Determination of Calcium and Phosphor in Bones Using Microwave Digestion and ICP-MS
Comparison of Manual and Automated Methods using ICP-MS

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Abstract—The determination of the elemental composition of environmental samples provides information for biological monitoring in environmental and occupational health. Concentrations of selected elements contained in human bones may show the current state of health, diseases, intoxications or food pattern. Various studies show age independent concentrations of Calcium (Ca) and Phosphor (P) in human bones, but a correlation between osteoporosis and a decreased Ca concentration is reported. The combination of inductively coupled plasma and mass spectrometry (ICP-MS) enables fast multi-elemental measurements with high accuracy and relative low material consumption. In this study, three methods for the determination of P and Ca in bone materials using ICP-MS are presented and compared. This includes two manual methods and one method for automated sample preparation and subsequent analysis in an automated system, which enables an accurate and fast determination of P and Ca in bone materials. All methods were validated and tested using commercially available animal bones. For the methods presented, the repeat accuracy was determined with 39-46 pig bone samples. A concentration range of P with 93.5-100.5 g/kg and of Ca 191.2-203.2 g/kg with was determined with coefficients of variation (CV) in the range of 1.56-3.17%. The measurement precision showed CV values of 1.07-2.08%. A fully automated system for sample preparation, sample transportation, measurements using ICP-MS and subsequent data processing was realized and validated.

Index Terms—Laboratory automation, automated sample preparation, ICP-MS, microwave digestion, bone material, human health, environmental monitoring.

I. INTRODUCTION

The elemental composition of human bones may provide comprehensive information about the state of health, chronic or acute diseases, intoxications as well as diet or food pattern. Following this, elemental concentrations can be used as indicators of nutritional status and for biological monitoring in environmental and occupational health [1]. Osteoporosis is a bone disease characterized by a decreased bone mineral density which results in an increased tendency to fall and bone fractures with advancing age [2], [3]. In the United States suffer more than 10 million people from this disease and the annual costs are more than 13.5 billion dollars [3]. A link between the progression of arterial calcification and bone loss as well as bone fractures is reported. It has been suggested that a relationship exists between osteoporosis and cardiovascular disease [4]. In general, human bone material consists of 35%

mineral salts with the main components calcium and phosphor in form of hydroxyapatite ($Ca_{10}(OH)_2(PO_4)_6$). The inorganic matrix also contains carbonate and citrate as well as small amounts of magnesium, sodium, potassium, chloride, fluoride, and various trace elements. The organic matrix (20%) contains collagen, marrow, fat, and non-collagen proteins. The water content in bones is about 45% [5], [6].

For the characterization of the nature and the condition of bone a set of properties is used: content and composition of the organic and the inorganic matrix, mineral crystal size and mechanical strength [3]. The most important clinical indication of osteoporosis is the bone mineral density [7]. The determination of the elemental composition of bones is a further way to investigate the nature of this disease and its causes. In various studies was shown that the concentrations of Ca and P not depend on the age of the human [2], [8]. But a correlation between osteoporosis and a decreased Ca concentration was reported [1], [2], [8]–[10].

II. DETERMINATION OF BONE PROPERTIES AND ELEMENTAL COMPOSITION OF BONE MATERIAL

In the characterization of bone tissues and in the determination of the current condition a multitude of techniques are available. Classic non-invasive techniques are dual-energy X-ray absorptiometry (DEXA), quantitative computed tomography (QCT), and qualitative ultrasound (QUS). For the determination of mineral content and mineral density a method using X-ray attenuation is reported [10], [11]. Further techniques to study mineralized tissues are diffraction methods using neutrons or electrons as probes and vibrational spectroscopy such as Raman and Fourier transform infrared (FT-IR) spectroscopy [12]. For the determination of the elemental bone composition a multitude of techniques is available, whereby multi-elemental procedures are preferred [13]. Prior to the analysis, adhering tissue and fat has to be removed from the bone [8], [10], [14]. To transform the solid sample into a liquid phase and for decomposing the organic matrix a wet digestion [15], [16] or a microwave digestion [5], [16], [17] is often performed. The most popular techniques for analyzing Ca are spectroscopic techniques such as atomic absorption spectroscopy (AAS) [18], [19], flame AAS (F-AAS) [9], [15], [17], [20], inductively coupled plasma atomic emission

spectroscopy (ICP-AES) or ICP optical emission spectroscopy (ICP-OES) [2], [8], [19], [21], [22]. In P determination usually ICP-AES measurements are performed [1], [2], [8], but also a sensitive procedure using inductively coupled plasma mass spectrometry (ICP-MS) has been reported [23].

