UNIVERSITY OF PARDUBICE

FACULTY OF CHEMICAL TECHNOLOGY

DEPARTMENT OF ANALYTICAL CHEMISTRY

MULTI-ELEMENT ANALYSES OF SAMPLES WITH COMPLEX MATRIX BY ICP-oa-TOF-MS

DISSERTATION

(ANNOTATION)

AUTHOR: **Tereza Šídová, MSc.**

SUPERVISOR: **Assoc. Prof. Jitka Šrámková**

2017

Summary

The presented work was focused on the development of new methods for multi-element analyses of coal fly ashes, infant formula, pet food and otters tissues using the ICP-oa-TOF-MS spectrometer GBC OptiMass 8000. These samples are known to be difficult to analyze when using instrumentation with low resolving power due to the high content of matrix elements. Even though the achievable resolution of ICP-oa-TOF-MS is higher compared to quadrupole mass spectrometers (common R for $^{238}U^{+}$ ~ 2000) it is not sufficient to overcome the spectral interferences originating from the various polyatomic species produced from matrix components in the plasma. These interferences seriously influence the determination of number of elements with mass smaller than 100 m/z. Although, the reaction/collision cell technology in combination with a time of flight analyzer was recently introduced to analytical practice by GBC Scientific Equipment company, this technology is not accessible to the first and second-generation of instruments being currently the most widely used in routine analytical practice. Application of mathematical correction methods to attenuate the interference to manageable level may be used for these instruments, however it is less useful at ultra-trace level. In this work the modification of the sample preparation procedure (microwave-assisted extraction or digestion) by adding ammonium fluoride or nitric acid and hydrogen peroxide to investigated sample matrices in order to decrease the matrix effects prior to ICP-oa-TOF-MS analysis is introduced. The main advantage of this approach is that it serves as a cheap, rapid and simple solution for overcoming the spectral effects from different polyatomic species on the determination of desired elements. Another benefit is that using this approach the interference of the matrix is decreased significantly and accurate analysis with the use of the matrix matched calibration method can be performed for all elements of interest. Moreover, with the use of Rh as the internal standard all the elements are determined simultaneously without the need for specific setting for individual analytes which may be another benefit over the Q-ICP-MS or HR-ICP-MS methods. Proposed methods also avoid handling the corrosive and toxic acids like HF, H_2SO_4, HCl or $HClO_4$ being commonly used during preparation of investigated samples and prevent the potential risk of analyte loss due to the creation of volatile compounds like SiF_4, BF_3, etc. in the presence of HF. The described methods are environmentally friendly and correspond to green chemistry trends. On the other hand, the detection limits are limited by the quality of analytical reagents and additionally depending on the selected method some elements cannot be determined properly with the proposed methodology due to the problems with co-precipitation and/or inclusion in the precipitated matrix. Despite the above mentioned drawbacks, for ICP-oa-TOF-MS instrumentation this procedure is currently the only solution to attenuate the interferences to a manageable level. The developed methods were employed for analysis of real samples, specifically to 21 powdered infant formulas, 149 otter´s tissues and 51 pet foods. Design of experiments in a form of full or fractional factorial design was utilized to find the optimal conditions for preparing various types of samples. The influence of the observed factors and their interactions were evaluated by Pareto´s charts of standardized effects and response surface. The resulting data sets were evaluated by univariate and multivariate statistical methods. The accuracy of the proposed methods was validated by standard reference materials or standard addition using analytical recoveries.

1. Introduction

Matrix effect and spectral interferences are recognized as main problems that influence ICP-MS analyses. Spectral interferences are caused by isobaric and polyatomic species which can overlap isotopes of interest in the mass spectrum. Monoisotopic and polyatomic ions originating from elements of solvent, atmospheric or plasma gases and /or sample matrix [1, 2] can be resolved by high resolution spectrometry (HR-ICP-MS) or by collision / reaction cell technology in case of quadrupole mass spectrometry (Q-ICP-MS). Matrix effects are induced by a high concentration of matrix elements, which also influences the analyte sensitivity [3]. Simple dilution of the sample is the easiest way how to solve matrix effects, but the concentration of the analyte of interest often does not allow such an approach so isotope dilution [4], standard addition method [5, 6], matrix matched standard calibration [7] or widely used internal standardization [5, 8] have to be performed. Separation methods by means of electrothermal vaporization [9], solvent and solid phase extraction [10], chromatographic methods [11], precipitation and/or coprecipitation [12, 13], etc. have been proposed to decrease and/ or minimize the influence of matrix elements. Thanks to that matrix effect is reduced as well as spectral interfereces originating from the matrix of the sample. The careful optimization of sample preparation (including sample pre-treatment, choice of reagents, condition of extraction or decomposition, etc.) can prevent the occurrence of matrix effects and spectral interferences. The conventional procedure for the quantitative analysis of real samples is based on a preparation procedure employing microwave digestion (MWD) [14-18] or microwave-assisted extraction (MAE) [19-21] prior to the ICP-MS measurement. For this purpose HNO_3, H_2O_2, HF, H_2SO_4, HCl or $HClO_4$ have been employed for preparation of environment, biological and industrial samples [16, 22-24]. Improvements in sample preparation techniques have recently focused on time efficiency, as well as simplicity and the ability to use dilute [25-27] and less toxic reagents with regards to green chemistry trends [28]. Factorial design is one of the chemometric tools used to optimize the parameters of sample preparation, including digestion or extraction procedure and other steps [29, 30].

Microwave digestion is currently the most frequently involved procedure during preparation of coal fly ash which mainly consists of Si, Al, Fe and Ca [22]. Employing MAE instead of MWD shortens the time of sample preparation and some interfering major components of matrix remain in solid phase that is filtrated prior to the ICP-MS analysis. Reagents such as HNO_3, HCl, H_2O_2 [23] and their mixtures are usually required

for decomposition of aluminosilicate matrix as well as highly toxic HF and explosive $HClO_4$ [22]. The use of HF as a reagent may also damage glass components of the spectrometer as well as it may lead to formation of several volatile fluorides like As, B, Se, Sb, Hg and Cr which may be lost during the sample preparation step [31-34]. The later problem occurs even if the closed systems are employed for sample preparation [32-34]. The addition of H_3BO_3 [35-37] may solve some drawbacks being attributed to the use of HF, but the addition of H_3BO_3 increases the salinity of the sample, price of the analysis and disables the determination of boron in the sample. Based on the literature [38] ammonium fluoride reacts with various Si compounds to form complex, thermally stable fluorides such as $(NH_4)_2SiF_6$ thus the potential risk of analytes loss due to the creation of volatile species is eliminated. Therefore, new possibilities for degradation of the silicate matrix were investigated by employing NH_4F. Multi-element analysis of coal fly ash is described in several works utilizing HR-ICP-MS [39, 40] or Q-MS [37, 41], however, such a method has not been published for ICP-oa-TOF-MS yet.

The nebulization of raw milk samples is influenced by the size of the droplets [42] and content of fat [43], which attribute to poor sensitivity and accuracy of the analysis [44]. Methods for direct analysis of milk samples involve slurry sampling [45], flow injection analysis [46] or matrix modification by precipitation [47]. The last method [47] was previously validated in our laboratory where the filtrate was analyzed after precipitation of Ca^{2+} and proteins by oxalic and nitric acid, respectively [47]. However, determination of some elements (Sr, Cu, Bi, Pb, Ag, Th or Hg) was probably affected by the formation of insoluble oxalates [48] and due to their co-precipitation and / or inclusion in the precipitated matrix [47]. Therefore, in order to perform complete analysisdigestion of milk is usually done by HNO_3 and H_2O_2 [24, 49, 50]. Samples of milk were analyzed employing HR-ICP-MS [24, 51] and Q-ICP-MS [50, 52, 53] together with collision [54-56] and dynamic reaction cell [15, 57-60] with NH_3 [58], Ar [59], He [20, 54, 61] and H_2 [62, 63] as collision / reaction gases.

Dried pet food consists of many ingredients including those of animal origin, plant derived materials, and minerals and vitamins [19]. Minerals in form of salts for example Cu as $CuSO_4$, Se and Na as Na_2SeO_3 are added to improve the nutrient requirements. The composition of dried pet food samples can lead to high content of total dissolved solids in the mineralization solution and hamper the ICP-MS measurement due to interferences. Some drawbacks can be eliminated by employing a V-groove nebulizer [3] or simple dilution

of the sample. Few studies dealing with the analysis of pet foods were published [19, 64-66] and covered the period of 2010–2016. Pet food samples were analyzed by flame atomic absorption spectrometry (FAAS) [64], inductively coupled plasma optical emission spectrometry (ICP-OES) [19] and ICP-MS with DRC [65, 66]. For ICP-oa-TOF-MS such a work has not been published yet.

Analysis of otter tissues was carried out in cooperation with the charitable trust ALKA Wildlife [67]. One of the factors influencing the death of otters is environmental pollution caused by persistent pollutants and toxic trace elements [68, 69]. The possibilities of preserving and archiving the unique biological samples provided by ALKA Wildlife for further analysis were investigated. It was found that lyophilization [18, 70, 71] is suitable for this purpose. Lyophilization is a process where a product is dried by removing the water or other solvents under low temperature and pressure. Besides lyophilization, drying to constant mass is possible [16, 72, 73] to obtain dry matter of sample. Decomposition of biological samples can be done by dry ashing in muffle furnace [71], ashing in low pressure oxygen plasma [72], high pressure ashing [74], microwave digestion [16, 18, 68], etc. Severe loss of sensitivity was observed during ICP-MS analysis as a result of instrumental drift and matrix effects originating from measurement of imperfectly digested organic samples [16]. Residual carbon can be monitored as an indicator of degree of decomposition in the development of the procedure. In terms of suppression of polyatomic interferences HR-ICP-MS [17] was used as well as Q-ICP-MS with collision reaction cell and NH_3, H_2 and He [75, 76] for analysis of biological tissues.

In this work, the attention was focused on the development of environmentally friendly procedures for ultra-trace analysis of coal fly ash, powdered infant formula, pet food, and tissues of Eurasian otters by ICP-oa-TOF-MS OptiMass 8000. Possible interferences of real samples were predicted by interference study where the influence of increasing amount of matrix element on determination of elements was observed. The modification of sample preparation procedure by employing NH_4F, HNO_3, and H_2O_2 was investigated. The parameters of the investigated methods including sample preparation steps, the amount and concentration of reagents, the mass of the sample, and the ICP-MS and ICP-OES settings were optimized to obtain precise and accurate results.

2. Experimental

2.1. Reagents and standards

All solutions were prepared using deionized water purified by means of Milli-Q® Reference (Merc Millipore, Germany) pure water system to 0.5 μS cm^{-1}. Standard stock solution of 1 ± 0,002 g L^{-1} or 10 ± 0,002 g L^{-1} of Rh, Li, B, Be, C, Al, Ti, V, Cr, Mn, Ni, Co, Zn, Cu, Ga, Ge, As, Se, Rb, Rh, Sr, Zr, Mo, Ru, Pd, Ag, Cd, Sn, Sb, Te, Ba, Ta, W, Re, Pt, Hg, Tl, Pb, Bi, Th, Cs, Hf, U, Mg, K, Ca, Na and Fe were purchased from Analytika Ltd. (Czech Republic) or SCP Science Ltd. (Canada). Commercially available standard containing 100 mg L^{-1} Ce, La, Nd and Pr and 20 mg L^{-1} Dy, Er, Eu, Gd, Ho, Lu, Sc, Sm, Tb, Tm, Y, Yb was obtained from SCP Science. Sub-boiled nitric acid was prepared from nitric acid (65%, w/w) of Selectipur quality (Penta Ltd., CR). Hydrogen peroxide (30%, w/w), NaCl, KCl, $CaCl_2$, $MgCl_2$, HCl (36%, w/w), H_2SO_4 (96%, w/w) and H_3PO_4 (85%, w/w) were purchased from Fluka Ltd. (Switzerland). Ammonium fluoride, p.a. was obtained from Sigma-Aldrich Ltd. (USA).

