High throughput method for multielemental analysis of horse hair by oaTOF-ICP-MS

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Abstract

An approach for high throughput reliable multielemental analysis of trace elements in a large number of horse hair samples was designed. Suitability of time-of-flight mass spectrometer (oaTOF-ICP-MS) for fast determination of unlimited numbers of isotopes in the low volume samples was demonstrated. Due to quasi-simultaneous capability of the oaTOF-ICP-MS the large number of highly valuated data with unaffected isotopic ratio in a very short time could be obtained. The choice of horse hair was obvious because of easy reachability and clear conception about horse nutritional habits and stabling. Such large data set with preserved isotopic ratios is ideal for statistical evaluation which could reveal some interesting interconnection between elemental composition of horse hair and the way of stabling, feeding, etc. Statistical treatment of the data is not a part of this study and will be presented later. We collected one hundred horse hair samples from horse stables through Czech Republic. Samples were washed by optimized washing process to eliminate exogenic contamination prior to digestion and following analysis. A determination of 36 elements (As, Au, B, Be, Cd, Ce, Co, Cr, Dy, Er, Eu, Ga, Gd, Ge, Ho, La, Li, Lu, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Sm, Ta, Tb, Te, Tm,

U, V, Y, Yb) in horse hair by oaTOF-ICP-MS was optimized. A throughput of 100 samples with unlimited numbers of isotopes per 6 h was achieved. Proposed very fast multielemental method preserves isotopic ratios, and therefore, is undoubtedly highly suitable for statistical studies. Detection limits of the proposed method ranged from 0.13 μg kg^{-1} (Eu, Gd, Tm) to 27.9 μg kg^{-1} (Au), except for Ni (48.5 μg kg^{-1}) that is probably affected by contamination raised from nickel cones.

Keywords

High throughput method
Multielemental analysis
ICP-MS
Horse hair

Introduction

An analysis of hair is plentifully utilized to assess an impact of the environment or diet habits on the state of health of organism (biomonitoring of pollutant's exposition and bioaccumulation studies). History of hair analysis goes back to the nineteenth Century. Hair tissue presents a very specific tool to consider an intoxication of organism by various elements (As, Cd, etc.) or by organic compounds such as PCDD, PCDF, PCB, PAH, THC, barbiturates and so on. Trace elements, minerals, drugs, toxins and their metabolites are incorporated into the structure of hair during life which can be determined even after long time. Usually a hair stem presents information about exposition of drugs or other chemicals several months or years retrospectively and a hair root reveals an actual exposition (Dunnett and Lees 2003).

Borgese et al. (2010) demonstrated the accumulation of Pb from Ayurvedic drug in human hair. Kolacz et al. (1999) found up to 10 times higher contents of heavy metals in the wool of Merino ewes grazing on polluted pastures than in control group. Patkowska et al. (2009) reported significant differences in contents of elements in sheep wool depending on breed and origin. Chojnacka et al. (2006) found the effect of living habits stronger than the influence of both sex and breed on element contents in human hair.

A contamination of hair can be divided into an exogenous and an endogenous contamination. An endogenous contamination originates from food or environment (air, dust, smoke, cosmetics). Endogenous contaminants are a result of a long-term exposure which enters an organism by several ways such as respiration, alimentary route, or through a skin and they are built into the hair

structure during growth. Exogenous alternatively surface contaminants stick on a skin or hair and can be easily removed by washing processes (Asano et al. 2006). For the long-term contamination, hair profiles are suitable while analysis of hair roots can demonstrate acute intoxication (Rodushkin and Axelsson 2000). Hair is a non-invasive and readily available matrix easily treatable without any special storage requirements (Esteban and Castano 2009).

One of the most important steps in any analysis is a way of sample preparation. It can affect the results of analysis from 20 to 30% (Kazi et al. 2009). Gentle, but sufficient removing of an exogenous contamination prior an analysis is emphasizing. Practically, a lot of washing procedures were tested using Triton (1:200), isopropyl alcohol and acetone (Asano et al. 2002). Washing in an alkaline solution tetramethylammonium hydroxide was employed, as well as (Rodrigues et al. 2008), washing in various concentrated (0.1–2%) solution of Triton (Sobanska 2005) or using a combination of nitric acid and hydrofluoric acid (Dunnett and Lees 2003). The most widespread approach of sample preparation of hair is washing in nitric acid and in the Triton followed by microwave digestion in various mixtures of HNO_3 and H_2O_2, HNO_3 and HCl, HNO_3 and HF (Rodushkin and Axelsson 2000; Rao et al. 2002) whose choice depends on an analytical detection technique used.

