3	2,0 (2%)

Table 3. Comparative Results of Zn Concentration ( $\mu\text{gg}^{-1}$ ) Obtained by TXRF of Slurries, TXRF of Digested Samples , FIAAS of Slurries and FAAS of Digested Samples. The values in parenthesis indicate the relative standard deviation. \*\* The value corresponds to digested sample. Cf: Frontal Cortex; Ce: Cerebellum

### 3.1.3 Determination of Copper

The slurries for Copper determination should have a concentration higher than 20% w/V, due to the low concentration of the analyte in the samples. Slurries with concentration less than 20% (w/V) have an analyte concentration bellow the lowest point of the working calibration range ( $1 \mu\text{g mL}^{-1}$ ). Values of slurry concentration higher than 24% (w/V) have the lack of matrix effects, transport effects and capillary tube obstruction among others. The nitric acid should be added to the slurries in order to extract efficiently the analyte to the aqueous phase and to enhance the nebulisation and atomization processes in the spray chamber and into the flame. See table 4.

The copper determination was performed by direct FIAAS analysis and by the standard addition method (see reference 78), due to the high concentration (w/V) of the slurries. As shown in table 4 no significant differences were found between results using standard addition method and the direct method. The correlation coefficient between results was 0.994 the slope 0.99 and intercept 0.017. The t-Student test at 95% confidence level was  $t=$

0.11,  $p=0.92$ . These results demonstrate that even for slurries at 24% w/V no matrix effects are observed. This fact is a consequence of the addition of the nitric acid and the analyte extraction to the aqueous phase.

The precision of the results for the FIAAS-Slurry method was between 3% of RSD and 11% of RSD. Nevertheless precision values higher than 5% RSD were not observed for digested samples. Then the parameter is affected by the matrix.

The accuracy was evaluated by comparison to the technique of TXRF, as shown in table 5. When FIAAS method is compared to TXRF using the t-Student test at 95% of confidence level ( $t=0$ ;  $p=1$ ), demonstrating the good agreement between the results obtained by both techniques.

Sample	Cu Concentration $\mu\text{g g}^{-1}$ (%RSD)	
	Standard Addition	Calibration Curve
Ce1	0.93*(10%)	0.92(4%)
Ce3	0.68(6%)	0.71(4%)
Te5	0.52(6%)	0.52(7%)
Cf1	0.78(3%)	0.80(11%)
Ce4	0.98(5%)	0.94(10%)
Ce2	0.94(3%)	0.92(4%)
Cf4	0.72(2%)	0.71(3%)
Cf3	0.72(5%)	0.62(3%)
P1	1.55(3%)	1.50(3%)
P2	1.58(2%)	1.59(1%)

Table 4. Concentration of Cu ( $\mu\text{g g}^{-1}$ ) in Crude Brain Slurry Samples Determined Using Standard Calibration Curve vs. Standard Addition Method of Determination. The values in parenthesis indicate relative standard deviation.

Sample	Concentration of Cu ( $\mu\text{g g}^{-1}$ )	
	TXRF	FIAAS
Cf1	0.8 +/-0,1	0.77*(3%)
Cf4	0.8 +/-0,1	0.72 (2%)
Ce3	0.9 +/-0,1	0.68 (6%)
Cf2	0.6 +/-0,1	0.69 (9%)
Ce1	1.1 +/-0,1	0.93*(10%)
Te3	0.5 +/-0,1	0.52 (6%)
Ce2	1.0 +/-0,1	0.93 (3%)
Te2	0,6 +/-0,1	0.56 (6%)

Table 5. Copper Levels in Crude Brain Slurries by Standard Addition-FIAAS Method vs. TXRF. The values in parenthesis indicate relative standard deviation.



### 3.2 Analysis of onion samples

#### 3.2.1 Optimization of experimental conditions

*Recovery efficiency in acid medium:* It is expected that the partial extraction of the analyte to the supernatant occurs when acid is added to a slurry. The atomization efficiency is enhanced, in addition due to the fact that the acid helps to decrease the particle size.