In contrast to the single-elemental technique AAS, ICP-AES, ICP-OES and ICP-MS allow the simultaneous analysis of the relevant elements. These multi-elemental techniques show differences in the detection limits depending on the element. Usually, the gas (e.g. Ar), material and sample consumption in ICP-MS is lower than in ICP-AES or ICP-OES. For this reason, ICP-MS is an interesting technique for determining different elements in bone material using an appropriate sample preparation including microwave digestion and dilution. In ICP-MS analysis microwave digestion methods selected should ideally remove or avoid the addition of compounds or elements which results in spectral, non-spectral interferences and/or in formation of polyatomic ions generated in the plasma [16], [24]–[26]. The use of a chemical inert collision gas such as He in a collision cell of an ICP-MS reduces these undesired effects [27], [28].

III. Elemental Analysis in the Field of High-Throughput Screening

Since the past decades high-throughput screening processes have gained great significance in pharmaceutical and biotechnological industries and clinical laboratories [29]. The field of activities are not only drug discovery [30] but also agricultural, food and environmental analysis [29]. This requires the development of fast and cost effective measurement procedures. Other important challenges are the integration into automation systems and the implementation of suitable software solutions for data evaluation. To reduce the high costs of many reagents and storage capacity, the trend is toward increasing miniaturization [29], which enables less consumption of sample, solvents and chemicals [31] and decreases the costs for waste disposal [32]. In this study, three methods for P and Ca determination in bone materials using ICP-MS are presented. The first sample preparation method is a common manual procedure used in the majority of solid environmental samples. This method was miniaturized and adapted for a later integration in an automation system and was manually validated. The third method includes the miniaturized procedure and its execution on a fully automated system including automated sample and standard preparation as well as subsequent ICP-MS analysis.

A. Chemicals and Standards

All solutions for calibration, samples, and rinsing were prepared using Rotipuran®Supra nitric acid (69%), Rotipuran® Supra hydrochloric acid (35%) both from Carl Roth (Karlsruhe, Germany) and ultrapure water. The microwave digestion was performed with undiluted nitric acid, and the calibration solutions were prepared with nitric acid (1%, v/v). For ICP-MS analysis two rinsing solutions were used. The first solution contains nitric acid (5%, v/v) and hydrochloric

acid (1%, v/v). The second rinsing solution contains nitric acid (1%, v/v) analog to the dissolved digestion samples. The following single ICP-MS standards were purchased from Merck (Darmstadt, Germany): P, Ca, Lu, and Re. Beside commercially available pig bones used in method validation, the P and Ca content of animal bones from chicken, duck, goose, lamb, and cow was determined. Chromasolv® ethanol (99.8%, HPLC purity) for removing tissue residuals was purchased from Sigma-Aldrich (Seelze, Germany). The recovery rate was determined using $Ca_3(PO_4)_2$ ($\geq 96\%$) from Sigma-Aldrich, (Steinheim, Germany) for spiking the bone material. All solutions for microwave digestion, calibration, rinsing and samples were exclusively arranged and stored in vessels from polytetrafluoroethylene (PTFE), polyethylene (PE) or polypropylene (PP).

B. Instrumentation and Parameters

The solid bone samples were prepared using the microwave device Mars 5 from CEM (Kamp-Lintfort, Germany). Therefore, CEM XP1500 vessels with a volume of 50 mL (max. 55 bar) as well as CEM Xpress vessels with a volume of 25 mL and 75 mL (self-venting, no pressure limit) were used with PTFE magnet stirrers from VWR (Darmstadt, Germany).

After addition of weighted bone meal and nitric acid, the samples were predigested for a period of 20 min. One blank sample was included at every digestion run. The basic microwave digestion was developed analogue to references [33]–[35] with the following parameters: temperature-time ramp for 20 min with a final temperature of 180 °C (356 °F), then 25 min hold time at 1,200 W if more than six vessels used on the microwave turntable. The magnetic stirrers in each vessel were used with a medium speed (stage 2 of 3). Following this, the vessels were cooled to room temperature and uncapped.

The analysis was performed with an ICP-MS 7700x von Agilent Technologies (Waldbronn, Germany) with the following parameters: radio frequency power 1,550 W, sample depth 10 mm, carrier gas 0.65 L/min, nebulizer pump 0.10 rps, spray chamber temperature 13 °C (55.4 °F), and dilution gas 0.40 L/min.