Nowadays, for the elemental analysis of hair, the most common methods utilized are ICP-OES (inductively coupled plasma optical emission spectrometry) (Rao et al. 2002; Chojnacka et al. 2005), ET-AAS (atomic absorption spectrometry with electrothermal atomization) (Ribeiro et al. 2000; Baysal and Akman 2010) and ICP-MS (inductively coupled plasma mass spectrometry) (Chojnacka et al. 2005; Madejón et al. 2009). Another analytical technique employed for hair analysis is PIXE (particle inducted X-ray emission spectrometry) which does not require complicated sample preparation (Asano et al. 2006), or INAA (Instrumental neutron activation analysis) which involves a minimum sample handling as well (Armelin et al. 2001). LA-ICP-MS (laser ablation ICP-MS) method can be used for a rapid identification and screening of toxic and nutritious elements in hair and does not need time-consuming sample preparation. Steely et al. (2007) used LA-ICP-MS for analysis [75]As, [64]Zn and [208]Pb in human hair to differ them according to their origin and demonstrated that LA-ICP-MS can be used to obtain rapid qualitative and quantitative identification of arsenic in a single strand of human hair as a bio-indicator tissue.

Chandola et al. (1983) presented first results of horse hair analysis using ICP-OES to study elements bioaccumulation. Asano et al. (2006) investigated relationship between elements concentrations in horse hair and atrial fibrillation using PIXE method. He proved significantly higher contents of Ca and Zn and

Zn/Cu ratio in the ill group hair in comparison with the control group and showed connection of increased Ca and Zn concentrations in hair and heart illness. In another study, they evaluated sex, breed, colour and age of riding horses as factors influencing concentrations of trace elements in mane hair and compared to human hair samples (Asano et al. 2005a, b).

Rodrigues et al. (2008) used ICP-MS method for monitoring the nutritional status of essential elements and assessing exposure to toxic elements. They found the relationship between levels of trace elements in human hair and whole blood or plasma and concluded that human hair is not an appropriate biomarker for evaluating Cu, Mn and Sr deficiency or Pb exposure. Dunnett (2005) evaluated using of HPLC-MS and GC-MS methods in hair analysis as a potential diagnostic technique to retrospectively monitor exposure to a range of environmental, dietary and other toxins.

An analysis of horse hair is connected with horse riding where it can be useful for horse health assessment or as a proof of banned compounds (Anielski et al. 2005). Elemental composition of horse hair can be investigated from many aspects such as dependence on the state of health, age, colour, stabling, and feeding.

The aim of our study was to develop fast multielemental method for horse hair analysis which would provide a reliable data with unaffected isotopic ratios. Obtained extensive data with preserved isotopic ratios are highly valued for statistical treatment.

Experimental

Instrumentation

Washing of samples was carried out with an ultrasonic bath, Powersonic UCC 1 (Czech Republic). Samples were digested with the Microwave digestion system Speedwave MWS-2 with high pressure PTFE vessels DAK-60 K (the maximal pressure 100 barr, the maximal temperature 300 °C) and Speedway SW-4 with high pressure PTFE vessels DAP-40+ (the maximal pressure 55 barr, the maximal temperature 260 °C, rotor up to 24 vessels) all Berghof, Germany. The multielemental analysis of mineralized hair samples was performed with the oaTOF-ICP mass spectrometer Optimass 8000 (GBC Scientific Equipment Pty Ltd., Australia) equipped with the concentric nebulizer Micro Mist coupled to low volume (cinnabar) thermostated (10 °C) cyclonic spray chamber (all Glass expansion Pty. Ltd., Australia). Furthermore, deionised water was purified using the SG Ultra Clear system (SG, Hamburg, Germany). Nitric acid used in the

work was purified by sub-boiling distillation equipment (BSB 939 IR, Berghof, Eningen, Germany).

