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Scientific paper

CADMIUM AND LEAD IN GREY WOLF LIVER SAMPLES: OPTIMISATION OF A MICROWAVE-ASSISTED DIGESTION METHOD

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A microwave-assisted digestion method for the determination of cadmium (Cd) and lead (Pb) by inductively coupled plasma mass spectrometry (ICP-MS) was optimised on certified reference material (CRM) (bovine liver, BCR-185R) and wolf liver samples. Different factors influencing digestion efficiency (temperature, time, composition of the digestion mixture, sample mass) were tested. Validation included linearity (up to 200 μg L^{-1} for Cd and Pb), detection (0.003 μg L^{-1} for Cd and 0.035 μg L^{-1} for Pb), and quantification (0.008 μg L^{-1} for Cd and 0.081 μg L^{-1} for Pb) limits. Good agreement between measured and certified values was achieved in all conditions, with recoveries ranging from 94 % to 111 % for Cd and from 95 % to 105 % for Pb. The precision of the method, expressed as relative standard deviation, was up to 3 % for Cd and 8 % for Pb. The best digestion parameters (260 °C, 30 min, 1 mL HNO_3+4 mL H_2O, 0.1 g of CRM) based on accuracy and precision were applied on two wolf liver samples to evaluate the need for the pre-digestion step (freeze-drying) and appropriate mass of the sample. Freeze-drying improved precision and minimising the tissue mass to 0.1 g reduced the matrix effect. Using these optimised digestion conditions, we determined Cd and Pb in 40 wolf livers collected in Croatia, and their medians (0.055 μg g^{-1} and 0.107 μg g^{-1}, respectively) were in the range of previously reported data for the grey wolf.

KEY WORDS: *inductively coupled plasma mass spectrometry, reference material bovine liver, toxic metal*

Animals positioned on the top of the food web, such as large carnivores, are excellent for monitoring environmental heavy metal contamination (1-4). Among the most common contaminants (As, Cd, Cr, Cu, Hg, Pb, V, Zn), cadmium (Cd) and lead (Pb) stand out, as they bioaccumulate, have a long biological half-time, and cannot be degraded. As the primary uptake route of Cd and Pb is diet (5, 6), large carnivores could be at risk of chronic toxicity.

Grey wolf (*Canis lupus* L., 1758) is one of the three large carnivores (the other two being the lynx and brown bear) inhabiting 32 % of the Croatian territory (7). This species has been protected by Croatian law since 1995 (8). According to the Wolf Management Plan for Croatia (7), carcasses of accidentally or purposely killed wolves are collected by the Zagreb University Faculty of Veterinary Medicine, and collaboration with the Faculty's experts has given us unique opportunity to study element levels in a relatively large number of wolves. Although wolves in Croatia are a part of a larger Dinaric-Balkan population stretching from Slovenia to Greece and Bulgaria (9), reporting on element status has so far been limited to a study of four animals from Croatia

(10). In addition, there is only one report from the whole Eurasian continent (north-western Russia), where almost all liver data for Cd and Pb were under the limit of detection (11).

The foundation of every quality monitoring are reliable results obtained from sufficiently sensitive analytical methods. Tissue digestion has long been recognised as a critical step in assuring as reproducible and accurate element quantification as possible, without loss of elements (12).

Our study was motivated by the blatant scarcity of element data for the wolf population, but the primary aim was to identify microwave-assisted digestion parameters that would ensure optimal quantification of Cd and Pb in animal tissues with inductively coupled plasma mass spectrometry (ICP-MS).

MATERIALS AND METHODS

Instrumentation

An UltraCLAVE IV microwave digestion system (Milestone S.r.l., Sorisole, Italy) with integrated software and the cryoLAB cooling system, equipped with 40 Quartz vessels (12-mL capacity) and Teflon caps was used for sample decomposition. A Hetosicc lyophilisator (Heto, Birkerød, Denmark) was used for freeze-drying of wolf liver samples.