2.2. Instrumentation

The digestion of the samples was carried out in the microwave system Speedwave™ MWS-3^+ (Berghof, Germany) with the maximum total output of microwave generator 1450 W. The device was supplemented by Multi Tube (MT) system from the same company where 3 MT PFA tubes (volume 10 mL) are placed into digestion vessel DAC-100 (volume 100 mL). Freeze dryer ScanVac CoolSAFE from LaboGene ApS (Denmark) for dehydration of samples by sublimation of ice at low pressure and temperature was used to obtain dry matter content of sample and long term preservation of otter tissues. Content of mercury was determined by single purpose atomic absorption spectrometer AMA 254 from Altec, Ltd. (CZ) in lyophilized otter liver tissue, infant formula and pet food.

Optimass 8000 ICP-oa-TOF-MS (GBC Scientific Equipment Pty, Ltd., Australia) was used for ICP-MS measurements and was equipped with Ni sampling and skimmer cones, concentric nebulizer MicoMist and 70 mL temperature controlled (10 °C) spray chamber (Glass Expansion). The detail description of Optimass 8000 is summarized in ref. [77]. SmartGate ion blanking system for rejection of unwanted species was set according to the needs of the analyses as is listed in tables 1–4. Detailed operating conditions are given

in ref. [47]. Using these conditions sensitivity of 15,000 count s^{-1} and resolution of 1600 was achieved for 1 µg L^{-1} $^{238}U^{+}$. The calibration for ICP-oa-TOF-MS ranged from 0 to 50 µg L^{-1} Li, B, Be, Al, Ti, V, Cr, Mn, Ni, Co, Cu, Ga, Ge, As, Se, Rb, Sr, Zr, Mo, Ru, Pd, Ag, Cd, Sn, Sb, Te, Ba, Ta, W, Re, Pt, Hg, Tl, Pb, Bi, Th, Cs, and Hf. The calibration range was for elements Ce, La, Nd, Pr, and U from 0 to 2 µg L^{-1} and for Dy, Er, Eu, Gd, Ho, Lu, Sc, Sm, Tb, Tm, Y, Yb from 0 to 0,4 µg L^{-1}.

Table 1 SmartGate setting for analysis of coal fly ash

Range m/z	Interfering ion
11.5 – 57.4	$^{12}C_2^+$, $^{14}N^+$, $^{16}O^+$, $^{16}O^1H^+$, $^1H_2{}^{16}O^+$, $^{24–26}Mg^+$, $^{28}N_2^+$, $^{14}N^{16}O^+$, $^{32–34}S$, $^{35–37}Cl$, $^{40}Ar^+$, $^{40}Ar^1H^+$, $^{40}Ar^{16}O^+$, $^{38}Ar^{17}O^1H^+$, $^{56}Fe^+$
63.4 – 68.0	$^{63}Cu^+$, $^{65}Cu^+$, $^{64}Zn^+$, $^{68}Zn^+$
79.4 – 81.7	$^{40}Ar_2^+$

Table 2 SmartGate setting for analysis of infant formula

Range m/z	Interfering ion
11.5–57.4	$^{12}C_2^+$, $^{14}N^+$, $^{16}O^+$, $^{16}O^1H^+$, $^1H_2{}^{16}O^+$, $^{24–26}Mg^+$, $^{28}N_2^+$, $^{14}N^{16}O^+$, $^{32–34}S$, $^{35–37}Cl$, $^{40}Ar^+$, $^{40}Ar^1H^+$, $^{40}Ar^{16}O^+$, $^{38}Ar^{17}O^1H^+$, $^{56}Fe^+$
63.4–68.0	$^{65}Cu^+$, $^{64}Zn^+$, $^{68}Zn^+$
79.4–81.7	$^{40}Ar_2^+$

Table 3 SmartGate setting for analysis of dry pet food

Range m/z	Interfering ion
11.5–57.4	$^{12}C_2^+$, $^{14}N^+$, $^{16}O^+$, $^{16}O^1H^+$, $^1H_2{}^{16}O^+$, $^{24–26}Mg^+$, $^{28}N_2^+$, $^{14}N^{16}O^+$, $^{32–34}S$, $^{35–37}Cl$, $^{40}Ar^+$, $^{40}Ar^1H^+$, $^{40}Ar^{16}O^+$, $^{38}Ar^{17}O^1H^+$, $^{56}Fe^+$
63.4–68.0	$^{63}Cu^+$, $^{65}Cu^+$, $^{64}Zn^+$, $^{68}Zn^+$
79.4–81.7	$^{40}Ar_2^+$

Table 4 SmartGate setting for analysis of lyophilized otter tissues

Range m/z	interfering ion
11.5 – 48.2	$^{12}C^+$, $^{13}N^+$, $^{16}O^+$, $^{17}OH^+$, $^{18}H_2O^+$, $^{18}N_2^+$, $^{30}NO^+$, $^{32}O_2^+$, $^{32}S^+$, $^{40}Ar^+$, $^{41}ArH^+$, $^{80}Ar^{2+}$
55.5 – 57.5	$^{56}ArO^+$, $^{56}Fe^+$
79.4 – 80.7	$^{40}Ar_2^+$
62.6 – 65.1	$^{63}Cu^+$, $^{65}Cu^+$
84.5 – 85.3	$^{85}Rb^+$

Rhodium (1 µg L^{-1}) as an internal standard was added to all solutions (blank, samples and calibration standards) to compensate instrumental drift and matrix effects during ICP-oa-

TOF-MS analysis. Internal standard rhodium was chosen as the most appropriate element due to its absence in all matrices, average ionization potential, and m/z in the middle of the mass spectrum.

Carbon residues in otter tissues and matrix elements in pet food were analyzed by ICP optical emission spectrometer GBC Integra XL 2 (GBC Scientific Equipment) [78], for detail information of instrument settings see ref. [79]. The range of calibration was 0–200 mg L^{-1} for K, 0–20 mg L^{-1}, 0–50 mg L^{-1} C; 0–5 mg L^{-1} Cu, Fe, Mn, 0–1 mg L^{-1} Ba and 0–10 mg L^{-1} for Ca, Na and P.

Double beam atomic absorption spectrometer SensAA (GBC Scientific Equipment) was used to determine Na, Ca, Mg, K, Fe and Zn in infant formula and Na and Mg in otter liver tissue and Si in coal fly ash. The setup parameters of the spectrometer and the calibration ranges are shown in table 5.

Table 5 Operating conditions of AAS/AES spectrometer

Analyte	Wavelength (nm)	Lamp current (mA)	Spectral width (nm)	Flame (Flow rates – L min^{-1})	Calibration range (mg L^{-1})
Mg [a]	285.2	5.0	0.2	C_2H_2 (1.5)/air (10)	0–0,4
Ca [a]	422.7	5.0	0.5	C_2H_2 (1.5)/air (10)	0–20
K [b]	766.5	6.0	0.5	C_2H_2 (1.5)/air (10)	0–20
Na [b]	589.0	5.0	0.5	C_2H_2 (1.5)/air (10)	0–20
Zn [a]	213.9	5.0	0.2	C_2H_2 (1.5)/air (10)	0–0,8
Fe [a]	248.3	7.0	0.2	C_2H_2 (1.5)/air (10)	0–4
Si [a]	251.6	15.0	0.2	C_2H_2 (5.1)/N_2O (10)	0–100

[a] Absorption measuring mode
[b] Emission measuring mode

2.3.List of analyzed samples

2.3.1. Certified reference materials

Bovine Liver SRM® 1577c (National Institute of Standards & Technology, USA), Milk Powder NCR ZC73015 (China National Analysis Center for Iron and Steel, China), Spiked Skim Milk Powder – Trace elements BCR RM No. 150 (Institute for Reference Materials and Measurements, Belgium), BCR®-032 Natural Moroccan Phosphate Rock (Institute of Reference Materials and Measurements IRMM, Belgium), Sludge from city water treatment WH-T No. 12-3-14 (Slovak Institute of Metrology, Slovak Republic), BCR®-060 Aquatic Plant (Lagarosiphon major, IRMM, Belgium), Reference material No. 12-2-03 P-Alfapa Essential and toxic elements in Lucerne (Slovak Institute of Metrology, Slovak

Republic), NCS DC 73350 Leaves of Poplar (China National Analysis Center for Iron and Steel, China), GBW 07603 and GBW 07602 Bush twigs and leaves (Institute of Geophysical and Geochemical Exploration, Langfang China), Constituent Elements in Coal Fly Ash SRM® 1633b (National Institute of Standards &Technology, USA) a Fly Ash CTA-FFA-1 (Institute of Nuclear Chemistry and Technology, Poland).

2.3.2. Real samples

Twenty-one samples of powdered infant milk based on cow's milk and one soybean-based infant milk sample were obtained in the period 2015–2016. Most of these formulas come from multinational companies (Danone, Hipp, Nestlé, etc.) and are available worldwide. Samples of dry pet food were received from different breeders during year 2016, and the samples included different brands of international companies and local producers of dry pet food. In sum, 149 samples of otter tissues (*Lutra lutra*) were provided by ALKA Wildlife (Czech Republic) [67]. Tissue samples were obtained from dead otters from the territory of the Czech Republic in the years 2006–2015. Records of age, sex, cause of death, date and location of death were made for each individual otter. Tissues were stored in a PE bag and were frozen at -18 °C.

2.4.Sample preparation

2.4.1. Optimization of MAE procedure for samples with variable amount of silicate matrix

Prior to multielement analysis of fly ash by ICP-oa-TOF-MS the MAE procedure of samples with variable amount of silicate matrix was optimized. The quantity of **50**, 100 or 150 mg of sample (BCR®-032 Natural Moroccan Phosphate Rock) were weighed into 10 mL MT-tubes along with 2, **5** or 7 mL NH_4F (100 g L^{-1}). The tubes were then placed in a 100 mL Teflon digestion vessel DAC-100 with 20 mL of NH_4F (100 g L^{-1}). The level of NH_4F is higher in the 100 mL digestion vessel than in the PFA MT-tubes. By this the vapor pressures are compensated and evaporation of the solution from the MT-tubes is prevented [80]. Using the following program Si from samples was extracted: 5, 10 or **15** minutes at 140, 160, 180 °C (with ramp set at 3 min, 50% generator power). Selected values of each parameter used for the microwave assisted extraction of all samples are highlighted. After

MAE resulting extract was filtered through a 0.45 μm syringe filter (Whatman Autovial) and diluted with deionized water to a volume of 10 mL.

2.4.2. Multielement analysis of coal fly ash

The certified reference material Fine Fly Ash CTA-FFA-1 was employed for optimization of the MAE procedure. A portion of approximately 250 mg of this sample was at first ground in the vibration mill Wig-L-Bug 30 (Crescent Dental Mfg. Co.) for reassurance of the sample homogeneity. Then, 10 mg of the CTA-FFA-1 were weighted into the MT-tubes and NH_4F was added at constant volume of 5 mL with different concentration of NH_4F being 60, 100 and **140** g L^{-1}, respectively, which corresponds to the absolute amount of 300, 500 and **700** mg of NH_4F in MT-tubes. These MT-tubes were placed into outer digestion vessel, where 20 mL of appropriate concentration of NH_4F were added. The level of NH_4F is higher in the outer vessel than in the PFA tubes. Extractions were performed at the temperature of 160, 180 or **200** °C held for 5, **10** or 15 minutes (with a ramp set at 5 min at 50% power MWS-3^+). Selected values of each parameter used for the MAE of all samples are highlighted. The resulting solutions were filtered through a 0.45 μm syringe filter and diluted to 10 mL with deionized water.