Reagents and standards

All reagents used were of an analytical-reagent grade. Nitric acid 65% (v/v) (LachNer, Neratovice, Czech Republic) was purified in sub-boiling distillation equipment as above. For washing processes, Triton X-100 for medicinal purposes (Merck, Darmstadt, Germany) and Acetone (Lach-Ner, s.r.o., Neratovice, Czech Republic) were used. All calibration standards were prepared from single and multi-element stock standards (As, Au, B, Be, Cd, Co, Cr, Ga, Ge, Li, Ni, Pb, Pd, Pt, Rb, Sb, Sc, Ta, Te, U, V, Y,) at concentration 1 ± 0.002 g L^{-1} or at 100 ± 0.02 mg L^{-1} (Ce, La, Nd, Pr) and 20 ± 0.02 mg L^{-1} (Dy, Er, Eu, Gd, Ho, Lu, Sc, Sm, Tb, Tm, Y, Yb) (Analytika Co., Ltd., Praha, Czech Republic; SCP Science, Canada; Merck, Germany). Reference material Human Hair NCS ZC 8100 (China National Analysis Centre for Iron & Steel 2005) was used for validation.

Sample preparation

A pack of one hundred of horse hair samples was obtained from stables through Czech Republic. They were taken from horses with different gender, colour, age, stabling and feeding during August and September 2011. Sampling was done with a support of horse owners. 32 sampled horses originated from horse farm Siglavy (České Budějovice), 23 from Jeníkov and 45 horses came from private horse breeders in a vicinity of Pardubice. All samples were cut up by a ceramic scissors to avoid a metal contamination and treated by a washing procedure, to remove an exogenous contamination, in an ultrasonic bath. Three different washing procedures were tested (see Table 1).

Table 1

The summary of washing agents and procedures

Step/washing procedure	Time in the sonic bath and agents used		
	1	2	3
1	10 min acetone	10 min acetone	**10 min acetone**
2	10 min water	10 min water	**10 min water**
3	10 min 1% Triton	10 min 0.1 M HNO_3	**10 min 0.1 M HNO_3 in 1% Triton**
4	10 min water	10 min water	**10 min water**

Step/washing procedure	Time in the sonic bath and agents used		
	1	2	3

Bold washing procedure number 3 was chosen as the best

Washed and dried (laboratory temperature, 24 h) samples were digested in a high pressure microwave digestion system MWS-2 and SW4 (optimized for high throughput): ~0.2 g of sample was accurately weighted into Teflon vessels, 7 mL of concentrated HNO_3 was added and the mixture was let opened for 20 min. In first step, temperature was hold on 150 °C for 15 min with power 80%, in the second step, temperature was kept on 205 °C for 25 min with power 80%, then digests were cooled and filled up to 50 mL. Digests were further five times diluted for ICP-MS analysis and internal standard Rh and In were added to be 5 and 1 µg L^{-1}, respectively, in final solution. The certified reference material was digested and made up in the same way as horse hair samples.

Multielemental standards for ICP-MS analysis: three calibration standard sets were prepared at twelve concentration levels. The set "A" contained 20, 15, 10, 5, 3, 2, 1, 0.5, 0.25, 0.1, 0.05 and 0.01 µg L^{-1} of As, Au, B, Be, Cd, Co, Cr, Ga, Ge, Li, Ni, Pb, Pd, Pt, Rb, Ru, Sb, Se, Ta, Te, U, V. The set "B" contained 2, 1.5, 1, 0.5, 0.3, 0.2, 0.1, 0.05, 0.025, 0.01, 0.005 and 0.001 µg L^{-1} of Ce, La, Nd, Pr. The set "C" contained 0.4, 0.3, 0.2, 0.1, 0.06, 0.04, 0.02, 0.01, 0.005, 0.002, 0.001, and 0.0002 µg L^{-1} of Dy, Er, Eu, Gd, Ho, Lu, Sc, Sm, Tb, Tm, Y, Yb. All standards were stabilized with 0.6 mL 65% w/v HNO_3/100 mL of solution and contained internal standards In and Rh in the final concentration 5 and 1 µg L^{-1}, respectively. The standard blank containing 0.6 mL HNO_3/100 mL and internal standards In and Rh (5 and 1 µg L^{-1}, respectively) was prepared.