Liver Cd and Pb were determined using an Agilent 7500cx ICP-MS (Agilent Technologies, Waldbronn, Germany) equipped with an integrated autosampler, a Peltier cooled (at 2 °C) Scott-type quartz spray chamber, a MicroMist nebuliser, a collision cell, and Ni cones. Details about the operating conditions and measuring parameters are summarised in Table 1.

We measured ^{114}Cd the most abundant Cd isotope. Although, known for its isobaric overlap with ^{114}Sn isotope, preliminary measurements showed that Sn was not quantifiable in liver samples. Germanium and rhodium isotopes ^{72}Ge and ^{103}Rh were used as internal standards to correct for instrumental drift and matrix effects. Initially we added either ^{45}Sc, ^{72}Ge, ^{74}Ge, ^{103}Rh, or ^{193}Ir as internal standards that closely match the ionisation energy or atomic mass of Cd and Pb to correct for signal instabilities. The concentration of the internal standards in the samples introduced to ICP-MS was 5 μg L^{-1}.The best accuracy and precision of measurement in reference material BCR-185R ("no gas mode") was obtained with ^{72}Ge as internal standard for ^{114}Cd and ^{103}Rh for ^{208}Pb.

Our laboratory facilities, designed for routine measurement of trace elements, operate under positive pressure maintained by the HVAC (heating, ventilating, and air conditioning) system combined with HEPA filters.

Reagents and standards

Ultrapure water (18 MΩ cm) obtained with a GenPure system (TKA, Germany) was used for dilution of all solutions and samples. All standard solutions were prepared from a 1000 mg L^{-1} stock ICP multi-element standard solution IV (Merck, Darmstadt, Germany). Analytical grade nitric acid (65 %, Merck, Darmstadt, Germany) was used after purification by sub-boiling distillation in an ultrapure quartz apparatus (subPUR, Milestone, Italy). Hydrogen peroxide (30 %, Kemika, Zagreb, Croatia) was of analytical grade. All quartz vessels and polypropylene containers were cleaned with detergent solution, soaked in 10 % HNO_3 for 24 h, and rinsed with ultrapure water two times.

Samples

The efficiency of different digestion procedures was evaluated, and the optimised method validated using certified reference material of bovine liver BCR-185R from the Institute for Reference Materials and Measurements, Geel, Belgium.

The optimised method was then used to prepare different mass aliquots of two wolf liver tissues, both fresh and freeze-dried. Liver was chosen over other tissues due to sufficient supply and satisfactory preliminary measurements (which included muscle, liver, and kidney cortex, data not shown). Frozen wolf liver samples, separately packed in BD Falcon™ tubes (Becton, Dickinson and Company, New Jersey, NJ, USA) after carcass inspection, were supplied by the Biology Department of the Zagreb University Faculty of Veterinary Medicine. The samples were collected from the Croatian regions of Gorski kotar, Lika, and Dalmatia between November 2008 and October 2010. Prior to analysis, the samples were slightly thawed and rinsed with deionised water. Secondary contamination was avoided by removing the surface liver tissue (0.5 cm thick) using stainless steel scissors. Two wolf liver samples, supplied in sufficient amount, were freeze-dried to simulate the texture of certified reference material (BCR-185R), also prepared by freeze-drying, but from bovine liver.

Multivariate optimisation of the digestion method

Optimisation was conducted with BCR-185R by changing the following factors that can influence the efficiency of digestion on an UltraCLAVE IV: (1) maximum digestion temperature: 230 °C, 245 °C, or 260 °C; (2) digestion time at maximum digestion temperature: 15 min, 30 min, or 40 min; (3) concentration of the nitric acid: (4.4, 8.8, or 13.2) mol L^{-1} (*i.e.* 1+4, 2+3, 3+2 dilution of the conc. HNO_3 with H_2O); (4) hydrogen peroxide additions [final concentrations: (3.9, 7.8, or 11.7) mol L^{-1}] to nitric acid and water (*i.e.* 0.6+1+3.4, 1.2+2+1.8, 1.8+3+0.2); (5) sample mass: 0.1 g, 0.2 g, or 0.3 g. Hydrogen peroxide was added to the samples gradually to avoid excess foaming and heating due to abundant release of molecular oxygen and carbon dioxide in exothermic reaction with plenty of organic matter present in samples.