Taking this fact into account, diluted nitric acid was employed at concentration levels of 0, 5, 10 y 15% v/v, in water. The signal increases due to the HNO<sub>3</sub> effect, being the optimal value 5%, with 100% of recovery for the determined elements. A slight suppression of the signal was observed for HNO<sub>3</sub> (10 y 15%) and in consequence lower % recovery for Mn, Zn and Fe. The addition of HNO<sub>3</sub>, induces a predigestion of the solid phase, decrement of particle size and almost the total extraction of the analytes into the aqueous phase. (See table 6). The elements Ca and K are extracted to the aqueous phase with deionized water.

Element	% of recovery			
	0 % V/V HNO <sub>3</sub>	5 % V/V HNO <sub>3</sub>	10 % V/V HNO <sub>3</sub>	15 % V/V HNO <sub>3</sub>
Fe	30	101	103	104
Mn	34	100	98	96
Zn	33	102	80	85
Cu	25	98	94	93
K	100			
Ca	100			

Table 6. Percent of recovery in onion bulb samples after ultrasonic extraction procedure as function of the nitric acid concentration (% V/V).

*Optimization of FIAAS parameters:* the optimized FIAAS parameters were pump flow rate (3.5 mL/min), suction flow rate set 0.2 units bellow pump flow rate (3.3 mL/min). As it is deduced from table 7 there are not significant differences in the recovery, but the highest rate tested ensures a minor residence time of the sample in the nebuliser and chamber, and in consequence a lesser memory effect. The optimal sample volume was fixed at 300 µl. It was found that sample volumes higher than 350 µl did not allow to a significant enhancement. In this case there is not compensation of the matrix effect by dispersion and the signal was similar to that of the classical FAAS analysis. See Figures 4 and 5.

Element	% of recovery			
	2 mL/min	2.5 mL/min	3 mL/min	3.5 mL/min
Fe	86	89	88	89
Mn	92	93	93	93
Cu	97	98	97	99
Zn	100	101	101	103
Ca	100			
K	100			

Table 7. Percent of recovery for Fe, Mn, Cu, Zn, Ca and K as function of the pump flow rate in the analysis of onion bulb leachates by FIAAS. Nitric acid (5% v/V) and sample loop volume 350 µL.

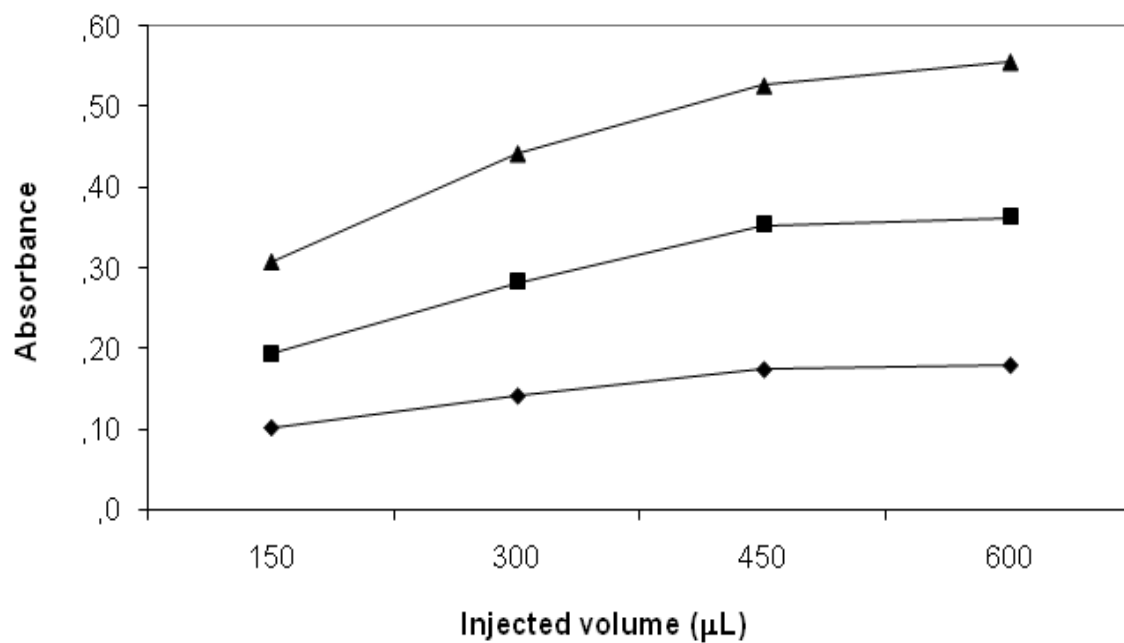


Fig. 4. Relative Absorbance signal as function of the injected sample volume and nitric acid concentration. 5% V/V (♦), 10 % V/V (■) and 15% v/v (▲).