2.4.3. Analysis of powdered infant formula

Amount of 500 ± 5 mg infant formula in triplicate were weighed into 100 mL digestion vessel prior to adding 5 mL HNO_3 (65%, w/w) and 2 mL H_2O_2 (30% w/w). The mixture was microwave heated following 5 stage digestion program: (i) 10 min at 170 °C and 60% power, (ii) 15 min at 220 °C and 70% power and (iii-v) 1 min at 100 °C and 10% power. Resulting transparent solution was diluted to 25 mL with deionized water.

2.4.4. Analysis of dry pet food

Dry pet food in portion of 1 g was placed into sterile glass containers and deep frozen at -80 °C for 24 hours then lyophilized using the following temperature program: Step I: pressure 0 kPa for 1 hour; Step II: 0,1 kPa for 24 hours; Step III: 0 kPa pressure for 4 hours. Step II was optimized for 3 different periods of time 12, 24 and 48 hours. As will be discussed below, the chosen time was 24 hours. Approximately 400 mg of dry pet food was weighed

and placed into a microwave digestion vessel with 2.5 mL of HNO_3 (65%, w/w) and 1 mL of H_2O_2 (30% w/w) and 3.5 mL deionized water to comply with the minimum set volume of the digestion vessel. The samples were subjected to a microwave program consisting of five steps: (i) 10 min at 170 °C and 60% power, (ii) 15 min at 200 °C and 60% power and (iii-v) 1 min at 100 °C and 10% power. Resulting transparent solutions were diluted to 25 mL with deionized water.

2.4.5. Analysis of tissues from Eurasian otters

About 0.5 g of thawed well blotted tissue was placed into sterile glass containers and deep frozen at -80 °C for 24 hours then lyophilized using the following temperature program: Step I: pressure 0 kPa for 1 hour; Step II: 0,1 kPa for 24 hours; Step III: 0 kPa pressure for 4 hours. Step II was optimized for 3 different period of time 12, 24 and 48 hours. As will be discussed below, the chosen time was 24 hours. Lyophilized samples were weighed (50 or **200** mg) to each MT-tubes and mixture of **1 mL HNO_3 (65%, w/w) + 1 mL of H_2O_2 (30% w/w)** or 1 mL HNO_3 (65%, w/w) + 0.4 mL H_2O_2 was added. MT-tubes were transferred to a digestion vessel (100 mL) with 15 mL mixture of HNO_3 and H_2O_2 ratio according the mixture used in MT-tubes. Selected values of each parameter used for decomposition of all samples are highlighted. The samples were subjected to a microwave program consisting of five steps: (i) 5 min at 130 °C and 20% power, (ii) 10 min at 160 °C and 40% power, (iii) 15 min at 200 °C and 60% power and (iv-v) 1 min at 100 °C and 10% power. As will be discussed below, step (iii) was optimized in the range of 180–220 °C and 5–20 min, the selected value was 15 min at 200 °C. The resulting colorless solutions were diluted to 10 mL with deionized water.

3. Results and discussion

3.1. Coal fly ash

3.1.1. Utilization of NH_4F for aluminosilicate matrix

Utilization of NH_4F for aluminosilicate matrix was evaluated by analysis of variety of samples such as plants, fertilizers and sludge samples [81] than the potential of ammonium fluoride for extraction of number of elements from fly ash samples prior to ICP-oa-TOF-MS analysis was investigated for the first time. Optimization of procedure was performed

by BCR®-032 certified reference material for analysis of samples with variable amount of silicate matrix. The optimal conditions were set in order to ensure robustness of the method and the following parameters were chosen as optimal: 50 mg sample extraction or in case of coal fly ash 10 mg volume 5 mL and extraction time 15 min at 180 °C. Under these conditions the reference materials of BCR®-032 Natural Moroccan Phosphate Rock, Sludge WH-T No. 12-3-14, Aquatic Plant BCR®-060, P-Alfapa CRM No. 12-2-03, Leaves of poplar NCS DC 73350, Bush twigs and leaves GBW 07603 and GBW 07602, Coal Fly Ash SRM® 1633b a Fly Ash CTA-FFA-1 were prepared and silicon was measured by AAS. Table no. 6 summarized the obtained data, where in all cases the quantitative extraction of Si from sample matrix was achieved. Lower value of Si in certified reference material of Aquatic Plant BCR®-060 (28.5 g kg^{-1}) is only indicative and the measured value (24.0 ± 0.7 g kg^{-1}) was close to previously reported concentration (24 , 9 ± 0.4 g kg^{-1}) [82]. For detailed description of this procedure see ref [81].

Table 6 Results of measurements of Si in selected certified reference materials

	Silicon			
Reference material	**Certified** [a] **(g kg^{-1})**	**Found** [a] **(g kg^{-1})**	**R** [b] **(%)**	**RSD** [c] **(%)**
Fertilizer BCR®-032	9.8 ± 0.56	9.9 ± 0.3	101	1.5
Sludge WT-H No. 12-3-14	82 [d]	77.4 ± 1.3	94	1.0
Aquatic plant BCR®-060	28.5 [d]	24.0 ± 0.7	84	1.5
P-ALFALFA	3 [d]	3.1 ± 0.3	103	4.8
Leaves of poplar NCS DC 73350	7.1 ± 0.8	6.6 ± 0.3	93	2.2
Bush twigs and leaves GBW 07603	5.8 ± 0.4	5.7 ± 0.2	98	1.8
Bush twigs and leaves GBW 07602	6.0 ± 0.7	5.8 ± 0.2	97	1.7
Fine Fly Ash CTA-FFA-1[e]	224.8 ± 9.2	216.3 ± 4.1	96	1.0
Coal Fly Ash NIST SRM 1633b [e]	230.2 ± 0.8	230 ± 18	100	3.9

[a] Mean ± 2 SD (n = 3); [b] Recovery expressed as ratio measured value to certified × 100; [c] standard deviation of three independent measurements; [d] Indicative value; [e] Amount of sample 10 mg.

3.1.2. Optimization of MAE for multielement analysis of coal fly ash by ICP-oa-TOF-MS analysis

The effects and significance of the variables in the extraction process were evaluated using Pareto's charts, including a vertical line that corresponds to the 95% limit which indicates statistical significance. From all of the elements present in the calibration standards, accurate and precise results were obtained for 11 of them based on the analysis of CRMs as will be discussed later in the text. The example of a Pareto's chart is given for tungsten.

As can be seen from figure 1, for the extraction of W, the extraction efficiency is improved by improving the extraction time and temperature. The time of the extraction has the most positive effect on the extraction efficiency. In addition, significant interaction between the extraction temperature and the mass of the extractant was observed (see figure 1). The significant variables for the other elements are given in table 7. As can be seen from figure 2, sufficient extraction temperature and time for determination of W are 180 °C and 10 min, respectively (the certified value for W is 10.5 ± 1.1 mg kg^{-1}). Finally, 200 °C was chosen as at this temperature good agreement between the certified and obtained value was observed for all the elements of interest.

Table 7 Statistically significant variables evaluated using Pareto's charts for the selected elements

Element	Variable	Element	Variable
Li, Rb, U	Temperature, Time, Amount of NH_4F	**As, W**	Temperature, Time,
Be, Se, Cs, Tl	Time, Amount of NH_4F	**Sb, Ni**	Amount of NH_4F

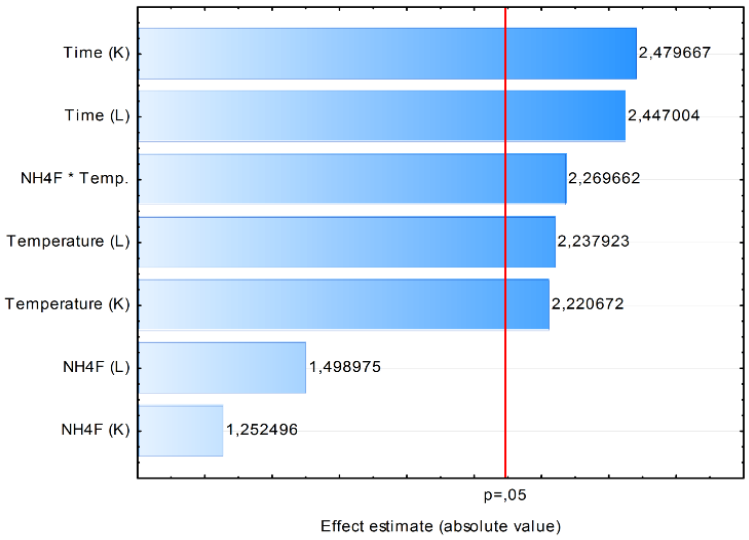

Figure 1 Pareto chart of the standardized effects of the fractional factorial design 3^{4-1} (n = 3). The L and K letters indicate linear and quadratic effect of the factor, respectively.

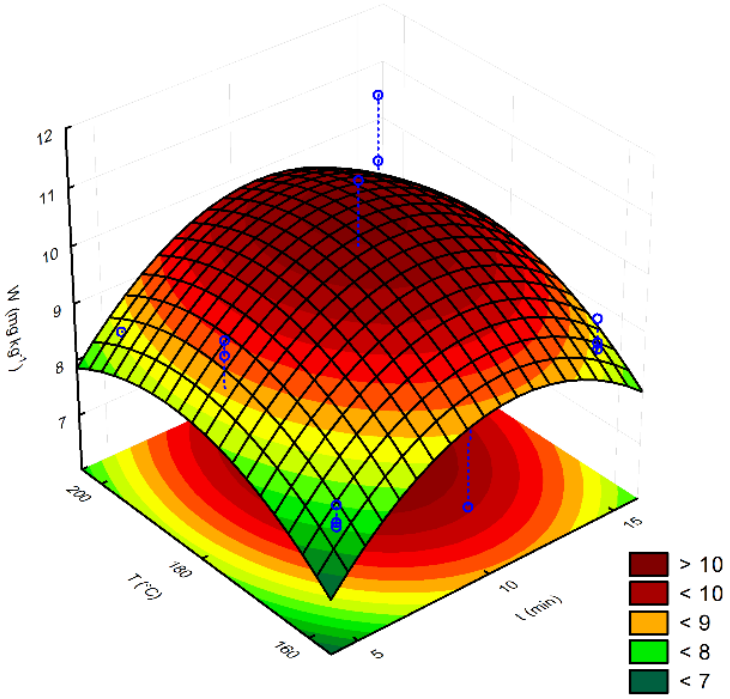

Figure 2 Response surfaces from 3^{4-1} design

In order to find the best mathematical models that correlate the variables and the analytical response, all possible subsets regression available in QC.Expert^{TM} program were performed. For each possible combination of model terms the following criteria: MEP (mean squared prediction error), coefficient of determination (also referred to as the R^2 value), Akaike's information criterion (AIC) or F-statistic were monitored, as a good model should have large value of F and R^2, small MEP value and small AIC value [83, 84]. Based on these criteria, a suitable mathematical model was constructed to fit the data and the reality of the situation the best (data not shown here).

3.1.3. Analytical performance

Two certified reference materials were employed to assess the trueness and precision of the proposed method: Coal fly ash CRM 1633b and Fine fly ash CTA-FFA-1. From all of the tested elements good agreement between found values and certified ones were found for 11 elements Li, Be, Ni, As, Se, Rb, Sb, Cs, W, Tl and U (see table 8). Statistical comparison by paired *t*-test (QC expert) showed no significant difference between certified and found values at significance level of 0.05 for both CRM CTA-FFA-1 ($p = 0.226$) and SRM 1633b ($p = 0.482$). The precision of the method was calculated as relative standard

deviation (RSD) of three independent measurements (each done in 10 replicates) varied from 0.2% to 8.2% for CRM CTA-FFA-1 and for SRM 1633b from 1.5% to 7.9% in dependence on element (see table 8). No significant matrix effects during the determination of ultra-trace elements in fly ash were observed, which was confirmed by comparison of the slopes of aqueous calibration with the slopes of the standard addition method (data not shown here). Most of the matrix elements remain in solid phase and were filtrated prior ICP-MS analysis thus the possibility of matrix effects or polyatomic interferences was reduced.