Due to the high cost of CRM we could not afford the full factorial design, but only a few combinations to optimise digestion. The influence of every digestion parameter on Cd and Pb recovery from CRM was tested while other variables remained unchanged. The significance of parameter (e.g. temperature) influence on digestion efficiency was established using the one-way analysis of variance (ANOVA). Student's *t*-test was used to determine differences between parameter subgroups (e.g. between 230 °C and 260 °C).

Samples were digested using a modified program UC-10 for the digestion of fresh meat from the UltraCLAVE Application Note (13). The operating program included five steps. While all samples underwent the same 1st (3.5 min, 700 W, 70 °C, 100 bar), 2nd (15 min, 1000 W, 180 °C, 100 bar), and 5th step (40 min, 0 W, 30 °C, 20 bar), temperature in the 3rd ramp step (10 min, 1000 W, 140 bar), and temperature and time in the 4th step (1000 W, 140 bar) were alternated to determine optimum conditions. After completion of the temperature program and release of the pressure inside the reaction chamber, quartz vessels were allowed to cool to room temperature. The content of the digestion vessels was quantitatively transferred to polypropylene tubes and diluted to 6 mL with ultrapure water. Two method blank samples were run with each batch of samples during the optimisation to identify possible contamination during digestion and analysis.

Table 1 *ICP-MS Agilent 7500cx operating conditions and measurement parameters*

RF Power	1550 W
Makeup gas flow	0.2 L min^{-1}
Carrier gas flow	0.94 L min^{-1}
Sample Depth	8.3 mm
Torch-H	0.4 mm
Torch-V	0.2 mm
Nebulizer pump	0.08 rps
Extract lens 1 voltage	0.5 V
Extract lens 2 voltage	-126 V
Integration time	0.5 s
Monitored isotopes	^{114}Cd and ^{208}Pb

Optimal digestion conditions [Step 3: ramp (180 to 260) °C in 10 min; Step 4: hold at 260 °C for 30 min] were then applied for the digestion of the wolf liver samples, while optimising sample mass to meet the criteria of reproducibility, but with as low total dissolved solids (TDS) in digested solution as possible. About 0.1 g, 0.2 g or 0.3 g of freeze-dried and 0.3 g, 0.6 g or 0.9 g of fresh wolf liver in four replicates each were digested with 4.4 mol L^{-1} nitric acid.

All samples for Cd and Pb analysis were additionally diluted by factor of 10 with ultrapure water before analysis. Three replicate measurements were made for each sample. Blank concentration values were subtracted from measured sample concentrations.

RESULTS AND DISCUSSION

Analytical performance

Typical equations for calibration curves for Cd and Pb were Y=20212X+44; R^2=1 and Y=33932X+175; R^2=1, respectively. They were constructed using external standards and stretched well within the wide linear dynamic range of ICP-MS (up to 200 μg L^{-1}). Limits of detection (LOD), calculated as average concentration of the blanks plus three times the standard deviation of the blanks (n=12) were 0.003 μg L^{-1} for Cd and 0.035 μg L^{-1} for Pb. Limits of quantification (LOQ) calculated for each element as average concentration of the blanks plus ten times the standard deviation were 0.008 μg L^{-1} for Cd and 0.081 μg L^{-1} for Pb. The relatively higher LOD and LOQ for Pb could be the result of secondary contamination during sample preparation without a clean bench.

The accuracy of the digestion method, expressed as recovery, was assessed by comparing results obtained under variable conditions of digestion with certified values for BCR-185R. Good agreement between measured and certified values was achieved in all conditions, with recoveries ranging from 94 %

to 111 % for Cd and from 95 % to 105 % for Pb (Table 2). Measured and certified values were compared using Student's *t*-test. Statistically significant differences, where found, are indicated by superscripts in uppercase next to accuracy (Table 2).

The precision of the method, expressed as relative standard deviation (RSD), was up to 3 % for Cd and 8 % for Pb (Table 2).