Argon was used as plasma, carrier, and dilution gas. In the analysis of P and Ca a multitude of polyatomic interferences is occurring. Table 1 gives an overview about most commonly appearing polyatomic interferences [23], [24]. In the collision

Isotope	Abundance [%]	Interferences
^{31}P	100	$^{14}N^{16}O^1H^+$, $^{15}N^{15}N^1H^+$, $^{15}N^{16}O^+$, $^{14}N^{17}O^+$, $^{13}C^{18}O^+$, $^{12}C^{18}O^1H^+$, $^{30}Si^1H^+$
^{40}Ca	96.97	$^{40}Ar^+$
^{42}Ca	0.64	$^{40}Ar^1H^2$
^{43}Ca	0.145	$^{27}Al^{16}O^+$
^{44}Ca	2.06	$^{12}C^{16}O_2$, $^{14}N_2^{16}O^+$, $^{28}Si^{16}O^+$
^{46}Ca	0.003	$^{14}N^{16}O_2^+$, $^{32}S^{14}N^+$
^{48}Ca	0.19	$^{33}S^{15}N^+$, $^{34}S^{14}N^+$, $^{32}S^{16}O^+$

TABLE I
Interferences for P and Ca Isotopes in ICP-MS [23], [24]

cell, a helium flow was used with 4.3 mL/min to prevent polyatomic interferences. Furthermore, the concentration of nitric acid in the final sample solutions was reduced to 1% (v/v) to avoid the generation of polyatomic ions containing nitrogen and to reduce resulting interferences [23].

The measurements were performed with three replicates and a peak pattern of six points. Table 2 shows the analysis parameters and integration times for the elements analyzed. The rinse time was set to 55 sec at 0.3 rps of the nebulizer pump using the first rinsing solution, followed by 45 sec at 0.4 rps with the second rinsing solution. The automated sample introduction was performed using the ASX-500 autosampler (Cetac, Omaha, NE).

C. Sample and standard preparation

Adhering tissue was roughly manually removed from the bones. Fat and tissue residues were eliminated by absolute ethanol overnight. After this, the bone material was placed in an ultra-sonic bath about 15 min and rinsed with ultrapure water and absolute ethanol. The bones were roughly crashed and dried to constant weight at 60°C. Finally, the bone fragments were milled using the MM2000 ball mill from Retsch (Haan, Germany).

1) Standard manual method

About 250 mg of the milled sample and 8 mL nitric acid were added in XP1500 (vol. 50 mL) or Xpress vessels (vol. 75 mL). The samples were predigested for a period of 20 min under the laboratory hood, and then the microwave digestion was performed. The clear sample solutions were transferred to a volumetric flask (vol. 100 mL) and filled with ultrapure water. To reduce the acid concentration, the samples were further dissolved with ultrapure water (ratio 1:8, v/v) using a volumetric flask (vol. 100 mL). After this, the analyte concentration was adjusted by dissolving with nitric acid (1%, v/v) in a ratio of 1:10 (v/v). The calibration solutions were prepared in nitric acid (1%, v/v) with P and Ca concentrations of 0.1, 0.5, 1, 5, and 10 mg/L.

2) Miniaturized manual method

Re was used in the miniaturized method as second ISTD for correcting evaporation effects during the sample preparation and was added to nitric acid in a concentration of 50 mg/L. In the final diluted sample solution the resulting Re concentration was 50 µg/L. About 62.5 mg of the milled sample were weighted into Xpress vessels (vol. 25 mL) and 2 mL nitric acid was added followed by the microwave digestion procedure. After this, the sample solutions were transferred to a volumetric flask (vol. 100 mL) and filled with ultrapure water. The

samples were dissolved with ultrapure water (ratio 1:2, v/v) to reduce the acid concentration. The analyte concentration was adjusted by dissolving with nitric acid (1%, v/v) in a ratio of 1:10 (v/v). The calibration solutions were prepared in nitric acid (1%, v/v) with concentrations for P and Ca of 0.1 - 10 mg/L and for Re of 1, 5, 10, 50, and 100 µg/L.