Optimization of instrumental parameters

The operating conditions of the ICP-MS analysis were adjusted to compromise a sensitivity and resolution of the instrument for ^{238}U, as well as to obtain the minimal LaO^+/La^+ and UO^+/U^+ ratios: the sample flow rate 0.4 mL min^{-1}, plasma power 1250 W, plasma, auxiliary and nebulizer gas flow rate were 11, 0.6 and 0.98 L min^{-1}, respectively, and multiplier gain 2850 V. Average parameters of the ion optics and the detector part shows Table 2. The sensitivity of 60,000 counts s^{-1} for 1 µg L^{-1} (mass integrated peak) and resolution of 2100 was reached for ^{238}U. The mass calibration was achieved using responses from ^7Li, ^{114}In and ^{238}U. The external aqueous calibration with internal standard In and Rh was used for quantification. A peak area mode, 5 s data acquisition time and three replicates were used for measurement. A rinsing time 120 s between

samples has been used to avoid cross-contamination. Chosen unwanted ranges of m/z were excluded (10–44.5; 55–57 and 78–81 amu) using the device "smart gate". To avoid non-spectral interferences, In and Rh as internal standards were utilized.

Table 2

The working conditions of oaTOF-ICP-MS analysis

Ion optics		Pulse shaping	
Parameter	**Value**	**Parameter**	**Value**
Skimmer	−1000 V	Fill	−31 V
Extraction	−900 V	Fill bias	0.50 V
Z1	−900 V	Fill grid	0 V
Y mean	−150 V	Pushout grid	−580 V
Y deflection	0 V	Pushout plate	455 V
Z lens mean	−1150 V	Blanker	200 V
Z lens deflection	0 V	Spectral frequency	33 kHz
Lens body	−180 V	Reflectron	690 V
Multiplier gain	2850 V	Measurement	Pulse
Integration window	Auto	Mode	Counting/analog

Results and discussion

Optimization of the sample preparation procedure

Before a decomposition step, removing of exogenous contaminants from a hair surface is necessary. A washing procedure has to be effective in washing down of outer impurities and gentle in order not to interfere in an internal hair volume. In general, there is not any cleaning strategy to strictly separate an exogenous out of endogenous contaminants. Three procedures consisted of consequent washing with nitric acid, acetone and Triton X-100 or their mixtures and distilled water were tested and are summarized in the Table 1. In this case, a working sample of non-dyed black woman hair was used. Washing sequence consisted of four steps with different washing agents. The processes utilized acetone, 0.1 M HNO_3, 1% Triton X-100 and ultrasonic bath. One gram of sample was supplemented with 50 mL of an appropriate washing solution in a 100 ml plastic bottle and inserted into an ultrasonic bath for a defined time. Following washing process has been assessed as the best: 10 min sonication in

acetone, 10 min in water, 10 min in 0.1% HNO_3 together with 1% Triton and finally 10 min sonification in water. Washed and dried samples were digested using the above mentioned procedure and analyzed using ICP-MS method. Added volumes of washing agents were always 50 mL.

The choice of optimal washing process was based on an analysis of rinse waters and washed/digested hair samples. In the rinse waters were evaluated levels of rinsed elements. In the digested hair samples were determined contents of remaining elements. Comparison of our results obtained from the tested washing processes showed that the best approach is procedure including the washing step with a mixture of 0.1 M HNO_3 and Triton X-100. To develop an appropriate washing strategy proved to be very difficult. Even with large number of data obtained the statistical evaluation of the washing processes tested did not bring a clear verdict. Final decision was made on the basis of best recoveries for five parallel samples washed. The best washing strategy yields the highest recoveries ranged in 93–105% for all the elements determined. We are aware this approach does not have to be necessary the best for the accurate information about elemental composition but it should be sufficient enough to have at the worst the results biased in the same way in all samples. It is believed such washing procedure should not interfere valuable information obtained by following statistical treatment.

The choice of suitable isotopes was a very important step due to differences in sample matrices. Working isotopes have been selected with a regard to possible isobaric overlaps of interfering ions with the same mass. Their choice was carried out using both a spectral library integrated in equipment software, and mass spectra of samples. Table 3 presents isotopes used.