Optimisation of microwave-assisted digestion

Colourless digestion solutions without visible precipitate and high recovery results for Cd and Pb in BCR-185R (Table 2) indicated that samples from all experiments were completely digested. The only exceptions were digestion solutions with the highest mass of CRM and fresh/freeze-dried wolf liver, which were light yellow. According to Kingston et al. (14) and Wasilewska et al. (15), yellow colour indicates the presence of a large amount of organic substances in the digest. However, even a clear solution can give no reliable information about the amount of remaining organic compounds.

All our parameter combinations showed satisfactory recoveries and precision (Table 2) and even though some results significantly differed from the reference value, they all fell within the certified range for both Cd and Pb.

Temperature

The temperature of decomposition influences how complete the degradation of organic matter will be. In our study, digestion efficiency did not differ significantly between temperature subgroups (Table 2). Good recoveries and precision for Cd and Pb were obtained at all studied temperatures. Our optimal temperature was 260 °C, as higher temperatures improve the oxidising ability of nitric acid (14). Higher temperature applied in the fourth step of our digestion increased the pressure (Δt=30°C; Δp=25 bar). This pressure build-up in the digestion vessel during the final phase of decomposition was probably caused by CO_2 development from carbon contained in the sample, generation of NO/NO_2 in reaction with nitric acid, and the vapour pressure of water and nitric acid (15, 16).

Time

After the first three steps of microwave digestion, in which temperature reached 260 °C in less than 30 min, we measured the hold time at this temperature. We set a relatively long ramp time to prevent the occurrence of pressure and temperature spikes resulting from very exothermic and pressure-intensive reactions. Good accuracy and precision were observed at all three proposed times of digestion. Although our results have shown that even 15 min at 260 °C were enough to effectively decompose our samples, we opted for 30 min as optimum time to ensure as low total organic carbon (TOC) as possible. According to Wasilewska et al. (15), time of optimised digestion reduced by 30 % significantly increases the TOC value.

Composition of digestion mixture

Nitric acid

Nitric acid is the most common digestion reagent for organic matrices, used to decompose samples before quantitative trace element measurements. In our third experiment (Table 2), we used different ratios of concentrated nitric acid and water (1+4, 2+3, and 3+2) to prepare different concentrations of nitric acid [(4.4, 8.8, or 13.2) mol L^{-1}] in digestion solution. The addition of water to the reaction mixture helps to solubilise ions and molecules released from the matrix when reaction solution gets saturated and prevents temperature and pressure spiking in rapid closed-vessel decompositions (14). Other benefits of diluted nitric acid versus concentrated acid as digestion reagent are discussed by Castro et al. (17). Increasing nitric acid solution concentration in our study resulted in lower recovery for Cd, even though the difference was significant only between the group with the lowest nitric acid concentration and the ones obtained with 8.8 mol L^{-1} (p<0.001) or 13.2 mol L^{-1} (p<0.01). The decrease in Pb recovery was not significant. In conclusion, the lowest acid concentration solution used for digestion (4.4 mol L^{-1}) showed the best recovery for both elements. In addition, low solution acidity prolongs the life of the sample and skimmer cones and prevents problems with pneumatic nebulisation and aerosol formation (17).

Nitric acid and hydrogen peroxide

Hydrogen peroxide is often used in combination with nitric acid for the digestion of biological matrices because of its good oxidising potential that increases with acidity, leading to improved metal extraction efficiency compared to extraction with nitric acid alone. In addition, hydrogen peroxide (35 %) improves

Table 2 *Accuracy (as percent recovery) and precision (as relative standard deviation) determined for cadmium and lead in CRM bovine liver under varying digestion parameters.*