3) Optimized miniaturized method for automation

The optimized miniaturized method also uses Xpress vessels (vol. 25 mL), about 62.5 mg of the milled sample, 2 mL nitric, and the microwave digestion procedure as described above. After this, 1 mL of the clear sample solution was added to 11.5 mL ultrapure water in a PP tube (vol. 50 mL). To reduce the acid content, the samples were further dissolved in a ratio of 1:8 also in a PP tube (vol. 50 mL). The analyte concentration was finally adjusted by dissolving with nitric acid (1%, v/v) in a ratio of 1:10 (v/v). The calibration solutions were prepared in the same way as in the miniaturized manual procedure described. Figure 1 shows a mass spectrum of a standard solution with the analytes and the two ISTDs.

IV. RESULTS

The focus of this study was to develop a fast miniaturized method for P and Ca determination in bone materials, which can be used in a fully automated process. A method comparison was done between the manual standard, the manual miniaturized and the automated procedure. Therefore, three different kinds of microwave vessels were tested. The validation of each method includes repeatability testing (intraday precision), the determination of the recovery rate, within-laboratory reproducibility (interday precision), measurement precision, limit of detection (LOD) and of quantification (LOQ), as well as method stability. Finally, various animal bone samples were analyzed using the optimized method.

In the case of the miniaturized methods, the concentration C of the analytes P and Ca were corrected using the measured and the expected concentration of the second internal standard Re as shown in the following equation:

$$C_{P,Ca \; corrected} = \frac{C_{P,Ca \; measured} \cdot C_{Re \; specified}}{C_{Re \; measured}} \quad (1)$$

Element	Isotopic mass	Integration Time [s]	Analyte/ISTD
P	31	0.12	Analyte
Ca	44	0.12	Analyte
Lu	175	0.30	1st ISTD
Re	185	0.30	Analyte (2nd ISTD)

TABLE II
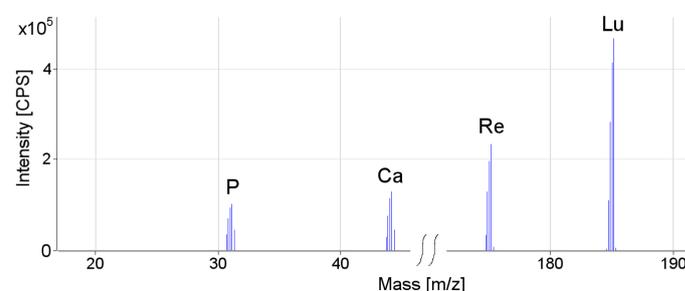ELEMENTS, ISOTOPIC MASSES AND INTEGRATION PARAMETERS

Fig. 1. Mass spectrum of a standard solution with element concentrations of 10 ppm for Ca and P, 100 ppb for Re, and 500 ppb for Lu.

A. Repeatability, within-laboratory precision, method stability

The repeatability testing was performed using 39-46 independent samples of bone meal. The results achieved in the three methods followed a normal distribution, which was verified by the David test; and the Horwitz criterion [36] was satisfied. The average values measured were in the range of 93.5-100.5 g/kg for P and 191.2-203.2 g/kg for Ca and were equivalent to the ecxpected values [8]. The coefficients of variation (CV) were in the range of 1.56-3.17%. Figure 2 shows the results achieved in the tree measurement methods. For determining the within-laboratory precision ten samples of the homogenized bone meal and one blank were prepared and measured on five consecutive days. The standard method showes average values for P of 94.1-128.22 g/kg and for Ca of 189.8-229.0 g/kg. The CV values at all days were in the range of 1.47-8.8%. The heterogenity of the bone samples itself can result in CV with a greater variation than 2-3% [37].
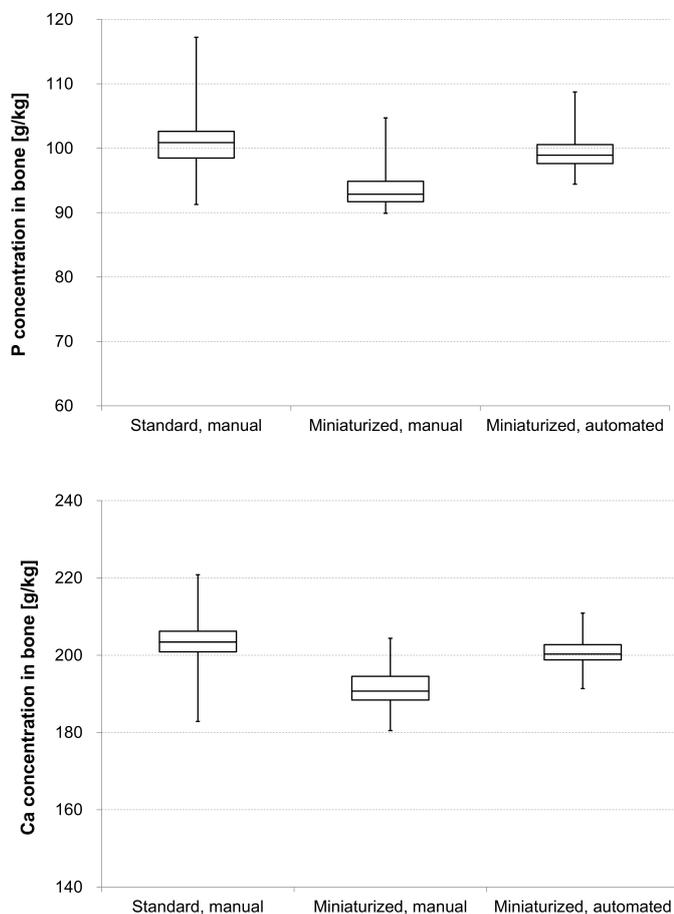

