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Comparison of selenium determination in bone tissue samples from lambs, piglets and calves by spectrophotometry and inductively coupled plasma – tandem mass spectrometry

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Selenium measurement in bone tissue or liver is the most accurate way to assess Se status for diagnostic purposes. This study was conducted in order to develop a methodology for the mineralization of bone tissue samples using acid mixture of HNO_3+HCl+HF and also to compare the spectrophotometric method with that using inductively coupled plasma – tandem mass spectrometry (ICP-MS/MS) for detection and quantification of Se. Detection by ICP-MS/MS was optimized by yttrium for Se^{78}, used as an internal standard. Selenium was determined quantitatively by both methods in samples taken from different organs from lambs, piglets and calves: shoulder blade (scapula), large shin bone (tibia) and spine (vertebral column). The results showed that ICP-MS/MS can be reliably used in place of a spectrophotometric method to quantify Se in bone tissue using an acid mixture of HNO_3+HCl+HF for sample mineralization.

Keywords: Se, Bone tissue, Acid mixture of HNO_3+HCl+HF, UV-Vis spectrophotometry, ICP-MS/MS

Abbreviations: AOAC – Association of Official Analytical Chemists; ICP–OES – inductively coupled plasma-optical emission spectrometry; ICP-MS – inductively coupled plasma-mass spectrometry; ETAAS – electrothermal atomic absorption spectrometry; FL – fluorometry; HG-AAS – hydride generation atomic absorption spectroscopy; ICP-MS/MS – inductively coupled plasma-tandem mass spectrometry.

INTRODUCTION

Today it is universally known that most diseases have a chemical origin and develop because of a surplus, deficit or imbalance of micro- and macroelements in the body [1]. Chemical elements are active centers of all enzymes, hormones, antibodies, etc. [2]. We have recently observed increased interest in the thorough investigation of trace element exchange in the human organism, as well as in animals under normal and pathological conditions. A new trend called microelementology develops in biomedical research [3, 4]. A Se deficit may occur in a variety of diseases, and its restoration can lead to normalizing the impaired functions of the body. Selenium has a specific therapeutic value. Se imbalance is associated with the pathogenesis of diseases going under the name of "Free radical diseases". Se is an essential trace component of body's antioxidant defenses against free radicals [1, 5]. Se is important for the normal activity of glutathione peroxidase. A trace element deficiency may affect the balance of pro- / antioxidant system, leading to weakening of antioxidant status and anti-cancer protection. The importance of Se for the normal development and functioning of the tissues of the human body is considered nowadays to be scientifically proven.

One of the selenium deficiency manifestations is its influence on the articular cartilage and bone tissue (Kashin-Beck disease). The incidence of Se deficiency is significantly greater than that of Se intoxication, so that veterinary laboratories are often concerned about the detection of low levels of Se in diagnostic assays. Se is an essential cofactor for sulfotransferase that performs the transfer of sulfur residue to glycosaminoglycan molecules. That is why Se participates in cartilage tissue metabolism and its components [6, 7]. Selenium status was assessed as an alleged factor in the etiology of certain cancers. Research has shown that there is a reverse correlation between cancer mortality and selenium status. In this context, the aim of our study is to develop a methodology for simultaneous determination of Se and bone tissue macro-and micronutrients by ICP-MS/MS, as well as to compare Se concentrations acquired by ICP-MS/MS with the values obtained for the same samples using UV-Vis spectrophotometry.

EXPERIMENTAL

Facilities

Selenium was determined in the samples using an ICP-MS/MS instrument equipped with an octopole reaction system positioned in-between two quadrupole mass analyzers and a JEOL JNM

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DELTA 500 spectrophotometer at a Se wavelength of 378.5 nm.