Experiment	Temperature / °C	Time / min	HNO_3 / mol L^{-1}	H_2O_2 / mol L^{-1}	Sample mass / g	Cd R.V.=0.544±0.017 µg g^{-1}			Pb R.V.=0.172±0.009 µg g^{-1}		
						Recovery* / %	RSD / %	$p^{\#}$	Recovery* / %	RSD / %	$p^{\#}$
1	**230**	30	4.4	-	0.3	102	2.1		103	5.1	
	245	30	4.4	-	0.3	100	1.3		97^{a}	1.2	
	260	30	4.4	-	0.3	100	0.5		101	5.1	
2	260	**15**	4.4	-	0.3	99	0.6	B^{a} C^{a}	98^{a}	1.5	
	260	**30**	4.4	-	0.3	100	0.5	A^{a}	101	5.1	
	260	**40**	4.4	-	0.3	101	1.1	A^{a}	101	8.3	
3	260	30	**4.4**	-	0.3	100	0.5	B^{c} C^{b}	101	5.1	
	260	30	**8.8**	-	0.3	96^{b}	0.7	A^{c}	96^{b}	1.2	
	260	30	**13.2**	-	0.3	94^{a}	2.4	A^{b}	97	5.5	
4	260	30	4.4	**3.9**	0.3	111^{c}	0.4	B^{c} C^{c}	105^{a}	2.6	$B^{c}C^{b}$
	260	30	8.8	**7.8**	0.3	100	1.7	A^{c}	95^{b}	1.7	A^{c}
	260	30	13.2	**11.7**	0.3	99	2.2	A^{c}	98	1.8	A^{b}
5	260	30	4.4	-	**0.1**	102	2.6		104	2.6	
	260	30	4.4	-	**0.2**	101	2.4		101	0.7	
	260	30	4.4	-	**0.3**	100	0.5		101	5.1	

**Lowercase superscript indicates significant result of t-test between mean (*N*=4) and certified reference value (R.V.): [a] p<0.05, [b] p<0.01, [c] p<0.001.*

*#Uppercase indicates significant result of t-test between measured means (*N*=4) of two subgroups; A: Significantly different from the first (of three) subgroup of matching digestion parameter (i.e. temperature, time, acid concentration…), B: Significantly different from the second subgroup of matching digestion parameter, C: Significantly different from the third subgroup of matching digestion parameter. Lowercase superscript indicates significance level of t-test: [a] p<0.05, [b] p<0.01, [c] p<0.001.*

the conversion of microwave energy into heat because of its high dielectric constant (14). In our study, the influence of nitric acid:hydrogen peroxide mixture concentrations on Cd and Pb recoveries (Table 2) was notable only between the first subgroup with the lowest nitric acid:hydrogen peroxide mixture concentrations (4.4 mol L^{-1} HNO_3:3.9 mol L^{-1} H_2O_2) and the other two mixtures (p<0.01). The lowest acid-hydrogen peroxide mixture yielded worse recoveries for Cd (p<0.001) than the matching acid solutions (4.4 mol L^{-1} HNO_3). As for Pb, it made no significant difference whether we used acid alone or an acid/peroxide mixture. Veschetti et al. (18) reported that different digestion mixtures applied on sewage-sludge had no effect on metal content, but that hydrogen peroxide added to nitric acid reduced the dissolved organic carbon content by 14 %. Although in our study hydrogen peroxide combined with the lowest concentrations of nitric acid yielded higher recoveries for Cd but lower accuracies, nitric acid alone was strong enough to completely digest the samples. One of the drawbacks when using hydrogen peroxide in closed-vessel decomposition system is a relatively small quantity of hydrogen peroxide that can be added because of the large quantities of molecular oxygen produced. This limits its efficiency compared to traditional open-vessel systems where large aliquots of fresh reagent are frequently added (14). Furthermore, gradual addition of hydrogen peroxide - to reduce foaming - prolonged the pre-digestion time of our samples for up to an hour (at 1.8 mL hydrogen peroxide) and consequently the whole sample preparation. An alternative would have been to use larger sample vessels that would contain the foam. Finally, hydrogen peroxide of analytical grade used in our experiments contained a high amount of Cd and Pb impurities (manufacturer declared it contained ≤1 µg g^{-1} heavy metals), which affected LOD. This is why we recommend the use of suprapure hydrogen peroxide. The above reasons tipped the scales against acid-hydrogen peroxide mixture and in favour of acid solution alone.