Fig. 2. Repeatability testing using the standard, the manual and the automated miniaturized measurement method.

In the manual minitaturized method the P average values were 95.7-103.6 g/kg and for Ca 188.08-204.8 g/kg. The CV values did not exceed 2.2%. The results of the automated method showed similiar values of 92.7-109.2g/kg P and 193.3-224.2 g/kg Ca. The CV values were in the range of 0.66-3.50%.

The method stability was determined with ten samples, which were divided in five parts. One part was immediatly measured. The other four parts were stored at room temperature at a dry place free from sunlight. The results showed stable values with similar average values for P and Ca. The CV values did not exceed 1.68% using the automated method.

B. Recovery rate, limit of detection, measurement precsision

The recovery rate was determined using ten pig bone samples with a weight of 250 mg, which were spiked with 50 mg $Ca_3(PO_4)_2$. This is a concentration offset of 40.0 g/kg P and 77.4 g/kg Ca. The concentrations measured were in good accordance with the expected bone concentration and the spiked value. To precise these recovery rate, in further measurements these values will be confirmed using certified reference material. Figure 3 shows the results for the measurements using the standard method. The values of the two other methods were similiar.

The limit of detection (LOD) and the limit of quantification (LOQ) were determined with ten blank samles and were calculated using the average value x and the standard deviation SD with the following equations analogue to previous works [33]–[35]:

$$LOD = \bar{x}_{Blank} + 3 \cdot SD_{Blank} \tag{2}$$

$$LOQ = \bar{x}_{Blank} + 10 \cdot SD_{Blank}. \tag{3}$$

The LOD was calculated for the measurement solutions in a volume concentration. The three methods presented showed a LOD for P in the range of 49-76 µg/L and for Ca of 17-64 µg/L. The LOQ for P was about 140-312 µg/L and for Ca about 141-181 µg/L. These results are in good accordance with values described in the literature. A LOD of 47-89 µg/L for Ca was reportet in the measurement solutions using ICP-MS [16] and a LOD of 10 mg/kg in bone materials using ICP-AES [2], [8].

The measurement precision was determined with ten measurements of one sample. In the three methods P concentrations of 95.33-109.50 g/kg and a Ca concentration of 185.23-219.33 g/kg were determined. The observed CV values were in the range of 1.07-2.08%.