Limits of detection of the proposed method were calculated according to IUPAC criteria based on two times the standard deviation of ten replicates of the blank for each isotope and taking into account sample weight (10 mg sample) and dilution 10 mL (see table 8). The LODs for all elements were below 1 mg kg^{-1} and were satisfactory for the determination of the selected elements at the concentration levels of the analytes certified in the reference materials.

Table 8 Determination of total concentration of selected elements in CRMs CTA-FFA-1 and SRM 1633b using the developed method

		CTA-FFA-1				SRM 1633b			
	LOD[a] (mg kg^{-1})	Certified[b] (mg kg^{-1})	Found[b] (mg kg^{-1})	R[c] (%)	RSD (%)	Certified[b] (mg kg^{-1})	Found[b] (mg kg^{-1})	R[c] (%)	RSD (%)
$^{7}Li^{+}$	0.079	128±22	133±4	104	1.6	N/A	187±24	N/A	6.3
$^{9}Be^{+}$	0.051	27	28±3	104	5.3	N/A	12.3±1.7	N/A	7.0
$^{60}Ni^{+}$	0.22	99±6	95±15	96	8.2	120.6±1.8	121.9±4.2	101	1.7
$^{75}As^{+}$	0.079	53.6±2.7	51.0±2.9	95	2.9	136.2±2.6	133.7±1.4	98	2.3
$^{78}Se^{+}$	0.46	4.6	4.7±0.7	102	6.9	10.2±0.2	10.2±1.6	100	7.9
$^{85}Rb^{+}$	0.51	185±5	184±11	99	3.0	140	143.1±4.2	102	1.5
$^{121}Sb^{+}$	0.036	17.6±2.5	16.8±1.6	96	4.9	6	6.2±0.6	103	4.5
$^{133}Cs^{+}$	0.003	48.2±2.3	45.6±2.3	95	2.5	11	10.0±0.9	91	4.7
$^{182}W^{+}$	0.047	10.5±1.1	11.1±0.7	106	2.9	5.6	5.6±0.2	100	2.2
$^{205}Tl^{+}$	0.003	N/A	7.3±0.2	N/A	0.2	5.9	5.5±0.2	93	6.3
$^{238}U^{+}$	0.001	15.1±0.8	14.3±1.5	95	5.1	8.8±0.4	8.4±0.2	96	5.8

N/A Not available; [a] LODs were calculated with 10 mg sample weight and 10 mL sample volume; [b] Mean ± 2 S.D. (n = 10); [c] Recovery (%) expressed as (the ratio of observed value obtained from an analytical process via a calibration graph/ the reference value) × 100.

Using the full capacity of the eight-position carousel in combination with the Multi Tube system allowed extracting 24 samples in one run, which took (including weighing, extraction, vessel cool down, and transfer of the extracts) approximately 80 min.

The extraction step took only 10 min, which is considerably less compared to conventional digestion procedures, which usually need 60 min for the digestion step.

3.2. Powdered infant formula

3.2.1. Accuracy and detection limits

Table 9 Results of selected element concentration in Certified Reference Material NCS ZC73015 Milk Powder

TOF-ICP-MS	LOD (µg kg^{-1})	Concentration [a] (mg kg^{-1})		R [b] (%)	RSD [c] (%)
		Certified	Found		
$^{7}Li^{+}$	0.455	40 [d]	39.43 ± 0.85	99	1.08
$^{59}Co^{+}$	0.710	30 ± 7	27.86 ± 1.24	93	2.23
$^{63}Cu^{+}$	0.515	510 ± 130	430 ± 21	84	2.44
$^{75}As^{+}$	13.68	31 ± 7	28.13 ± 3.41	91	6.06
$^{78}Se^{+}$	0.405	110 ± 30	97.76 ± 7.45	89	3.81
$^{85}Rb^{+}$	0.110	11600 ± 700	11999 ± 265	103	1.10
$^{88}Sr^{+}$	0.480	5300 ± 600	4941 ± 137	93	1.39
$^{98}Mo^{+}$	0.230	280 ± 30	297 ± 25	106	4.21
$^{121}Sb^{+}$	0.120	6 [d]	6.76 ± 0.68	113	5.03
$^{133}Cs^{+}$	0.047	34 ± 5	31.5 ± 1.24	93	1.97
$^{138}Ba^{+}$	0.740	1000 ± 300	956 ± 70	96	3.66
$^{139}La^{+}$	0.080	2.5 [d]	2.73 ± 0.33	109	6.04
$^{140}Ce^{+}$	0.055	4 [d]	3.35± 0.47	84	7.01
$^{208}Pb^{+}$	0.055	70 ± 20	50.6 ± 2.1	72	2.08
$^{238}U^{+}$	0.011	3 [d]	2.74 ± 0.15	91	2.74
AMA 254	**LOD (µg kg^{-1})**	**Concentration [a] (mg kg^{-1})**		**R [b] (%)**	**RSD [c] (%)**
		Certified	**Found**		
Hg	0.10	2.2 [d]	1.81 ± 0.28	82	7.73
FAAS/FAES	**LOD (mg kg^{-1})**	**Concentration [a] (mg kg^{-1})**		**R [b] (%)**	**RSD [c] (%)**
		Certified	**Found**		
Na	0.45	4700 ± 300	4845 ± 350	103	3.61
K	5.0	12500 ± 500	12428 ± 1479	99	5.95
Ca	6.0	9400 ± 300	9603 ± 367	102	1.91
Mg	2.5	960 ± 70	908 ± 17	95	0.94
Fe	4.0	7.8 ± 1.3	7.1 ± 0.2	91	1.41
Zn	2.0	34 ± 2	27.9 ± 0.3	82	0.61

[a] Mean value ± 2 SD (n = 3); [b] Analytical recovery R (%) expressed as (ratio found value /certified value) × 100; [c] Relative standard deviation (%) of three independent measurements; [d] Information values.

As mentioned previously the method for simple and direct analysis of milk was validated in our laboratory [47] but determination of some toxicologically important elements was not possible. Therefore the complete destruction with HNO_3 and H_2O_2 by microwave mineralization was done in this work. Internal standard rhodium was chosen as the most

appropriate element due to its absence in matrix, average ionization potential, and m/z in the middle of the mass spectrum. Commercially available SRM Milk Powder NCS ZC73015 was analyzed to evaluate trueness and precision of the proposed method (the results are shown in table 9). An aqueous calibration with Rh as an internal standard was used to analyze certified reference materials and samples. Calcium containing polyatomic ions ($^{43}Ca^{16}O^{+}$, $^{40}Ca^{35}Cl^{+}$, or $^{40}Ca^{37}Cl^{+}$) among others can be expected during analysis of milk that interfere the determination of $^{59}Co^{+}$, $^{75}As^{+}$, and $^{77}Se^{+}$. It can be seen from table 9 that for the elements $^{59}Co^{+}$ and $^{75}As^{+}$ the correct results were obtained. The isotope of $^{78}Se^{+}$ was chosen instead of $^{77}Se^{+}$ for determination of selenium in powdered milk samples. The precision of the proposed method expressed as relative standard deviation in terms of intra-day comparison was better than 12%. Detection limits were comparable to those previously published for the analysis of milk powder using Q-ICP-MS [20, 54, 58, 62].

3.2.2. Statistical data treatment

The method was used to analyze 21 powdered infant formulas (artificial baby milk). The obtained data (not shown here) were subjected to exploratory and discriminant analysis employing Statistica 12 (StaSoft Inc.). Based on results from the exploratory analysis the exponential transformation of the data was performed. Because of the nature of the experiment, the asymmetry cannot be eliminated by removing outliers. This data contains information of which loss could lead to a misinterpretation of the results and the loss of valuable information. Discrimination and canonical correlation analysis were done taking into account the category in which artificial nutrition is classified in the Czech Republic (premature formula, newborn formula, follow-up infant formula, toddler formula). The forward stepwise analysis was employed to identify subset of variables that best predict the discrimination between classes. Each class was discriminated by elements Li, Na, Sr, Co, Mg, Zn and Cu (table 10). This subset of elements was utilized for canonic correlation analysis.

Table 11 shows the grouping of elements to 4 classes. According to classification matrix (table 12) 100% of cases were correctly classified in each group by the current classification functions. Canonical correlation analysis was employed to assess the relationship between classes. There are 3 discrimination functions displayed in table 13 where first two of them were statistically significant. Values in table 14 represent the correlations between the variables and the discriminant functions.

Table 10 Results of Discrimination analysis after 4th step

	Wilks´ Lambda	Partial Wilks´ Lambda	Partial F values ((3,2))	p-value	Toler.	1-toler. (R^2)
Na	0.030707	0.093027	35.74832	0.000006	0.188041	0.811959
Sr	0.006859	0.416474	5.13741	0.018348	0.157637	0.842363
Li	0.022767	0.125474	25.55598	0.000029	0.027910	0.972090
Co	0.043864	0.065125	52.63558	0.000001	0.044149	0.955851
Mg	0.032425	0.088099	37.95296	0.000004	0.073434	0.926566
Zn	0.019123	0.149382	20.87890	0.000076	0.075398	0.924602
Cu	0.008292	0.344510	6.97647	0.006763	0.177780	0.822221

Table 11 Parameters estimation of classification function for each class

	Class 1 (p=0.09524)	Class 2 (p=0.47619)	Class 3 (p=0.28571)	Class 4 (p=0.14286)
Na	0.48	0.30	0.35	0.35
Sr	0.20	0.12	0.14	0.15
Li	-39.14	-25.28	-28.75	-28.96
Co	68.90	44.81	51.51	51.26
Mg	4.59	3.01	3.46	3.42
Zn	-30.84	-20.07	-23.09	-22.75
Cu	0.17	0.11	0.13	0.12
Absolute values	-2492.25	-1048.34	-1371.12	-1386.26

Table 12 Classification matrix: rows represent observed classification and columns predicted classification

	% (correct)	Class 1 (p=0,09524)	Class 2 (p=0,47619)	Class 3 (p=0,28571)	Class 4 (p=0,14286)
Class 1	100	2	0	0	0
Class 2	100	0	10	0	0
Class 3	100	0	0	6	0
Class 4	100	0	0	0	3
Total	100	2	10	6	3

Table 13 Chi square test of successive roots

Roots remove	Eigen- value	Canonical R	Wilks´ Lambda	Chi-Sqr.	df	p-value.
0	63.28586	0.992192	0.002857	84.94279	21	0.000000*
1	1.89365	0.808959	0.183639	24.57436	12	0.016974*
2	0.88187	0.684554	0.531386	9.16786	5	0.102553

* statistically significant parameter

Table 14 Factor structure matrix

	Na	Sr	Li	Co	Mg	Zn	Cu
Root1	-0.19698	-0.08247	-0.01422	-0.06265	-0.11577	-0.06791	-0.02582
Root2	0.31097	0.55170	0.17640	0.07523	0.07775	0.38032	-0.00087

The distribution of found classes is shown in figure 3 - Scatterplot of canonical scores. Milk nutrition for premature formula (Class 1) is plotted much further to the left in scatterplot, specifically sample number 1 and 8. Class 4 is differentiated by second discrimination function from others classes and includes samples number 12, 16 and 18. The largest group consists of toddler formula (Class 3) and follow-up infant formula (Class 2).