Sample mass

Two very important factors for complete oxidation of organic substances are the amount of organic

sample and striking the optimal ratio between sample mass and acid volume or vessel volume (16, 19). In our fifth experiment (Table 2), sample size had no influence on digestion efficiency and Cd and Pb recoveries. Even the lowest amount of BCR-185R sample (0.1 g), recommended by the producer as minimum analytical portion, yielded acceptable results. Similarly, Hassan et al. (19) reported significantly improved recoveries when sample mass to acid volume ratio was reduced 10 times. Wasilewska et al. (15) have shown that doubling the bovine liver sample mass from 0.2 g to 0.4 g resulted in twice as high TOC at temperatures from 220 °C to 250 °C. This is why we opted for the lowest, 0.1 g mass as optimal.

Wolf liver analysis

We applied the optimised digestion parameters to further study the effects of sample mass and an additional pre-digestion step (freeze-drying) on two wolf liver samples. Digestion with or without the freeze-drying step (Table 3) produced significantly different Cd and Pb levels only in liver 2 ($p<0.05$), which, regardless of sample mass, had higher element levels when fresh tissue was analysed. This inconsistency between the two wolf livers would have been overcome if more liver samples had been involved in the study, and we plan to carry out a more comprehensive research with more liver samples.

However, relative standard deviations (0.3 % to 4.3 % for freeze-dried livers, 1.4 % to 13 % for fresh livers) suggest that freeze-drying helps to improve precision. This is expected, since freeze-drying is widely used for minimising physical and chemical changes in samples during storage (20). However, in our study, freeze-drying prolonged sample preparation for 24 h.

In some liver samples, mass had a significant influence (ANOVA, Student's *t*-test) on element levels, whether the pre-digestion step was taken or not (Table 3), but element levels did not follow the increase in sample mass. A decision about tissue mass for digestion should take into account the following criteria: element content should be above the quantification limit; matrix effect should be as low as possible; and sample tissue should be available in sufficient quantities.

Table 3 *Cadmium and lead levels in freeze-dried and fresh grey wolf liver samples as a function of mass fraction (μg g^{-1}).*

Wolf sample	Sample mass / g	Cd / μg g^{-1}		Pb / μg g^{-1}	
		mean ± SD*	RSD / %	mean ± SD*	RSD / %
freeze-dried liver 1	0.1	0.106 ± 0.002	2.2	1.348 ± 0.029^{Ca}	2.1
	0.2	0.101 ± 0.004^{Ca}	4.3	1.323 ± 0.005^{Ca}	0.3
	0.3	0.108 ± 0.001^{Ba}	1.1	1.290 ± 0.019^{AaBa}	1.4
freeze-dried liver 2	0.1	0.214 ± 0.003	1.4	0.062 ± 0.000^{Cb}	0.5
	0.2	0.213 ± 0.009	4.0	0.062 ± 0.002	3.2
	0.3	0.218 ± 0.004	1.6	0.060 ± 0.001^{Ab}	1.4
fresh liver 1	0.3	0.092 ± 0.005	5.4	1.321 ± 0.042	3.2
	0.6	0.111 ± 0.014	13.0	1.411 ± 0.133	9.4
	0.9	0.099 ± 0.009	9.0	1.220 ± 1.127	10.4
fresh liver 2	0.3	0.233 ± 0.005^{BaCa}	2.3	0.066 ± 0.002	3.5
	0.6	0.219 ± 0.007^{Aa}	3.0	0.064 ± 0.001	1.4
	0.9	0.218 ± 0.007^{Aa}	3.4	0.063 ± 0.003	4.4

**Uppercase superscript indicates significant result of t-test between means (N=4) of two subgroups of the same wolf liver; [A]Significantly different from the first (of three) subgroup of sample mass, [B]Significantly different from the second subgroup of sample mass. [C]Significantly different from the third subgroup of sample mass. Lowercase superscript indicates significance level of t-test: [a] p<0.05, [b] p<0.01*