Table 1. ICP-MS/MS parameters used

Plasma and gas conditions	Value
RF power	1500 W
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	0.86 L min^{-1}
Carrier gas flow rate	0.9 L min^{-1}
Sampling depth	8.0 mm
Reaction or collision gas	hydrogen or helium
Reaction or collision gas flow rate	4.6 mL min^{-1}
Data acquisition Monitored isotopes (m/z)	Se (78)
Data point	3 points/peak
Integration time	0.3 s/isotope
Repetition	5 times

Reagents

Reagents were qualified as pure (Merck and Fluka). The standard solutions for ICP-MS/MS determination of Se with a concentration of 1000 mg kg^{-1} were supplied by Merck, Darmstadt Germany. Water was deionized in a Milli Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MΩ cm. All plastic and glassware were cleaned by soaking in dilute HNO_3 (1/9, v/v) and were rinsed with distilled water prior to use.

Samples

The samples of organ meat were bought in local supermarkets and butchers' shops of Stara Zagora (a city in Bulgaria). They were then transported to the laboratory of the Department of Chemistry, where they were dried in a fan oven at 60 °C for 48 h, their edible parts having been previously separated. Finally, samples were homogenized and kept in dark plastic polyethylene bottles at - 18 °C until analyzed.

Mineralization of samples

Part 1: We weighed 3.0 g of air-dried bone tissue to the nearest 0.01 g in a round-bottomed 100 mL flask and added 22.5 mL of HCI and 7.5 mL of HNO_3. We connected the flask to a reflux condenser and let it stand for no less than 16 h at room temperature, then gently heated to boiling for 2 h. After cooling and flushing the condenser with 25 mL of 12.5% nitric acid the sample was filtered and 100 mL of 12.5% nitric acid was added to the part of it in liquid phase.

Part 2: The undissolved component after the first phase was dried at 105 °C and quantitatively transferred into a 50 mL teflon vessel with a well-fitting lid. We added 5 mL of hydrofluoric acid and heated for 30 min at 140 – 150 °C. After cooling, we added 50 mL of saturated boric acid solution and transferred it to a 100-mL volumetric flask after which distilled water was added to the mark.

Microwave acid digestion method used for sample preparation

An amount of 0.2 g of samples was taken into digestion tubes and 5 mL of HNO_3 (65%), 1 mL of HCl (37%) and 3 mL of H_2O_2 (30%) were added. The samples were digested in a microwave closed system Multiwave 3000 (Anton Paar, Germany) according to the following heating program: (1) 15 min ramp to 120 °C, (2) 20 min ramp to 200 °C, and (3) 20 min hold at 200 °C. After digestion, the samples were diluted up to 25 mL with 2 mL L^{-1} HNO_3. Duplicate analysis was performed on the samples. Blank digestion was carried out in the same way.

Statistical analysis

All statistical computing, tests and graphics were performed with the statistical software R version 3.5.1 (2018-07-02). The data were presented as mean value and standard deviation (SD). The results were analyzed through one-way analysis of variance (ANOVA) followed by Duncan's test with $p<0.05$. Multiple pair-wise comparisons of means among species were obtained by the Tukey HSD test with $p<0.05$ and box and whisker plots.

RESULTS AND DISCUSSION

It is well known that in making ICP-MS/MS quantitative measurements, the most commonly used is the method of line calibration (MLC). It is based on solutions obtained by diluting a mono or multi standard with 1% or 2% acid solution used in sample mineralization (Fig. 1).

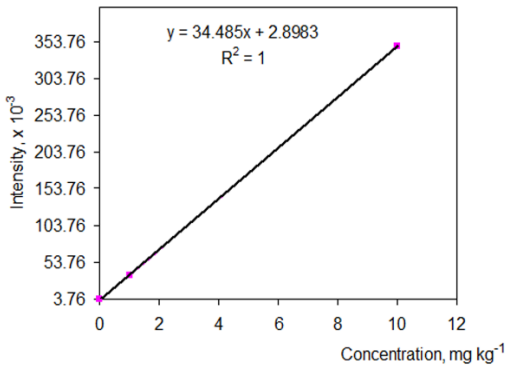

Fig. 1. Se calibration at 0.1, 1 and 10 mg kg^{-1}.

C_K unknown concentration was determined by the equation $I=M_2*C_K+B_2$, where I is the sample intensity.