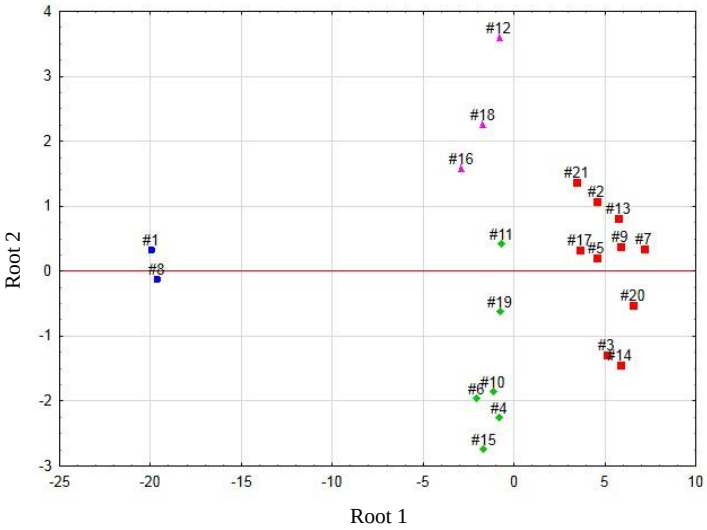

Figure 3 Scatterplot of canonical scores: (●) Class 1, (■) Class 2, (♦) Class 3, (▲) Class 4

3.3. Pet foods

3.3.1. Optimization of parameters for pet food analysis

Lyophilization efficiency was evaluated based on the differences in dry matter of sample. By comparison with the Student´s *t*-test no significant variance between the lyophilization time of 12 and 48 hours was found ($p = 0.362$) at the significance level of 0.05. To ensure sufficient robustness, a lyophilization g time of 24 hours was chosen. The proportion of water in the samples was not more than 5%. Decompositions of pet foods samples were at first performed with an already optimized procedure used for food decomposition [79] where a mixture of 65% HNO_3 and 30% H_2O_2 was applied. Then parameters such as the volume and concentration of nitric acid and hydrogen peroxide, the number of decomposition steps, the ramp and temperature hold, or the performance of the generator were optimized. In the resulting single-step MWD program, the temperature of 220 °C for 25 minutes with 30% (w/v) nitric acid and 5% (w/v) hydrogen peroxide was sufficient for the mineralization of the samples (400 ± 10 mg). Thus, the decomposition time

was reduced by about half, and at the same time reducing the acidity of the resulting solution, which is more suitable for inductively coupled plasma instrumentation [52]. During ICP-OES measurements elements were firstly analyzed at 2 wavelengths in order to detect possible spectral interferences in samples of beef, poultry and fish granules. Based on pair *t*-test (significance level 0.05) differences between two wavelengths were found insignificant ($p = 0.172$). According to results from analysis of CRM NIST 1577c Bovine Liver and pair *t*-test no spectral interferences were detected. Only one wavelength was employed for measurement thanks to that the time of analysis was shortened by half. The wavelength was selected based on its sensitivity. There was found no differences between slopes of aqueous calibration and standard addition or matrix matched standards and aqueous calibration could be used for the ICP-OES analysis. During ICP-oa-TOF-MS analysis the attention was focused on "SmartGate" settings. In case of analytical response higher than 10^7 counts (acquisition time 1s) in mass spectrum 6–238 m/z the signals were removed due to protection of detector. These signals related to matrix elements (Fe, Cu, Zn and Sr) in undiluted sample solution and in case of ten times diluted sample solution the signals of isotopes of Zn were extracted from mass spectrum. Internal standard rhodium was chosen as the most appropriate element due to its absence in matrix, average ionization potential, and m/z in the middle of the mass spectrum.

3.3.2. Accuracy

During ICP-MS analysis of dried pet food, it can be expected that determination of some elements will be complicated by the presence of polyatomic ions of Na, K, Ca, Mg, Cl, C, S, P, etc. Commercially available certified reference material NIST 1577c Bovine liver was analyzed to evaluate trueness and precision of the proposed method. The results are shown in table 15. Good agreement between certified and measured values was found even for the elements below m/z <100 the correct results were obtained. Also the solutions of samples were spiked, based on analytical recovery the trueness was evaluated and ranged from 86 to 115 %. The precision of the suggested method expressed as relative standard deviation in terms of intra-day comparison was better than 7%.

Table 15 Results of concentrations of selected elements in the certified reference material NIST 1577c Bovine Liver

TOF-ICP-MS	Concentration [a] (µg kg^{-1})		R [b] (%)	RSD [c] (%)
	Certified	Certified		
$^{7}Li^{+}$	12 [d]	12.8 ± 0.5	104	2.03
$^{52}Cr^{+}$	53 ± 14	55.4 ± 0.8	105	0.73
$^{58}Ni^{+}$	44.5 ± 9.2	45.8 ± 10.2	103	11
$^{59}Co^{+}$	0.300 ± 0.018 [e]	0.296 ± 0.006 [e]	99	1
$^{75}As^{+}$	19.6 ± 1.6	20.0 ± 2.4	102	6
$^{78}Se^{+}$	2.031 ± 0.045 [e]	1.994± 0.068 [e]	98	2.76
$^{85}Rb^{+}$	35.3 ± 1.1 [e]	33.8 ± 0.7 [e]	96	1.1
$^{88}Sr^{+}$	95.3 ± 4.2	99.3 ± 3.2	104	1.63
$^{98}Mo^{+}$	3.3 ± 0.13 [e]	3.3 ±0.16 [e]	100	2.45
$^{121}Sb^{+}$	3.13 ± 0.31	3.304 ± 0.28	106	4.31
$^{133}Cs^{+}$	21.7 ± 1.4	20.4 ± 0.88	94	2.15
AMA 254	**Concentration [a] (µg kg^{-1})**		**R [b] (%)**	**RSD [c] (%)**
	Certified	**Certified**		
Hg	5.36 ± 0.17	4.9 ± 0.42	92	7.06
ICP-OES	**Concentration [a] (µg kg^{-1})**		**R [b] (%)**	**RSD [c] (%)**
	Certified	**Certified**		
Na	0.2033 ± 0.0064 [f]	0.2024 ± 0.0029 [f]	100	1.5
P	1.175 ± 0.027 [f]	1.175 ± 0.0085 [f]	100	1
Ca	131 ± 10	126 ± 12	96	5.3
K	1.023 ± 0.064 [f]	1.026 ±0.076 [f]	100	3.7
Mg	620 ± 42	626 ± 12	101	1
Fe	198 ± 0.65	198 ± 1	100	0.3
Mn	10.46 ± 0.47	10.9 ± 0.13	104	1.3
Zn	181.1 ± 1	180 ± 5.6	99	1.55
Cu	275.2 ± 4.6	276.6 ± 4.43	101	1
Na	0.2033 ± 0.0064 [f]	0.2024 ± 0.0029 [f]	100	1.5
P	1.175 ± 0.027 [f]	1.175 ± 0.0085 [f]	100	1

[a] Mean value ± 2 SD (n = 3), [b] Recovery R (%) expressed as ratio measured concentration to certified × 100, [c] Relative standard deviation (%) of three independent measurement, [d] Information values, [e] mg kg^{-1}, [f] %.

3.3.3. Statistical data treatment

The method was used to analyze 48 dry pet foods and 3 treats for cats and dogs. The obtained data (not shown here) were subjected to exploratory, discriminant and factorial analysis. The assumption of normality was not fulfilled in case of selection of Hg, Na, Ca, Mg, Fe, Ba, Al, Li, V, Cr, Ni, Co, Ga, Nd, Eu, Gd, Dy, Ho, Er, Th and U in the first group of analyzed samples and in the second group for Li, Sb, Cs, Ce, Pr, Nd, Eu, Gd, Dy, Ho and Er. Before the discrimination analysis the Box-Cox transformation of data was performed. Based on forward stepwise analysis the best discriminatory variables were identified, the results after second step are summarized in table 16. Each class is discriminated

by elements Fe, Cr, Cs, Cu, Hg, Ba, Ca and P. In table 17, it is possible to observe the grouping of elements to 2 classes by cat or dog food. According to classification matrix (table 18) by the current classification functions 100% of cases were correctly classified.

Table 16 Results of Discrimination analysis after 2nd step

	Wilks´ Lambda	Partial Wilks´ Lambda	Partial F values ((3,2))	p-value	Toler.	1-toler. (R^2)
Fe	**0,462612**	**0,829133**	**8,65531**	**0,005288**	**0,489334**	**0,510666**
Cr	**0,480283**	**0,798627**	**10,59025**	**0,002249**	**0,449240**	**0,550760**
Cs	**0,527587**	**0,727021**	**15,77000**	**0,000275**	**0,429725**	**0,570275**
Cu	**0,585463**	**0,655151**	**22,10732**	**0,000028**	**0,392372**	**0,607628**
Hg	**0,427365**	**0,897515**	**4,79589**	**0,034133**	**0,691051**	**0,308949**
Ca	**0,564823**	**0,679092**	**19,84734**	**0,000061**	**0,105124**	**0,894876**
P	**0,510939**	**0,750709**	**13,94713**	**0,000560**	**0,111607**	**0,888393**
Ba	**0,445659**	**0,860673**	**6,79900**	**0,012574**	**0,617621**	**0,382379**

Table 17 Parameters estimation of classification function for each class

	Class 1 (p=0,84314)	Class 2 (p=0,15686)
Fe	126,985	138,345
Cr	-1,979	-7,439
Cs	-88,455	-76,788
Cu	2,764	0,493
Hg	-0,067	0,207
Ca	0,447	0,287
P	-0,047	-0,035
Ba	-10,735	-5,642
Absolute values	-386,325	-382,437

Table 18 Classification matrix: rows represent observed classification and columns represent predicted classification

	% (correct)	Class 1 (p= 0.84314)	Class 2 (p=0.15686)
Class 1	100	43	0
Class 2	100	0	8
Total	100	43	8

Factor analysis was utilized to define the underlying structure in a data matrix [85]. Matrix of eigenvalues $L L^T$ is summarized in table 19. According to data in table 19

the proportion of variance was about 80% for first two factors. Factor 1, 2 and 3 accounted approximately 90% of variance.

Table 19 Matrix of Eigenvalues and related statistics after Varimax rotation processed by software Statistica 12

Index	Eingenvalue	Individual %	Cumulative %
1	9.510083	59.43802	59.43802
2	3.021990	18.88744	78.32545
3	1.552608	9.70380	88.02926

Table 20 shows factor loadings of three-factor rotated solution and the values for each factor indicate how strongly the factor was affected by particular variables. Substantial loadings on the first factor appeared for elements Al, Cs, Ce, Pr, Nd, Eu and Gd. Factor 2 showed substantial factor loadings of Hg, As, Se and Sr and factor 3 of V, Dy, Ho and Er.

Table 20 Factor loadings after Varimax rotation

Variable	Factor 1	Factor 2	Factor 3
Hg	0.024443	**0.932638**	-0.079572
As	0.182527	**0.914631**	0.060864
Se	0.051811	**0.720220**	0.091508
Sr	0.032650	**0.847493**	0.060765
Al	**0.911116**	0.070993	0.255046
V	0.352531	0.262174	**0.760777**
Cs	**0.829504**	0.324166	0.207532
Ce	**0.942748**	0.059481	0.280941
Pr	**0.938114**	0.037017	0.302961
Nd	**0.924243**	0.052483	0.333038
Eu	**0.763512**	0.016075	0.592170
Gd	**0.733218**	0.042967	0.652730
Dy	0.469505	0.000397	**0.866349**
Ho	0.325856	-0.019726	**0.934930**
Er	0.231049	-0.004613	**0.966425**
Th	0.655235	-0.006874	0.627046
Expl. Var.	6.233102	3.132409	4.719170
Prp. Totl.	0,389569	0,195776	0,294948

3.3.4. Comparison with EU limits

According to previously published data [66] higher concentration of uranium and thorium were found in dry pet food samples in our case the concentration was above 0.1 mg kg^{-1}. The highest uranium concentration (2.72 mg kg^{-1}) was determined in sample no. 58

and sample no. 57 for thorium 0.235 mg kg^{-1}. In sample no. 57 were also found high concentration of Al, Cs, Ce, Pr, Nd, Eu, Gd, Hg, V, Cr and Ni. This was a sample of cat granules with trout and smoked salmon of the American brand Taste of Wild®. Concentration of Hg (2 mg kg^{-1}) and As (0,4 mg kg^{-1}) were in all cases below values set by Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed [86].