V. REAL ANIMAL SAMPLES

Using the methods presented, finally a multitude of animal samples was measured each with three replicates. Different bones from chicken, duck and goose (wings, legs), pig (ribs) lamb and cow (marrowbones) were investigated. The results show a general similarity between their content of P and Ca and the ratio of P and Ca. The marrowbone of the cow was the strongest material and has the highest P and Ca contents. Figure 4 shows the results achieved for various animal bones.
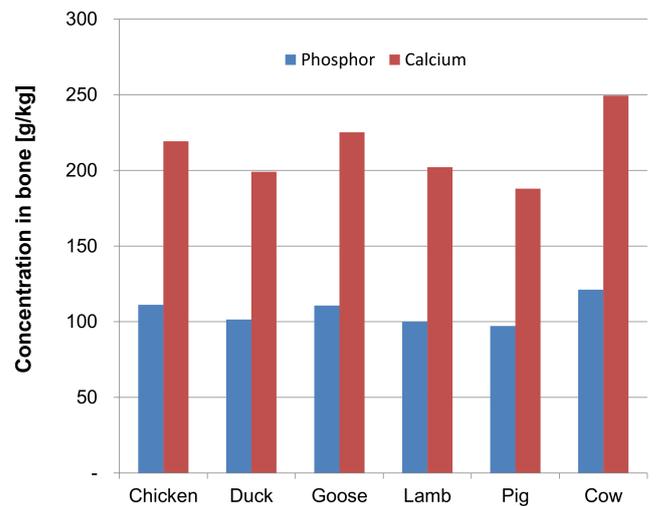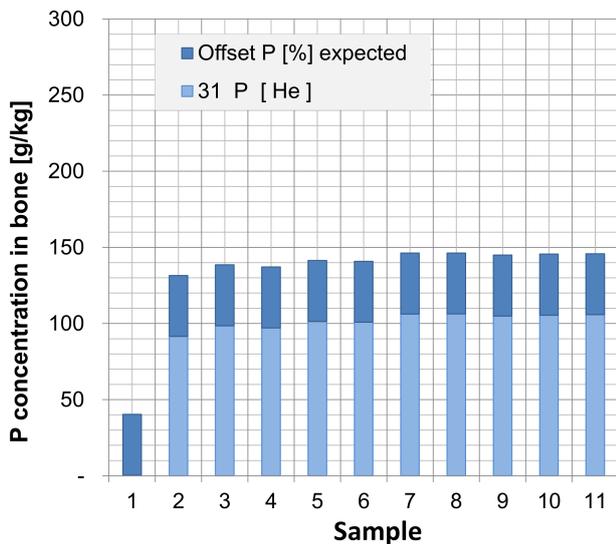
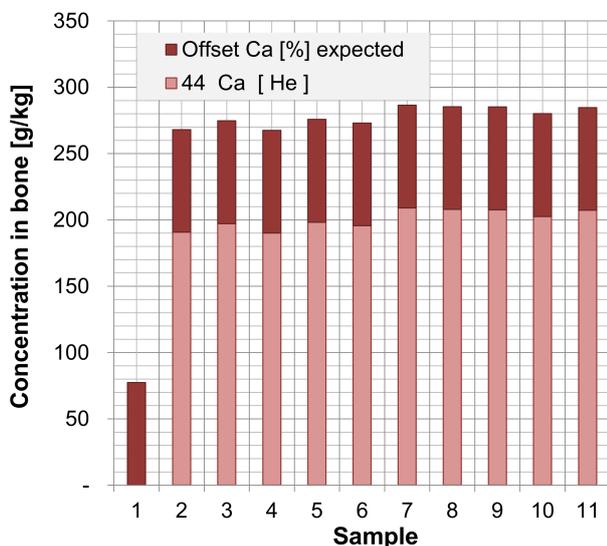Fig. 4.   P and Ca content of bones from different animals

Fig. 3.   Determination of the recovery rate using one spiked blank sample (1) and ten spiked bone samples (2-11).

VI. Concluding Remarks

High-throughput screening procedures in environmental analysis require fast and cost-effective measurement techniques. In this study, three sample and measurement methods were presented, which are characterized by different sample volume. The miniaturized methods enable a sample reduction by 75-82% and a reduction of digestion solution by 75%. Finally, about 90% of the solvents for dilution (water, 1% nitric acid) can be saved. Furthermore, due to the measurement value correction using the second internal standard Re the precision was increased. The automated method runs on an fully automated system including liquid handling, sample and material transport, analysis using ICP-MS and data processing. ICP-MS in combination with an automated sample preparation, enables

a sensitive and fast multi-element method for the determination of the major elements P and Ca in bones. Fast measurement methods are necessary in efficient exploring of chronic or acute diseases of bone or the skeletton.