Table 3

Isotopes utilized for horse hair analysis by oaTOF-ICP-MS

Element	Isotope (amu)	Element	Isotope (amu)	Element	Isotope (amu)	Element	Isot (am
As	75	Er	166	Ni	60	Ta	181
Au	197	Eu	151	Pb	206 + 207 + 208	Tb	159
B	11	Ga	71	Pd	106	Te	130
Be	9	Gd	158	Pr	141	Tm	169
Cd	114	Ge	74	Pt	194	U	238
Ce	140	Ho	165	Rb	85	V	51
Co	59	La	139	Sb	123	Yb	174

Cr Element Dy	52 Isotope (amu) 162	Lu Element Li	175 Isotope (amu) 7	Sc Element Sm	45 Isotope (amu) 152	Y Element Nd	89 Isot (am 142

amu atomic mass unit, Pb Σ 206, 207, 208 isotopes

◄   ►

Method validation

The validation of proposed method was achieved by analysis of certified reference material Human Hair NCS ZC 8100 and by standard addition technique. The results of analysis of reference material shows good match with certified values (see Table 4). Standard addition technique as a second validation method for elements non-certified in reference material was employed. Deviation of directions of calibration curves and curves plotted from standard additions ranged in 10% for all the determined elements except for Au (see Table 5).

Table 4

oaTOF-ICP-MS analysis of the reference material NCS ZC 81002b (human hair)

Element	Isotope (amu)	Certified (mg kg^{-1})	Found (mg kg^{-1})	Recoveries (%)
As	75	0.198 ± 0.023	0.186 ± 0.006	91–97
Cd	114	0.072 ± 0.010	0.085 ± 0.007	108–128
Co	59	0.153 ± 0.015	0.146 ± 0.042	68–123
Cr	52	8.74 ± 0.97	8.700 ± 3.10	64–135
Pb	206 + 207 + 208	3.83 ± 0.18	3.684 ± 0.33	88–105
Sb	123	0.12 ± 0.002	0.118 ± 0.026	77–120

Table 5

Validation of the proposed method by comparison of directions of calibration curves and curves plotted from standard addition technique

Directions				Directions			
Element	Calibration	STA	Δ (%)	Element	Calibration	STA	Δ (%)
As	0.0039	0.0039	0.0	Nd	0.0030	0.0031	3.2

Element	Calibration	STA	Δ (%)	Element	Calibration	STA	Δ (%)
Au Directions	0.0168	0.0220	234	Ni Directions	0.0083	0.0082	1.2
B	0.0010	0.0009	11	Pb	0.1357	0.1368	0.8
Be	0.0024	0.0025	4.0	Pd	0.0174	0.0163	6.7
Cd	0.0100	0.0100	0.0	Pr	0.0090	0.0091	1.1
Ce	0.0066	0.0067	1.5	Pt	0.0049	0.0050	2.0
Co	0.0430	0.0430	0.0	Rb	0.0401	0.0405	1.0
Cr	0.0335	0.0338	0.9	Sb	0.0122	0.0123	0.8
Dy	0.0007	0.0007	0.0	Sc	0.0000	0.0000	0.0
Er	0.0008	0.0008	0.0	Sm	0.0006	0.0006	0.0
Eu	0.0011	0.0011	0.0	Tb	0.0023	0.0023	0.0
Ga	0.0150	0.0153	2.0	Ta	0.1745	0.1759	0.8
Gd	0.0008	0.0008	0.0	Te	0.0029	0.0030	3.3
Ge	0.0053	0.0054	1.9	Tm	0.0025	0.0025	0.0
Ho	0.0024	0.0024	0.0	U	0.1833	0.1860	1.5
La	0.0060	0.0062	3.2	V	0.0369	0.0378	2.4
Li	0.0080	0.0083	3.6	Y	0.0011	0.0011	0.0
Lu	0.0024	0.0024	0.0	Yb	0.0008	0.0008	0.0

STA standard addition, Δ relative deviation of directions

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The limits of detection (LOD) of the used techniques were based on the triple of standard deviation of background counts for five blank solutions. The limits of detection of the whole method (LODm) were calculated from limits of detection for technique multiplied by sample dilution. Table 6 presents the limits of detection for both the technique and the whole method. Detection limits of the proposed method ranged from 0.13 µg kg^{-1} (Eu, Gd, Tm) to 27.9 µg kg^{-1} (Au), except for Ni (48.5 µg kg^{-1}) that is probably affected by contamination raised from nickel cones.