3.4. Tissue analysis of Eurasian otter

3.4.1. Optimization of parameters for otter tissue analysis

The attention was focused on careful optimization of sample preparation including lyophilization and microwave mineralization. Mixture of HNO_3 and H_2O_2 was used for the decomposition of the organic matrix. Using the smallest amount of decomposition mixture HNO_3 and H_2O_2 together with maintaining the maximum efficiency of mineralization was one of the goals of sample preparation. Three variables were optimized to obtain dry matter of otter tissues: amount of the sample (200, 350 and 500 mg), type of tissue (bone, fat and liver) and time of lyophilization (12, 24 and 48 hours). According to three level fractional factorial design 3^{3-1} Pareto´s chart was created (not shown here). There was obvious effect of the linear and quadratic effect for the factor of the type of sample. Amount of sample and lyophilization time were not found to be statistically significant within the given interval at the significance level of 0.05. Based on the response surface (fig. 4) the optimum parameters were set as follows: 500 mg of sample and 24 hours of lyophilization time.

The influence of homogenization of samples and amount of samples for analysis was investigated by observing the resulting concentration of Hg. The significant differences were not found between homogenized samples and not homogenized samples according to two factor ANOVA analysis. But the amount of the sample had a significant influence on determination. The difference was identified between the results for amount of the sample 5 and 10 mg of sample, no significant differences were observed between results for 10 and 15 mg of sample. For the analysis by AMA 254 the minimum amount of 15 mg sample was used.

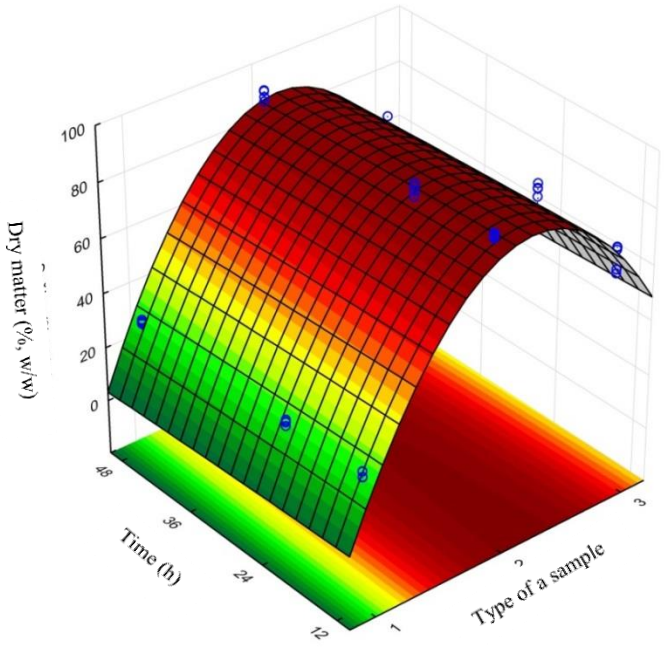

Figure 4 Response surface from 3^{3-1}

Condition of MWD was optimized by utilization of two level fractional factorial design with two replicates 2^{5-2}. The investigated parameters were: amount of sample (50 or 200 mg), type of sample (liver or fat tissue), decomposition temperature (180 or 220 °C), time of decomposition (5 or 20 min) and ratio of decomposition agent HNO_3:H_2O_2 (1:0,4 mL or 1:1 mL). The efficiency of microwave decomposition was evaluated by measurement of residual carbon in solution of sample. Concentration of carbon was determined by ICP-OES. The type of sample and the amount of reagents were identified based on the Pareto´s chart as statistically significant factors (see figure 5). Fat has a higher carbon content compared to liver tissue, hence the fat mineralization was less effective than liver tissue. Lower amount of residual carbon was measured with ratio of decomposition agent 1:1 mL HNO_3:H_2O_2. Optimal conditions were chosen: 50 mg sample, time and temperature of microwave mineralization for 15 minutes at 200 °C and decomposing agents 1 mL HNO_3 (65%, w/w) and 1 mL H_2O_2 (30%, w/w).

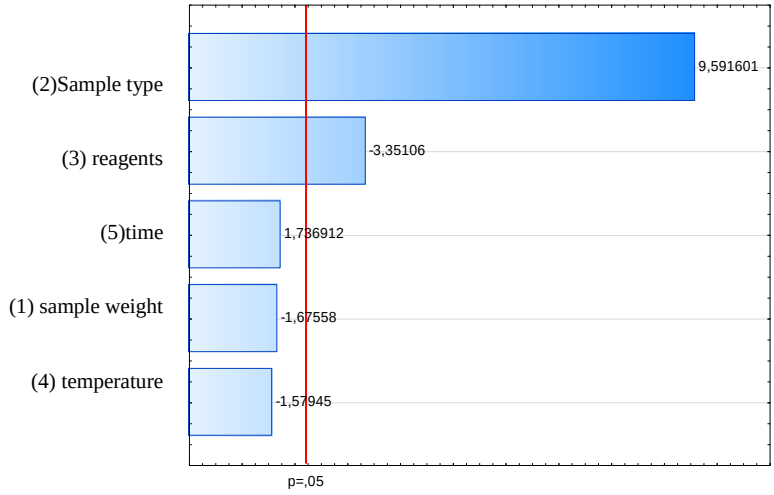

Figure 5 Pareto's chart of standardized effects of residual carbon determination in liver tissues depending on selected factors

3.4.2. Accuracy

Low concentration of most of elements in tissues of fat and bones was found and due to number of samples the complete analysis was done only for liver tissues as Livers organs that together with kidneys accumulate number of toxicologically important elements. Commercially available certified reference material NIST 1577c was analyzed to evaluate trueness and precision of the proposed method the results are shown in table 21. An aqueous calibration with Rh as an internal standard was used to analyze reference materials and samples. Good agreement between certified and measured concentration of elements was found. Solutions of sample were spiked and the trueness was evaluated by analytical recovery. The analytical recovery for all elements ranged from 85 to 107%. Accurate results were obtained even for elements such as Mn, Cu, As and Se which determination is problematic with quadrupole based spectrometer and collision cell technology [18, 75] or dynamic reaction cell [76] have to be used. Precision of elements expressed as relative standard deviation was better than 8%.

Table 21 Determination of selected elements in reference material NIST 1577c Bovine Liver

TOF-ICP-MS	Concentration [a] (μg kg^{-1})		R [b] (%)	RSD [c] (%)
	Certified	Found		
$^{7}Li^{+}$	12 [d]	12.5 ± 0.08	104	3.57
$^{55}Mn^{+}$	10.5 ± 0.5 [e]	10.5 ± 0.4 [e]	100	2.62
$^{59}Co^{+}$	300 ± 18	307 ± 32.4	102	2.27
$^{63}Cu^{+}$	275.2 ± 4.6 [e]	278.3 ± 9.1 [e]	101	3.27
$^{75}As^{+}$	19.6 ± 1.6	20.1 ± 0.17	103	3.03
$^{78}Se^{+}$	2.031 ±0.045 [e]	2.011 ± 0.05 [e]	99	0.92
$^{85}Rb^{+}$	35.3 ± 1.1 [e]	35.1 ± 0.8 [e]	99	1.54
$^{88}Sr^{+}$	95.3 ± 4.2	92.5 ± 2.11	97	2.41
$^{98}Mo^{+}$	3.3 ± 0.13 [e]	3.3 ± 0.07 [e]	101	1.6
$^{114}Cd^{+}$	97 ± 1.4	97.8 ± 5.4	101	2.16
$^{121}Sb^{+}$	3.13 ± 0.31	3.01 ± 0.3	96	5.28
$^{133}Cs^{+}$	21.7 ± 1.4	22.1 ± 0.8	102	2.04
$^{208}Pb^{+}$	62.8 ± 1.0	64.5 ± 12	103	6.63
AMA 254	**Concentration [a] (μg kg^{-1})**		**R [b] (%)**	**RSD [c] (%)**
	Certified	**Found**		
Hg	5.36 ± 0.17	4.9 ± 0.42	92	7.06
FAAS/FAES	**Concentration [a] (g kg^{-1})**		**R [b] (%)**	**RSD [c] (%)**
	Certified	**Found**		
Na	2.033 ± 0.064	1.93 ± 0.18	95	3.72
K	10.23 ± 0.64	9.72 ± 1.4	95	5.36
Mg	0.620 ± 0.042	0.612 ± 0.020	99	1.96

[a] Mean value ± 2 SD (n = 3); [b] Analytical recovery R (%) expressed as (ratio found value /certified value) × 100; [c] Relative standard deviation (%) of three independent measurements; [d] Information values ; [e] mg kg^{-1}.

3.4.3. Statistical data treatment

There were determined 33 elements in samples of 149 river otter tissues. Results were processed by exploratory and factor analysis. Based on exploratory analysis exponential transformation of data was performed. The highest concentration of Hg from dataset was identified in the sample no. 366 (18.9 mg kg^{-1}) and in the sample no. 478 (18 mg kg^{-1}). Critical concentration of Hg reported for otters was 30 mg kg^{-1} [87] in both cases (sample no. 366 and 478) the values of concentration Hg was not exceeded. Bad body condition and higher concentration of Pb for three samples of otters (no. 421, 419 and 431) indicated probable poisoning by Pb [67].

Table 22 Matrix of Eigenvalues and related statistics after Varimax rotation processed by software Statistica 12

Index	Eingenvalue	Individual %	Cumulative %
1	5,569855	55,69855	55,69855
2	2,421912	24,21912	79,91767

The factor analysis was used to reveal the internal structure in data set. Based on matrix of Eigenvalues (see table 22) first two factors describe 80% of data variability. The first factor is loaded by Co, Sb, Re, Pt, Tl and Th, and the second factor by variables of Pd, Hf, Ta and W. The described dependencies are also documented by a graph of factor loadings in figure 6. The scatter plot of factor scores for 149 samples can be seen in figure 7. Several clusters of samples were obvious there.The information of cadavers about age, cause of death, condition of organism is collected by ALKA Wildlife and data are being processed. It is not possible for now to confirm or excluded the relationship between samples in each cluster.

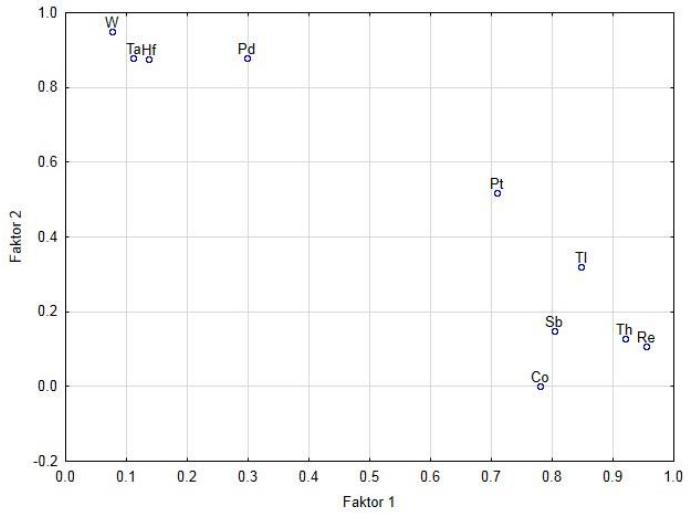

Figure 6 Factor loadings graph for 10 variables of concentration Co, Sb, Re, Pt, Tl, Th, Pd, Hf, Ta and W after data standardization

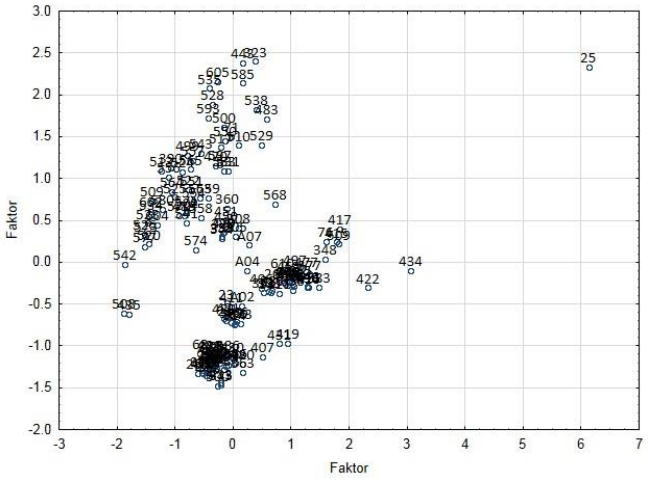

Figure 7 2D scatterplot of factor scores for 149 samples and 10 variables of concentration elements Co, Sb, Re, Pt, Tl, Th, Pd, Hf, Ta a W after data standardization