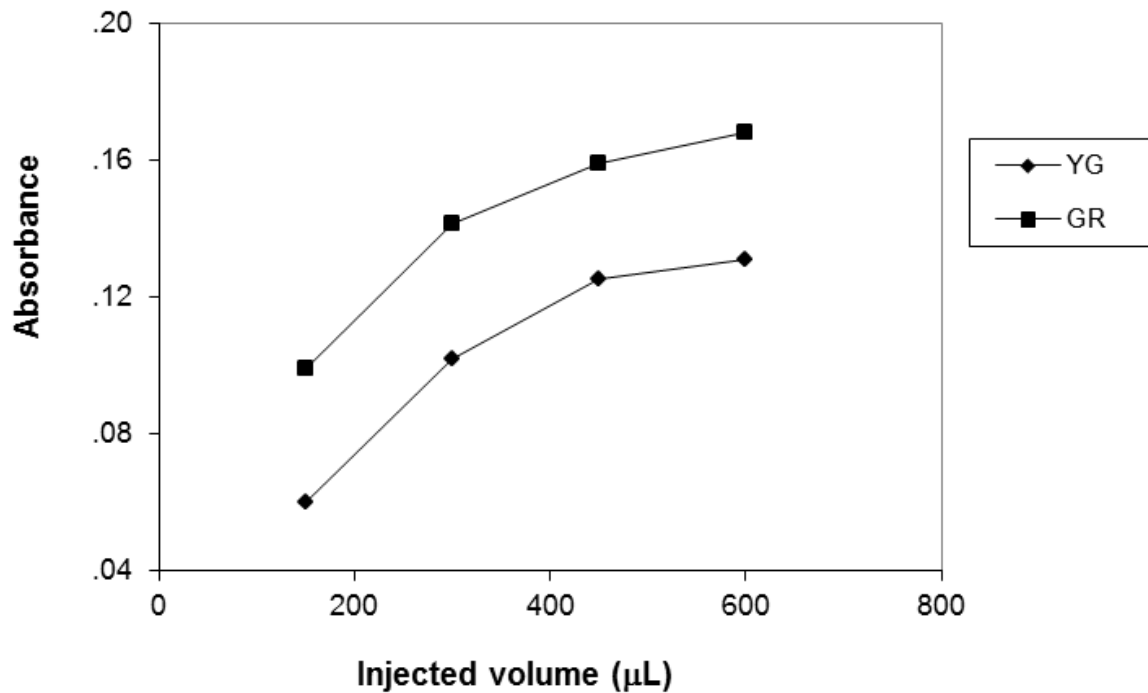


Fig. 5. Relative absorbance as function of the injected volume for leachates of the onion varieties Yellow Granex (YG) and Texas Grano 438 (GR). Fresh sample mass 5 g in a volume of 50 mL. Pump flow rate 3.5 mL/min.

### 3.2.2 Comparison of the results obtained by the preparation procedures using FAAS and the FIAAS method

It was found that in general, the dry ash procedure and the FIAAS method had not significant differences when the Sheffe test was applied in the comparison of the results for all the elements (See table 8). The results are higher than those of the wet ash procedure and the direct aspiration of the leachates by FAAS, with the exception of Potassium. This trend is the consequence of the stronger matrix effect in these procedures. In the wet ash treatment the simple mineralization could not be total as occurs with the wet ash. In the case of the direct aspiration of the leachates by FAAS, the particle size is higher and the matrix was not totally eliminated. The nebulisation and atomization processes were affected in a different way as the aqueous standards used for calibration. The FIAAS procedure and the subsequent dispersion reduced the matrix effects. The addition of nitric acid allowed to the extraction of the analites to the aqueous phase with the increment in the nebulisation and atomization efficiency as compared to the procedure when the leachate is directly aspirated by FAAS.