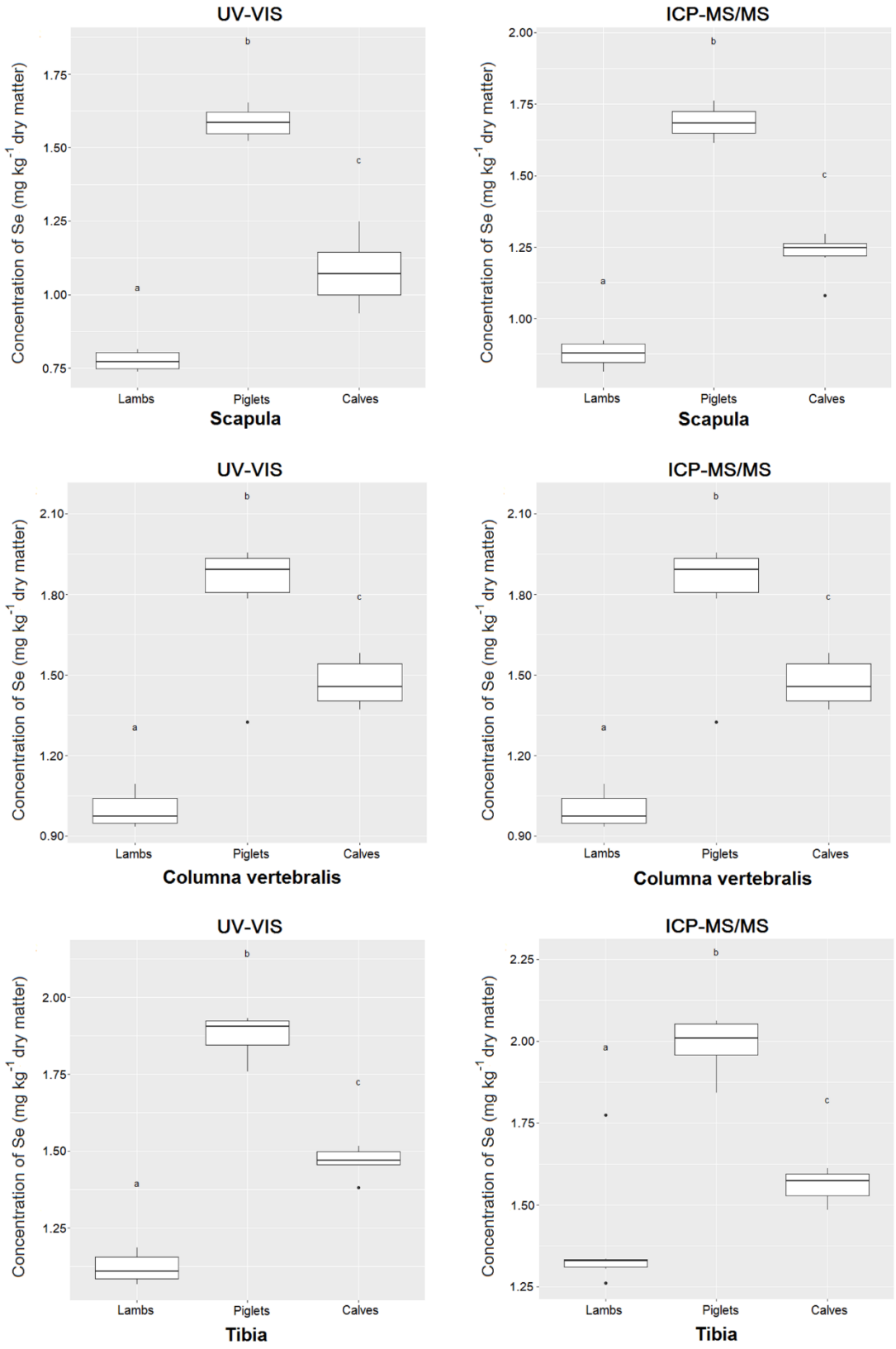

Fig. 2. Box and whisker plots representing the distribution of Se content in bone tissue as determined by UV-Vis spectrophotometry and ICP-MS/MS (n=6). The box represents the interquartile range, solid line within the box represents the median, and the whiskers represent the extremes of the distribution. Letters represent the results of Tukey's post hoc comparisons of mean values among the species ($p<0.05$).