4. Conclusions

In this work, analysis of coal fly ash, powdered infant formulas, pet food, and tissues of Eurasian otter by the ICP-oa-TOF-MS spectrometer GBC OptiMass 8000 was introduced. Results of analyses of 21 powdered infant formulas, 51 pet foods and 149 tissues of otters were evaluated by univariate and multivariate statistical methods. A novel, simple, efficient and environmentally friendly microwave-assisted extraction (MAE) method using ammonium fluoride as the extractant prior to multielement analysis of fly ash samples by ICP-oa-TOF-MS was developed. This method avoids handling the corrosive and/or toxic acids like HF, HCl or $HClO_4$ being commonly used during sample preparation of the fly ash samples. The optimum extraction parameters evaluated using a fractional factorial design were as follows: 10 mg of the sample extracted with 5 mL of 140 g L^{-1} NH_4F for 10 min at 200 °C. The optimized MAE procedure was successfully applied to the determination of 11 elements (Li, Be, Ni, As, Se, Rb, Sb, Cs, W, Tl and U) in two certified reference materials, namely Fine Fly Ash CTA-FFA-1 and Constituent Elements in Coal Fly Ash Standard Reference Material® 1633b. The obtained results were in a good agreement with the certified or comparative values with an overall precision better than 10% in all cases.

The complete decomposition of the matrix of powder infant formula was performed by microwave mineralization with mixture of HNO_3 and H_2O_2. The accuracy of the proposed method was evaluated by certified reference material NCS ZC73015. Good agreement

between certified and obtained values were achieved for 22 elements (Hg, Mg, Fe, Na, Ca, K, Zn, Mo, Co, Cu, Cs, Sr, Rb, U, As, Se, Pb, Sb, La, Ba, Cd and Li). Based on the discriminant analysis milk formulas were classified by category (premature formula, newborn formula, follow-up infant formula, toddler formula) with 100% correctness.

The individual steps of sample preparation of pet food were optimized. The temperature of 220 °C held for 25 minutes with 30% nitric acid and 5% hydrogen peroxide was sufficient for the mineralization of the samples. The accuracy was evaluated by analytical recoveries and by analysis of the certified reference material NIST 1577c Bovine Liver. Good agreement between the certified and measured values of analyte concentrations was found. The solutions of the samples were spiked and the trueness was evaluated in terms of analytical recovery and ranged from 86 to 115%. The method was used to analyze 51 pet food samples. The obtained data were subjected to exploratory, discriminant and factorial analysis. The assumption of normality was not fulfilled and before the discrimination analysis the Box-Cox transformation of data was performed. Each sample of pet food was correctly classified into two classes according to dog or cat dry food. Based on the factor analysis, no clear connection has been found between different feed types, composition, brands or quality.

The optimization of the sample preparation of otter tissues was processed with respect to the uniqueness of the sample. Optimal conditions of lyophilization were: 24 hours, 0.1 kPa for amount of sample 500 mg. The efficiency of microwave mineralization was evaluated by measuring the residual carbon using ICP-OES. Final parameters of the decomposition of the sample were set: 50 mg of lyophilized sample, 1 mL 65% HNO_3 and 1 mL 30% H_2O_2, temperature of 200 °C held for 15 minutes. The accuracy fortselected elements (Li, Ti, V, Mn, Ni, Co, Cu, Ga, Ge, As, Se, Rb, Sr, Zr, Ru, Pd, Ag, Cd, Sn, Sb, W, Pt, T1, Pb, Bi, Th, U, Mg, Na, and Hg) was validated by analytical recoveries and analysis of the certified reference material NIST 1577c Bovine Liver. Good agreement between certified and found values was obtained and the analytical recoveries ranged 85–107%. The data from analysis of 149 otter samples were subjected to factor analysis, where first two factors described 80% variability of the data and are influenced by Co, Sb, Re, Pt, Tl, Th, Pd, Hf, Ta, W. Complete information about individual otter was not available yet so it was not possible to identify or exclude connections between samples in one cluster.

5. References

[1] E.H. Evans, J.J. Giglio, J. Anal. At. Spectrom. **8**, (1993) 1.
[2] R. Thomas, *Practical Guide to ICP-MS*, Taylor and Francis Group New York, 2004, 0-8247-5319-4.
[3] C. Agatemor, D. Beauchemin, Anal. Chim. Acta **706**, (2011) 66.
[4] K.E. Murphy, S.E. Long, M.S. Rearick, O.S. Ertas, J. Anal. At. Spectrom. **17**, (2002) 469.
[5] R.F.J. Dams, J. Goossens, L. Moens, Microchim. Acta **119**, (1995) 277.
[6] P. Abbyad, J. Tromp, J. Lam, E. Salin, J. Anal. At. Spectrom. **16**, (2001) 464.
[7] S. Bürger, L.R. Riciputi, D.A. Bostick, J. Radioanal. Nucl. Chem. **274**, (2007) 491.
[8] J.C. Amarasiriwardena, N. Lupoli, S. Korrick, H. Hu, V. Potula, Analyst **123**, (1998) 441.
[9] M. Resano, M. Aramendia, F. Vanhaecke, J. Anal. At. Spectrom. **24**, (2009) 484.
[10] T. Duan, X. Song, P. Guo, H. Li, L. Pan, H. Chen, J. Xu, J. Anal. At. Spectrom. **22**, (2007) 403.
[11] H. Goenaga Infante, M. L. Fernandez Sanchez, A. Sanz-Medel, J. Anal. At. Spectrom. **14**, (1999) 1343.
[12] F. Ardini, E. Magi, M. Grotti, Anal. Chim. Acta **706**, (2011) 84.
[13] Y.-C. Sun, J. Mierzwa, C.-F. Lin, T. I. Yeh, M.-H. Yang, Analyst **122**, (1997) 437.
[14] D. Gunn, Spectroscopy **29**, (2014).
[15] N. Khan, I.S. Jeong, I.M. Hwang, J.S. Kim, S.H. Choi, E.Y. Nho, J.Y. Choi, B.-M. Kwak, J.-H. Ahn, T. Yoon, K.S. Kim, Food Chem. **141**, (2013) 3566.
[16] J.K. Friel, C.S. Skinner, S.E. Jackson, H.P. Longerich, Analyst **115**, (1990) 269.
[17] E. Engström, A. Stenberg, S. Senioukh, R. Edelbro, D.C. Baxter, I. Rodushkin, Anal. Chim. Acta **521**, (2004) 123.
[18] I. Varga, Á. Szebeni, N. Szoboszlai, B. Kovács, Anal- Bioanal. Chem. **383**, (2005) 476.
[19] S.S.L. da Costa, A.C. Lima Pereira, E. Andrade Passos, J.d.P. Hora Alves, C.A. Borges Garcia, R.G. Oliveira Araujo, Talanta **108**, (2013) 157.
[20] N. Mohd-Taufek, D. Cartwright, M. Davies, A.K. Hewavitharana, P. Koorts, P.N. Shaw, R. Sumner, E. Lee, K. Whitfield, Food Anal. Method **9**, (2016) 2068.
[21] A. Delazar, L. Nahar, S. Hamedeyazdan, S.D. Sarker, *Microwave-Assisted Extraction in Natural Products Isolation*, Natural Products Isolation, Humana Press, Totowa, NJ, 2012, pp. 89.
[22] K. Srogi, Anal. Lett. **40**, (2007) 199.
[23] V. Fernandez-Perez, L.E. Garcia-Ayuso, M.D. Luque de Castro, Analyst **125**, (2000) 317.
[24] F.A.R. Martino, M.a.L.F. Sánchez, A. Sanz-Medel, Anal. Chim. Acta **442**, (2001) 191.
[25] M. Costas, I. Lavilla, S. Gil, F. Pena, I. de la Calle, N. Cabaleiro, C. Bendicho, Anal. Chim. Acta **679**, (2010) 49.
[26] J.T. Castro, E.C. Santos, W.P.C. Santos, L.M. Costa, M. Korn, J.A. Nóbrega, M.G.A. Korn, Talanta **78**, (2009) 1378.
[27] W.P.C. Santos, J.T. Castro, M.A. Bezerra, A.P. Fernandes, S.L.C. Ferreira, M.G.A. Korn, Microchem. J. **91**, (2009) 153.
[28] P.T. Anastas, M.M. Kirchhoff, Acc. Chem. Res. **35**, (2002) 686.
[29] D.C. Montgomery, *Design and Analysis of Experiments* John Wiley & Sons, Nex York, 1991.
[30] G.E.P. Box, W.G. Hunter, S.J. Hunter, *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building* John Wiley & Sons, Nex York, 1978.
[31] D.D. Link, H.M.S. Kingston, Anal. Chem. **72**, (2000) 2908.
[32] R. Sah, P. Brown, Plant and Soil **193**, (1997) 15.
[33] R.N. Sah, P.H. Brown, Microchem. J. **56**, (1997) 285.