Element	Preparation Procedure							
	Dry Ash		Wet Ash		Ultrasonic Leaching-FAAS		Ultrasonic Leaching-FIAAS	
	YG	G	YG	G	YG	G	YG	G
Ca %	0.400*(0.6)	0.310 (1)	0.38(0.6)	0.30 (3)	0.37 (0.9)	0.30 (1)	0.400 (1)	0.34 (2)
K %	1.03 (1)	1.38(0.5)	1.06(0.6)	1.80(0.7)	1.07 (0.2)	1.53 (0.5)	1.00 (2)	1.40 (2)
Fe µg/g	27 (3)	45 (3)	28.2 (4)	41 (5)	21 (3)	39 (3)	20.0 (3)	40 (3)
Zn µg/g	16.7 (3)	19.3 (3)	15.2 (2)	17.7 (3)	11.7 (3)	15.4 (3)	17.3 (4)	19.5 (3)
Mn µg/g	17.1 (2)	40 (4)	12.9 (5)	34 (3)	14.1 (5)	36(4)	15.2 (4)	39 (4)

Table 8. Comparison of elemental concentrations in onion bulb samples using different methods by Flame Atomic Absorption Spectrometry. N=4. In parenthesis Relative Standard Deviation. YG: Yellow granex cultivar and G: Texas Grano 438.

### 3.2.3 Comparison to the TXRF technique

A good agreement was found between the results obtained by FIAAS and TXRF (See Figure 6). The results obtained by FAAS when the leachates are directly aspirated, were significantly lower ( $p=0.05$ ) with the exception of the element potassium, in the same way as in the wet ash and dry ash procedures, as explained before. It is important to point that the TXRF technique has not the matrix effects as the FAAS technique. The agreement between FIAAS and TXRF demonstrates the effectiveness of the proposed procedure of ultrasonic leaching and FIAAS analysis for the reduction of the matrix effects and its reliability in the analysis of onion bulb samples.