VII. Acknowledgement

References

[1] J. Yoshinaga, T. Suzuki, and M. Morita, *Sex- and age-related variation in elemental concentrations of contemporary Japanese ribs*, Sci. Total Environ., vol. 79, no. 3, pp. 209–221, 1989.
[2] T. R. Helliwell, S. A. Kelly, H. P. Walsh, L. Klenerman, J. Haines, R. Clark, and N. B. Roberts, *Elemental analysis of femoral bone from patients with fractured neck of femur or osteoarthrosis*, Bone, vol. 18, no. 2, pp. 151–157, 1996.
[3] D. Faibish, S. M. Ott, and A. L. Boskey, *Mineral changes in osteoporosis: A review*, Clin. Orthop. Relat. Res., no. 443, pp. 28–38, 2006.
[4] S. Gonnelli, C. Caffarelli, L. Tanzilli, C. Pondrelli, B. Lucani, B. M. Franci, and R. Nuti, *Effects of intravenous zoledronate and ibandronate on carotid intima-media thickness, lipids and FGF-23 in postmenopausal osteoporotic women*, Bone, vol. 61, pp. 27–32, 2014.
[5] M. V. Galiová, M. N. Fišáková, J. Kynický, L. Prokeš, H. Neff, A. Z. Mason, P. Gadas, J. Košler, and V. Kanický, *Elemental mapping in fossil tooth root section of Ursus arctos by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)*, Talanta, vol. 105, pp. 235–243, 2013.
[6] L. Tandon, G. V. Iyengar, and R. M. Parr, *A review of radiologically important trace elements in human bones*, Appl. Radiat. Isot., vol. 49, no. 8, pp. 903–910, 1998.
[7] M. Srivastava, S. K. Mandal, S. Sengupta, M. Arshad, and M. M. Singh, *Quantification of bone mineral density to define osteoporosis in rat*, J. Endocrinol. Invest., vol. 31, no. 5, pp. 393–399, 2008.
[8] N. B. Roberts, H. P. J. Walsh, L. Klenerman, S. A. Kelly, and T. R. Helliwell, *Determination of elements in human femoral bone using inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry*, J. Anal. At. Spectrom., vol. 11, no. 2, pp. 133–138, 1996.

[9] J. M. Burnell, D. J. Baylink, C. H. Chestnut III, M. W. Mathews, and E. J. Teubner, *Bone matrix and mineral abnormalities in postmenopausal osteoporosis*, Metabolism, vol. 31, no. 11, pp. 1113–1120, 1982.

[10] R. Lappalainen, M. Knuuttila, S. Lammi, and E. M. Alhava, *Fluoride content related to the elemental composition, mineral density and strength of bone in healthy and chronically diseased persons*, J. Chron. Dis., vol. 36, no. 10, pp. 707–713, 1983.

[11] P. Karjalainen, *A method for determination of the mineral content and mineral density of the distal radius using gamma ray attenuation,* Ann. Clin. Res., vol. 5, no. 4, pp. 231–237, 1973.

[12] D. Bazin, C. Chappard, C. Combes, X. Carpentier, S. Rouzière, G. André, G. Matzen, M. Allix, D. Thiaudière, S. Reguer, P. Jungers, and M. Daudon, *Diffraction techniques and vibrational spectroscopy opportunities to characterise bones,* Osteoporosis Int., vol. 20, no. 6, pp. 1065–1075, 2009.

[13] H. Zwanziger, *The multielemental analysis of bone. A review,* Biol. Trace Elem. Res., vol. 19, no. 3, pp. 195–232, 1989.

[14] E. M. Alhava, H. Olkkonen, J. Puittinen, and V. M. Nokso-Koivisto, *Zinc content of human cancellous bone,* Acta Orthop. Scand., vol. 48, no. 1, pp. 1–4, 1977.

[15] A. Hisanaga, M. Hirata, A. Tanaka, N. Ishinishi, and Y. Eguchi, *Variation of trace metals in ancient and contemporary Japanese bones,* Biol. Trace Elem. Res., vol. 22, no. 3, pp. 221–231, 1989.

[16] S. Ashoka, B. M. Peake, G. Bremner, K. J. Hageman, and M. R. Reid, *Comparison of digestion methods for ICP-MS determination of trace elements in fish tissues,* Anal. Chim. Acta, vol. 653, no. 2, pp. 191–199, 2009.

[17] J. Ščančar, R. Milačič, M. Benedik, and P. Bukovec, *Determination of trace elements and calcium in bone of the human iliac crest by atomic absorption spectrometry,* Clin. Chim. Acta, vol. 293, no. 1-2, pp. 187–197, 2000.

[18] J. B. Lambert, S. M. Vlasak, A. C. Thometz, and J. E. Buikstra, *A comparative study of the chemical analysis of ribs and femurs in woodland populations,* Am. J. Phys. Anthropol., vol. 59, no. 3, pp. 289–294, 1982.

[19] J. Yoshinaga, T. Suzuki, M. Morita, and M. Hayakawa, *Trace elements in ribs of elderly people and elemental variation in the presence of chronic diseases,* Sci. Total Environ., vol. 162, no. 2–3, pp. 239–252, 1995.