[34] S. Mann, D. Geilenber, J. A. C. Broekaert, M. Jansen, J. Anal. At. Spectrom. **12**, (1997) 975.
[35] A. Iwashita, T. Nakajima, H. Takanashi, A. Ohki, Y. Fujita, T. Yamashita, Fuel **85**, (2006) 257.
[36] S. Wu, Y.-H. Zhao, X. Feng, A. Wittmeier, J. Anal. At. Spectrom. **11**, (1996) 287.
[37] A.K. Das, R. Chakraborty, M.d.l. Guardia, M.L. Cervera, D. Goswami, Talanta **54**, (2001) 975.
[38] E.G. Rakov, E.I. Mel'nichenko, Russ. Chem. Rev. **53**, (1984) 851.
[39] I. Rodushkin, T. Ruth, D. Klockare, J. Anal. At. Spectrom. **13**, (1998) 159.
[40] H.-l. Xie, Y.-g. Tang, Y.-j. Li, L.-b. Li, J. Cent. South Univ. T. **14**, (2007) 68.
[41] X. Li, S. Dai, W. Zhang, T. Li, X. Zheng, W. Chen, Int. J. Coal Geol. **124**, (2014) 1.
[42] E. Coni, A. Stacchini, S. Caroli, P. Falconieri, J. Anal. At. Spectrom. **5**, (1990) 581.
[43] S.E. Emmett, J. Anal. At. Spectrom. **3**, (1988) 1145.
[44] J. A. Nobrega, Y. Gelinas, A. Krushevska, R. M. Barnes, J. Anal. At. Spectrom. **12**, (1997) 1243.
[45] C.-Y. Ho, S.-J. Jiang, J. Anal. At. Spectrom. **17**, (2002) 688.
[46] S. Stürup, A. Büchert, Fresenius. J. Anal. Chem. **354**, (1996) 323.
[47] L. Husáková, I. Urbanová, J. Šrámková, M. Konečná, J. Bohuslavová, Talanta **106**, (2013) 66.
[48] D.R. Lide, H.P.R. Frederikse, *CRC Handbook of Chemistry and Physics*, 75. vydání., CRC, Boca Raton 1994.
[49] R.S. Amais, A. Virgilio, D. Schiavo, J.A. Nóbrega, Microchem. J. **120**, (2015) 64.
[50] E. Coni, B. Bocca, B. Galoppi, A. Alimonti, S. Caroli, Microchem. J. **67**, (2000) 187.
[51] R. Melø, K. Gellein, L. Evje, T. Syversen, Food Chem. Toxicol. **46**, (2008) 3339.
[52] E.I. Muller, J.P. Souza, C.C. Muller, A.L.H. Muller, P.A. Mello, C.A. Bizzi, Talanta **156–157**, (2016) 232.
[53] D. Huynh, S.J. Zhou, R. Gibson, L. Palmer, B. Muhlhausler, J. Trace Elem. Med. Biol. **29**, (2015) 75.
[54] S. Dubascoux, M. Nicolas, C.F. Rime, J.R. Payot, E. Poitevin, J. AOAC Int. **98**, (2015) 953.
[55] K. Ljung, B. Palm, M. Grandér, M. Vahter, Food Chem. **127**, (2011) 943.
[56] N. Zand, B.Z. Chowdhry, F.B. Zotor, D.S. Wray, P. Amuna, F.S. Pullen, Food Chem. **128**, (2011) 123.
[57] V. Höllriegl, M. González-Estecha, E.M. Trasobares, A. Giussani, U. Oeh, M.A. Herraiz, B. Michalke, J. Trace Elem. Med. Biol. **24**, (2010) 193.
[58] S. D'Ilio, F. Petrucci, M. D'Amato, M. Di Gregorio, O. Senofonte, N. Violante, Anal. Chim. Acta **624**, (2008) 59.
[59] S.M. Azcarate, M. Savio, P. Smichowski, L.D. Martinez, J.M. Camiña, R.A. Gil, Talanta **143**, (2015) 64.
[60] R.S. Picoloto, M. Doneda, E.L.M. Flores, M.F. Mesko, E.M.M. Flores, P.A. Mello, Spectrochim. Acta B **107**, (2015) 86.
[61] M.L. Fernández-Sánchez, R.R. de la Flor St. Remy, H.G. Iglesias, J.B. López-Sastre, B. Fernández-Colomer, D. Pérez-Solís, A. Sanz-Medel, Microchem. J. **105**, (2012) 108.
[62] A. Sorbo, A.C. Turco, M. Di Gregorio, L. Ciaralli, Food Control **44**, (2014) 159.
[63] S. Fernández-Menéndez, M.L. Fernández-Sánchez, B. Fernández-Colomer, R.R. de la Flor St. Remy, G.D.C. Cotallo, A.S. Freire, B.F. Braz, R.E. Santelli, A. Sanz-Medel, J. Chromatogr.,A **1428**, (2016) 246.
[64] A. Duran, M. Tuzen, M. Soylak, Food Chem. Toxicol. **48**, (2010) 2833.
[65] D. Pick, M. Leiterer, J.W. Einax, Microchem. J. **95**, (2010) 315.
[66] R. Obenauf, L. Ernyei, R. Thomas, P. Atkins, W. Driscoll, Spectroscopy **26**, (2011).

[67] ALKA Wildlife, o.p.s., *Vydra říční* [online]. <http://www.alkawildlife.eu/>, citováno [23.1. 2017].
[68] S. Kang, J.-H. Kang, S. Kim, S.H. Lee, S. Lee, H.J. Yu, S.-J. Oh, J.-D. Park, K.-H. Nam, S.Y. Han, J.-D. Lim, D.-Y. Ryu, Ecotoxicology **24**, (2015) 1064.
[69] A.C. Gutleb, A. Kranz, G. Nechay, A. Toman, Bull. Environ. Contam. Toxicol. **60**, (1998) 273.
[70] S. Takahashi, I. Takahashi, H. Sato, Y. Kubota, S. Yoshida, Y. Muramatsu, Lab. Anim. **34**, (2000) 97.
[71] A. Kotulanová, J. Komárek, Acta Chim. Slov. **49**, (2002) 437.
[72] J. Locke, Anal. Chim. Acta **104**, (1979) 225.
[73] E. Kalisinska, N. Lanocha-Arendarczyk, D. Kosik-Bogacka, H. Budis, B. Pilarczyk, A. Tomza-Marciniak, J. Podlasinska, L. Cieslik, M. Popiolek, A. Pirog, E. Jedrzejewska, Ecotoxicol. Environ. Saf. **136**, (2017) 24.
[74] S. Zimmermann, C.M. Menzel, Z. Berner, J.-D. Eckhardt, D. Stüben, F. Alt, J. Messerschmidt, H. Taraschewski, B. Sures, Anal. Chim. Acta **439**, (2001) 203.
[75] J. Griboff, D.A. Wunderlin, M.V. Monferran, Microchem. J. **130**, (2017) 236.
[76] Perkin Elemer, Inc., *Determination of Copper and Iron in Liver Tissue Using the ELAN DRC ICP-MS* [online].
<http://icpms.kocaeli.edu.tr/yuklemeler/Karaci%C4%9Fer%20dokusunda%20bak%C4%B1r%20ve%20demir%20analizi.pdf>, citováno [21.11. 2016].
[77] R.E. Sturgeon, J.W.H. Lam, A. Saint, J. Anal. At. Spectrom. **15**, (2000) 607.
[78] GBC Scientific Equipment, *Integra XL* [online].
<http://www.gbcscientific.com/brochures/01082901.pdf>, citováno [26.1. 2017].
[79] L. Husakova, I. Urbanova, J. Sramkova, T. Cernohorsky, A. Krejcova, M. Bednarikova, E. Frydova, I. Nedelkova, L. Pilarova, Food Chem. **129**, (2011) 1287.
[80] Berghof Germany, *Multi Tube system for DAC-100* [online].
<http://www.onlinecas.com/Berghof/CATALOGUE%20BERGHOF/Micro-onde%20MWS3+/Catal_MWS3+_pdf/MTS_DAC-100_PT_en.pdf>, citováno [12.5. 2015].
[81] L. Husáková, I. Urbanová, T. Šídová, T. Cahová, T. Faltys, J. Šrámková, Int. J. Environ. Anal. Chem. **95**, (2015) 922.
[82] J. Sucharová, I. Suchara, Anal. Chim. Acta **576**, (2006) 163.
[83] J.M. M. Meloun, M. Forina, *PC-Aided Regression and Related Methods* Ellis Horwood, 1994.
[84] K. Kupka, *QC. Expert Profesional, Handbook*, TriloByte Statistical Software, Ltd., Pardubice, 2013.
[85] M. Meloun, J. Militký, *Kompendium statistického zpracování dat*, 3. vydání., Karolinum Praha, 2012, 978-80-246-2196-8.
[86] *Směrnice Evropského parlamentu a Rady 2002/32/ES ze dne 7. května 2002* [online].
<http://eur-lex.europa.eu/legal-content/CS/ALL/?uri=CELEX%3A32002L0032>, citováno [16.5. 2017].
[87] J. Lanszki, E. Orosz, L. Sugár, Chemosphere **74**, (2009) 741.

Publications and Contributions to Conferences and Seminars

Publications in International Scientific Journals:

1. Husáková L., Urbanová I., Šídová T., Mikysek T.: Multi-elemental analysis of sulfuric acid by oaTOF-ICP-MS after matrix modification with barium bromide. Analytical Methods *7* (2015) 5019. ISSN: 1759-9660.
2. Husáková L., Urbanová I., Cahová T., Šídová T., Faltys T., Šrámková J.: Evaluation of ammonium fluoride for quantitative microwave-assisted extraction of silicon and boron from different solid samples. International Journal of Enviromental Analytical Chemistry *95* (2015) 922. ISSN: 0306-7319.
3. Husáková L., Urbanová I., Šídová T., Šafránková M.: Simultaneous determination of 11 elements in fly ash by oaTOF-ICP-MS after closed-vessel microwave-assisted extraction with ammonium fluoride. Journal of Analytical Chemistry (2017). (Under review)
4. Husáková L., Urbanová I., Šafránková M., Šídová T.: Slurry sampling high-resolution continuum source electrothermal atomic absorption spectrometry for direct beryllium determination in soil and sediment samples after elimination of SiO interference by least-squares background correction. Talanta (2017). ISSN: 0039-9140

Papers Presented at International Scientific Conferences:

1. Šídová T., Husáková L., Urbanová I., Šrámková J.: Matrix modification for overcoming the spectral effects during oTOF-ICP-MS analysis of sulfuric acid. *21th Young Investigators' Seminar on Analytical Chemistry 2014*. Univerzita Pardubice 2014. ISBN 978-80-7395-810-7. s40. 25.6.-28.6.2014, Pardubice, Česká Republika. (Lecture)
2. Šídová T., Husáková L., Urbanová I.: Multi-element analysis of fly ash samples by oTOF-ICP-MS after microwave assisted extraction with NH_4F. *22nd Young Investigators Seminar on Analytical Chemistry 2015*. University of Łódź, 2015. ISBN 978-83-6125-44-8. s. 44. 30.6.–4.7.2015 , Łódź, Posko (Lecture).
3. Šídová T., Husáková L., Urbanová I.: Optimization of microwave-assisted extraction for fly ash elemental composition determination by oa-TOF-ICP-MS. *European Symposium on Atomic Spectrometry ESAS 2016.* Hungarian Chemical Society, 2016. ISBN 978-963-9970-65-6. s. 149. 31.3.–2.4.2016, Eger, Maďarsko (Poster).

Papers Presented at Local Scientific Conferences (in Czech):

1. Venclová J., Husáková L., Šídová t., Urbanová I.: Multiprvková analýza náhradní kojenecké výživy. Czech chemical society symposium series *15* (2017) 95. ISSN 2336-7210.
2. Šídová T., Husáková L., Urbanová I., Šrámková J.: Multielementární analýza kyseliny sírové metodou TOF-ICP-MS. *Monitorování cizorodých látek v životním prostředí XVI*. Univerzita Pardubice, 2014. ISBN 978-80-7395-821-3. s. 101. 9.-11.4.2014, Mladkov-Petrovičky. (Lecture)
3. Nováková L., Husáková L., Urbanová I., Šídová T., Beran V., Poledník L., Poledníková K., Šrámková J.: Využití frakcionálního faktorového plánování a exploratorní analýzy dat při optimalizaci přípravy vzorku a stanovení rtuti ve vzorcích jater, kostí a tuku vyder říčních termooxidační metodou. *Soutěž o nejlepší studentskou vědeckou práci v oboru analytická chemie o cenu Karla Štulíka*. Ostrava: Ostravská univerzita, 2015. ISBN 978-80-7464-362-0. s. 125 Ostrava. 4.-5.2.2015, Ostrava (Lecture)
4. Šídová T., Husáková L., Urbanová I., Cahová T., Šrámková J: Optimalizace mikrovlnné extrakce vzorků životního prostředí pro potřeby stanovení Si a B vybranými metodami atomové spektrometrie. *Monitorování cizorodých látek v životním prostredí XVII.* Univerzita Pardubice, 2015. ISBN 978-80-7395-926-5. s. 89. 8.-10.4.2015, Mladkov-Petrovičky. (Lecture)
5. Šafránková M., Husáková L., Šídová T., Urbanová I.: Stanovení Be ve vzorcích půd a sedimentů metodou HR-CS-ET-AAS. *Monitorování cizorodých látek v životním prostředí XVIII*. Univerzita Pardubice, 2016. ISBN 978-80-7560-005-9. s. 149. 30.3.-1.4.2016, Mladkov-Petrovičky.
6. Šídová T., Husáková L., Urbanová I.: Development of a simple and fast microwave-assisted extraction method for trace element determination in fly ash samples using inductively coupled plasma time-of-flight mass spectrometry, *68. sjezd chemiků*, Česká společnost chemická, 2016. ISSN: 2336-7202. s. 282. 4.-7.9. 2016, Praha. (Lecture)
7. Husáková L., Šafránková M., Urbanová I., Šídová T.: The use of fractional factorial design for optimization of direct determination of Be in soil and sediment samples by HR-CS-ET-AAS with slurry sampling, *68. sjezd chemiků*, Česká společnost chemická, 2016. ISSN: 2336-7202. s. 281. 4.-7.9. 2016, Praha. (Lecture)