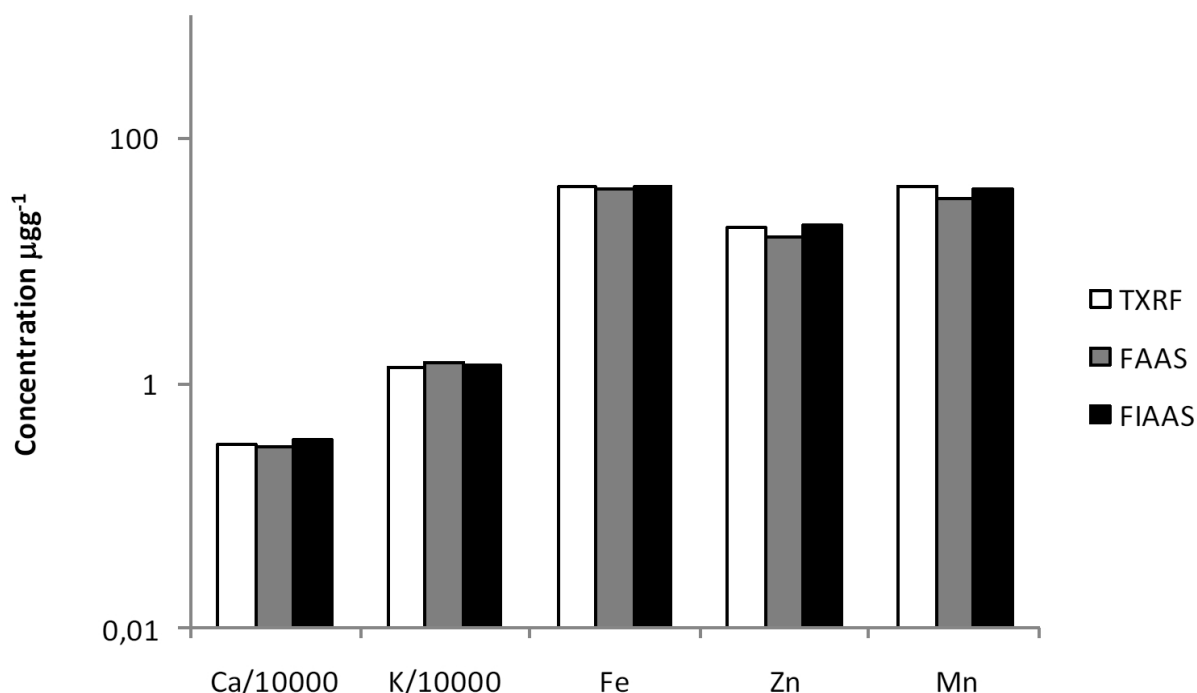


Fig. 6. Comparison of the results by FAAS and FIAAS to the TXRF technique for the leachates of onion bulb samples using ultrasonic aided extraction.

#### 4. Conclusions

A simplified sample preparation procedure was evaluated for the analysis of human brain samples. The feasibility of the preparation of crude brain slurry samples for Atomic absorption spectrometry analysis, in terms of precision and accuracy was demonstrated. These slurry samples must be properly diluted in 5% V/V HNO<sub>3</sub> to aid the analyte extraction to the aqueous phase, to carry out the determination of copper and zinc by flame atomic absorption spectrometry. Optimal slurry concentration was in the range of 2.3% w/V to 12.5% w/V for zinc determination while for copper the slurry concentration must be higher than 20% w/V for an appropriate detection and less than 24% w/V to avoid matrix interferences. A good agreement between results by TXRF and FIAAS confirmed the accuracy of the proposed sample preparation procedure. The mean precision for the zinc and copper determination was less than 5% for most of the samples.

The determination of K, Ca, Mn, Fe, and Zn in fresh onion bulb samples using ultrasonic extraction is a reliable procedure when a FIAAS set up is used. The process must be aided with nitric acid at a concentration level of 5% v/V with five g of homogenized fresh sample in 50 mL. It was demonstrated the substantial reduction of matrix effects if a FIAAS method is applied. The procedure is fast, simple and has lower cost compared to the wet ash and dry ash procedures. The accuracy was verified by comparison to the independent technique TXRF and demonstrated by the good agreement found. The precision for the ultrasonic extraction and FIAAS set up is less than 5% of relative standard deviation for all analyzed elements.

#### 5. Acknowledgments

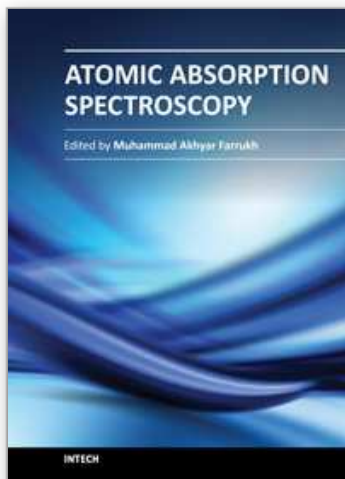
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## **Atomic Absorption Spectroscopy**

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Atomic Absorption Spectroscopy is an analytical technique used for the qualitative and quantitative determination of the elements present in different samples like food, nanomaterials, biomaterials, forensics, and industrial wastes. The main aim of this book is to cover all major topics which are required to equip scholars with the recent advancement in this field. The book is divided into 12 chapters with an emphasis on specific topics. The first two chapters introduce the reader to the subject, its history, basic principles, instrumentation and sample preparation. Chapter 3 deals with the elemental profiling, functions, biochemistry and potential toxicity of metals, along with comparative techniques. Chapter 4 discusses the importance of sample preparation techniques with the focus on microextraction techniques. Keeping in view the importance of nanomaterials and refractory materials, chapters 5 and 6 highlight the ways to characterize these materials by using AAS. The interference effects between elements are explained in chapter 7. The characterizations of metals in food and biological samples have been given in chapters 8-11. Chapter 12 examines carbon capture and mineral storage with the analysis of metal contents.

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