[20] J.-L. Riond, I. Goliat-von Fischer, B. Küffer, A. Toromanoff, and R. Forrer, *Influence of the dosing frequency of parathyroid hormone-(1-38) on its anabolic effect in bone and on the balance of calcium, phosphorus and magnesium,* Eur. J. Nutr., vol. 37, no. 2, pp. 183–189, 1998.

[21] H.-W. Kuo, S.-M. Kuo, C.-H. Chou, and T.-C. Lee, *Determination of 14 elements in Taiwanese bones,* Sci. Total Environ., vol. 255, no. 1-3, pp. 45–54, 2000.

[22] H. Kosugi, K. Hanihara, T. Suzuki, S.-I. Himeno, T. Kawabe, T. Hongo, and M. Morita, *Elemental composition of ancient Japanese bones,* Sci. Total Environ., vol. 52, no. 1–2, pp. 93–107, 1986.

[23] J. S. Becker, S. F. Boulyga, C. Pickhardt, J. Becker, S. Buddrus, and M. Przybylski, *Determination of phosphorus in small amounts of protein samples by ICP-MS,* Anal. Bioanal.Chem., vol. 375, no. 4, pp. 561–566, 2003.

[24] T. W. May and R. H. Wiedmeyer, *A Table of Polyatomic Interferences in ICP-MS,* At. Spectrosc., vol. 19, no. 5, pp. 150–155, 1998.

[25] H. Vanhoe, J. Goossens, L. Moens, and R. Dams, *Spectral interferences encountered in the analysis of biological materials by inductively coupled plasma mass spectrometry,* J. Anal. At. Spectrom., vol. 9, no. 3, pp. 177–185, 1994.

[26] S. H. Tan and G. Horlick, *Matrix-effect observations in inductively coupled plasma mass spectrometry,* J. Anal. At. Spectrom., vol. 2, no. 8, pp. 745–763, 1987.

[27] E. McCurdy and G. Woods, *The application of collision/reaction cell inductively coupled plasma mass spectrometry to multi-element analysis in variable sample matrices, using He as a non-reactive cell gas,* J. Anal. At. Spectrom., vol. 19, no. 5, pp. 607–615, 2004.

[28] F. Vanhaecke, C. Vandecasteele, and R. Dams, *Inductively Coupled Plasma Mas-Spectrometryfor the Determination of Aluminium, Calcium, Chlorine, Iron, Magnesium; Manganese, and Sodium in Fresh Water,* Anal. Lett., vol. 25, no. 5, pp. 919–936, 1992.

[29] T. Chapman, *Lab automation and robotics: Automation on the move,* Nature, vol. 421, no. 6923, pp. 661, 663, 665-666, 2003.

[30] A. Smith, *Screening for drug discovery: The leading question,* Nature, vol. 418, no. 6896, pp. 453–459, 2002.

[31] J. Hong, Y. Miki, K. Honda, and H. Toita, *Development of the automated cleanup system for the analysis of PCDDs, PCDFs and DL-PCBs,* Chemosphere, vol. 88, no. 11, pp. 1287–1291, 2012.

[32] J. B. Quintana, M. Miró, J. M. Estela, and V. Cerdà, *Automated on-line renewable solid-phase extraction-liquid chromatography exploiting multisyringe flow injection-bead injection lab-on-valve analysis,* Anal. Chem., vol. 78, no. 8, pp. 2832–2840, 2006.

[33] H. Fleischer and K. Thurow, *Determination of Total Mercury Content and Trace Metal Analysis in Wood Materials Part 1: ICP-OES Using Mercury Cold Vapor,* Am. Lab., vol. 45, no. 8, pp. 6–9, 2013.

[34] H. Fleischer and K. Thurow, *Determination of Total Mercury Content in Wood Materials - Part 2: ICP-MS - A Multi-element Method,* Am. Lab., vol. 45, no. 9, pp. 6–11, 2013.

[35] H. Fleischer, E. Vorberg, and K. Thurow, *Determination of Total Mercury Content in Wood Materials - Part 3: Miniaturization Using ICP-MS,* Am. Lab., pp. in press, 2014.

[36] W. Horwitz, *Evaluation of Analytical Methods Used for Regulation of Foods and Drugs,* Anal. Chem., vol. 54, no. 1, pp. 67A-76A, 1982.

[37] J. M. Burnell, D. J. Baylink, C. H. Chestnut III, M. W. Mathews, and E. J. Teubner, *Bone matrix and mineral abnormalities in postmenopausal osteoporosis,* Metabolism, vol. 31, no. 11, pp. 1113–1120, 1982.