Table 6

Limits of detection of technique and method

Element	LOD (µg L^{-1})	LODm (µg kg^{-1})	Element	LOD (µg L^{-1})	LODm (µg kg^{-1})	Element	LOD (µg L^{-1})	I (
As	0.0212	26.6	Eu	0.0001	0.13	Pd	0.0137	1
Au	0.0223	27.9	Ga	0.0024	3.02	Pr	0.0008	1
B	0.0022	2.76	Gd	0.0001	0.13	Pt	0.0015	1
Be	0.0042	5.25	Ge	0.0033	4.10	Rb	0.0010	1
Cd	0.0090	11.3	Ho	0.0001	0.16	Sb	0.0051	6
Ce	0.0006	0.74	La	0.0009	1.13	Sc	0.0069	8
Co	0.0019	2.40	Lu	0.0003	0.32	Sm	0.0004	0
Cr	0.0038	4.72	Nd	0.0006	0.75	Tb	0.0001	0
Dy	0.0002	0.21	Ni	0.0388	48.5	Ta	0.0027	3

LOD limits of detection of technique oaTOF-ICP-MS, *LODm* limits of detection of the w

Throughput and method evaluation

One hundred of horse hair samples were collected and prepared for multielemental analysis by oaTOF-ICP-MS according to above mentioned procedures. First step of sample preparation was the washing process. As the best washing strategy was chosen process incorporated detergent Triton. Decision has been made according to highest recoveries for five replicates of one tested sample (93–105%). Reproducibility of the mentioned washing strategy was very high and it is believed that valuable information which could be obtained from isotopic ratios still persists. Typical washing throughput was 20 samples per hour. Washed samples were let dried 24 h (laboratory temperature). Following microwave digestion was optimized to have throughput 24 (23 samples and 1 blank) positions per hour. As an analytical technique inductively coupled plasma time-of-flight mass spectrometry was utilized. In comparison with other technique oaTOF-ICP-MS has an advantage of very low detection limits and very fast analysis with fully isotopic information in one time. This is particularly important when a large number of low volume samples with a number of elements to be determined is analyzed. Another reason why to use such technique is very precise isotopic ratio obtained. When using ICP-OES for this kind of analysis one can have very fast multielemental method but insufficient detection limits for trace elements and impossibility to get isotopic information. In case when XRF techniques are employed you can avoid time-consuming sample digestion. Unfortunately, XRF does not provide isotopic

information and the detection limits are poor. Electrochemistry and AAS are not considered as fast methods, and therefore, mass spectrometry remaining to be suitable. Mass spectrometry methods are well-known to have superior detection limits and capability to measure isotopes. Quadrupole and sector field mass spectrometers have excellent detection power but they are not fast, and therefore, isotopic ratios can be affected because of fluctuation during analysis. Moreover, when analysing low volume samples, it is impossible to get fully isotopic information. Chosen oaTOF-ICP-MS has the features of very fast multielemental analysis with unaffected isotopic ratios and very low detection limits. All the samples were analyzed as duplicates and results were averaged. One sample sequence consists of 30 real samples (15 duplicates), calibration, recalibration after every ten samples and check sample after 20th sample. Ranges of determined elements are depictured in Fig. 1. Relative standard deviation for all samples and determined elements did not exceed 10%. This large data set was subsequently used for statistical evaluation which will be presented in the separate paper, not devoted to the analytical method. A sample consumption of 0.7 ml for determination of unlimited number of isotopes in one duplicate was accomplished. Acquisition time for one of the duplicate was three times 5 s. To avoid cross-contamination between samples a rinse time of 120 s has been used. We were monitoring response on all the utilized isotopes for highly concentrated sample and found that after 2 min of rinsing any signal dropped to the baseline. We achieved a high throughput method, one hundred of samples per six hours (without sample preparation). High throughput is very important for large batches of samples which are intended for biomonitoring and statistical studies.