Table 4 *Cadmium and lead levels (μg g^{-1}) in the liver of 40 grey wolfs from Croatia*

Wolf liver	Cd / μg g^{-1}	Pb / μg g^{-1}
N	40	40
Mean ± SD	0.084 ± 0.088	0.194 ± 0.263
Median	0.055	0.107
Range	0.0092 to 0.414	0.037 to 1.30

Once we had optimised all digestion parameters, we tested the method on 40 grey wolf liver samples. Table 4 shows somewhat higher Pb than Cd levels, but in a narrower range. Our findings matched well with an earlier report for four animals from Croatia (10) and with the results for the arctic wolf (subspecies of grey wolf) from Yukon (1). Data about wolves from north-western Russia (11) are very scarce because of high method LODs, which were three and four orders of magnitude higher than ours for Pb and Cd, respectively. The only three detectable levels of Pb in the study of Shore et al. (11) were all higher than our highest Pb measurements.

CONCLUSION

Microwave-assisted digestion is a powerful method for the preparation of tissue samples for environmental monitoring, both fresh and freeze-dried. It ensures total recovery of low-level Cd and Pb from the matrix and subsequent quantification with ICP-MS. Our optimised operating mode (260 °C for 30 min with 4.4 mol L^{-1} nitric acid added to 0.1 g of CRM bovine liver or freeze-dried wolf liver) ensured as accurate and precise quantification of Cd and Pb as possible. Freeze-drying, although time consuming, has shown to improve precision. A comprehensive multi-element study of various wolf tissues is planned in the future to explore possible age and sex differences and element interactions within wolf tissues.

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Sažetak

KADMIJ I OLOVO U UZORCIMA VUČJE JETRE: OPTIMIZACIJA METODE RAZGRADNJE UZORAKA UZ POMOĆ MIKROVALOVA

Certificirani referenti materijal (CRM) govedskih jetara (BCR-185R) i uzorci vučjih jetara koristili su se za optimizaciju metode razgradnje uzoraka prije mjerenja kadmija (Cd) i olova (Pb) masenom spektrometrijom induktivno spregnute plazme (ICP-MS). Ispitivani su različiti uvjeti koji utječu na učinkovitost razgradnje (temperatura, vrijeme, sastav otopine za razgradnju, masa uzorka). Validacijom je obuhvaćeno ispitivanje linearnosti (do 200 μg L^{-1} Cd/Pb), granice detekcije (0,003 μg L^{-1} za Cd, 0.035 μg L^{-1} za Pb) i kvantifikacije (0,008 μg L^{-1} za Cd, 0,081 μg L^{-1} za Pb). Postignuto je dobro slaganje izmjerenih i certificiranih vrijednosti u svim ispitivanim uvjetima uz izračunati raspon iskorištenja 94 % do 111 % za Cd i 95 % do 105 % za Pb. Najveća relativna standardna devijacija, kao mjera preciznosti, iznosila je 3 % za Cd i 8 % za Pb. Najbolji uvjeti za razgradnju (260 °C, 30 min, 1 mL HNO_3+4 mL H2O, 0,2 g CRM), izabrani prema točnosti i preciznosti, primijenjeni su na uzorke vučjih jetara pri procjeni odgovarajuće mase uzorka i potrebe za uvođenjem dodatnog koraka prije razgradnje (liofilizacije). Liofilizacija poboljšava preciznost pa je preporučujemo pri pripremi uzoraka tkiva. Također, najmanja masa uzorka izabrana je ne samo zbog bolje preciznosti nego i zbog najmanjeg utjecaja matrice te potrebne količine tkiva. Medijani Cd (0,055 μg g^{-1}) i Pb (0,107 μg g^{-1} mokre mase) dobiveni analizom 40 uzoraka vučjih jetara skupljenih u Hrvatskoj bili su u rasponu vrijednosti objavljenih u literaturi za sivog vuka.

KLJUČNE RIJEČI: *masena spektrometrija induktivno spregnute plazme, otrovni metal, referentni materijal, govedska jetra, vučja jetra*

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