Ideally, analytical methods must be able to accurately determine selenium in various matrix samples at low concentrations from micrograms per kilogram to several hundred micrograms per gram. The methods approved by the AOAC are the best specific analysis procedures under controlled conditions [8-10]. Overall, the most widely used trace elements determination techniques in bone samples are FL [11], HG-AAS, UV-Vis spectrophotometry, ETAAS [12], X-ray fluorescence, ICP-OES [13-15] and ICP-MS [14-20]. Some of these methods can give good selectivity and sensitivity, but require very expensive reagents, as well as time-consuming and complicated procedures. In recent years there has been a trend of displacing the classical colorimetric method for determining selenium in biological materials by the modern ICP-MS/MS method [21-25]. The main advantages of the ICP-MS/MS method are associated with the measurement speed and the possibility for simultaneous determination of the mass of bone macro- and micronutrients. The application of this method is not easy because of the conflicting results when comparing it with the established in practice colorimetric one. This requires expanding the volume of research in this area and accumulation of more experimental results in terms of sample preparation methods of biological samples.

As seen from the results presented in Fig. 2, both methods (spectrophotometric and ICP-MS/MS) for selenium measurement in samples give comparable results. In samples from lambs, the UV-Vis spectrophotometry yielded 0.775 mg kg^{-1} in the scapula; 0.896 mg kg^{-1} in the vertebral column and 1.120 mg kg^{-1} in the tibia. Through ICP-MS/MS, the same samples gave Se amounts as follows: 0.874 mg kg^{-1} in the scapula; 0.997 mg kg^{-1} in the vertebral column and 1.388 mg kg^{-1} in the tibia. In samples from piglets, the UV-Vis spectrophotometry yielded 1.585 mg kg^{-1} in the scapula; 1.739 mg kg^{-1} in the vertebral column and 1.876 mg kg^{-1} in the tibia. Through ICP-MS/MS, the same samples gave Se amounts as follows: 1.686 mg kg^{-1} in the scapula; 1.792 mg kg^{-1} in the vertebral column and 1.985 mg kg^{-1} in the tibia. In samples from calves, the UV-Vis spectrophotometry yielded 1.028 mg kg^{-1} in the scapula; 1.328 mg kg^{-1} in the vertebral column and 1.466 mg kg^{-1} in the tibia. Through ICP-MS/MS, the same samples gave Se amounts as follows: 1.275 mg kg^{-1} in the scapula; 1.471 mg kg^{-1} in the vertebral column and 1.559 mg kg^{-1} in the tibia.

The published scientific information leads to the conclusion that the ICP-MS/MS method gives better results for the total selenium content in bones than the spectrophotometric method [26-29]. However, there are a lot of exceptions and conflicting results, so that no definite answer can be given. It should be borne in mind that in none of the sample mineralization studies hydrofluoric acid was used, i.e. the matrix did not pass into the solution, being necessary to use a mixture of HNO_3+HCl+HF. The use of a mixture of HNO_3+HCl+HF ensures total bone sample digestion while the methods described in literature use different acid mixtures without the participation of hydrofluoric acid in surveyed element extraction.

CONCLUSIONS

It can be concluded that:

- Sample mineralization for analysis is a critical step in obtaining accurate data about Se content. This is particularly important in the studies on biological materials, because most often they are not sufficiently homogeneous and usually contain variable matrices;
- Although there is some matrix interference, the correction factor introduction to the calibration curve results requires more experimental results and mathematical processing.

The ICP-MS/MS advantages over alternative methods include: application on various types of samples, high sensitivity, need for minimal sample preparation, small sample sizes, as well as both multi-element analysis and high performance.

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