Fig. 1

Concentration ranges (in logarithmic scale) of 36 elements determined in 100 horse hair samples
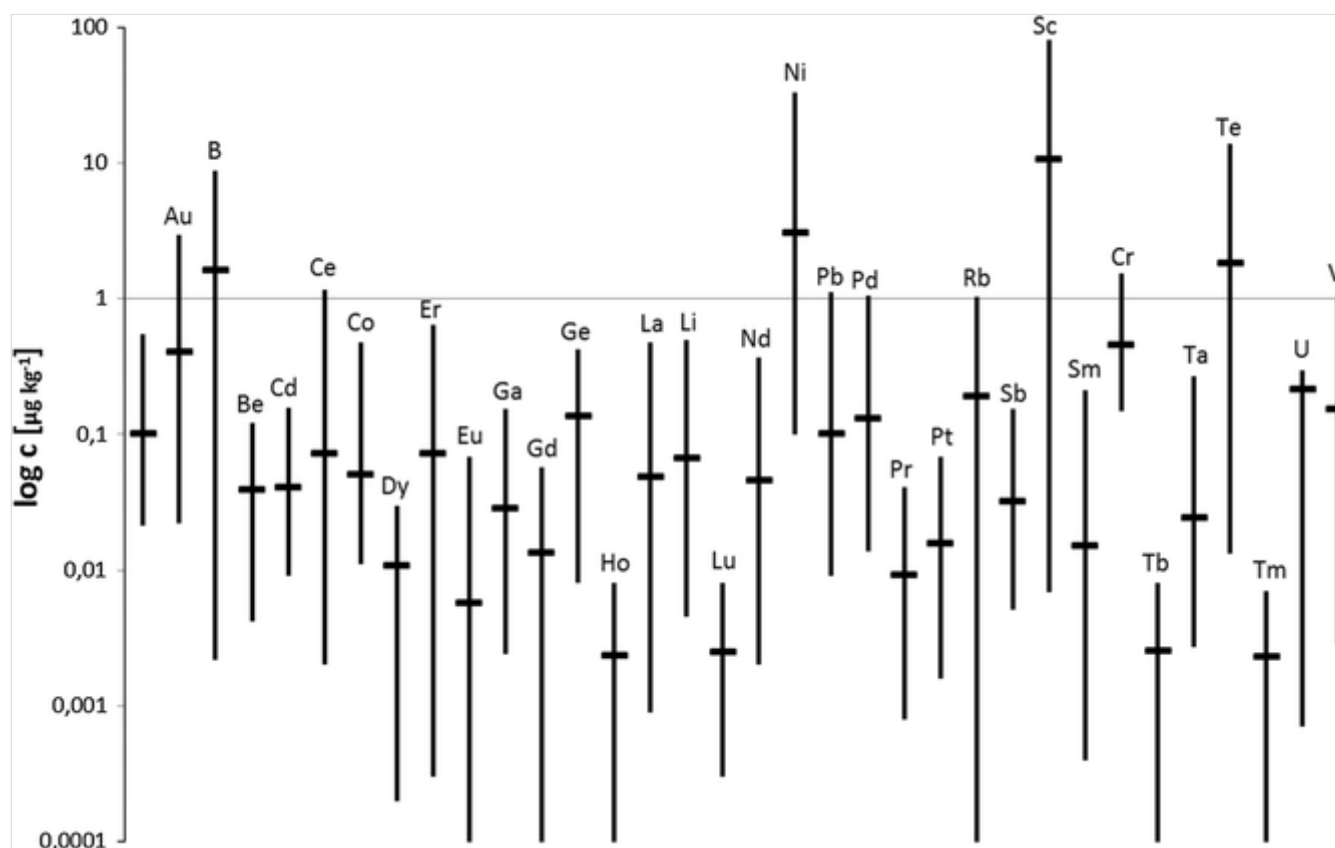
Conclusions

Horse hair represents a very interesting biological matrix which can reveal interesting facts about diet habits, environmental impact, etc. on individual or group of horses. Elemental composition of horse hair tissue can serve as a "fingerprint" of particular individual/group of horses treated by specific way (feeding, stabling, etc.) Information about isotopic ratios appears as a tool enable to reveal interesting facts about different breeding approaches.

The aim of this work was to develop a fast, high throughput and multielemental method for the analysis of biological samples (horse hair). Rapidity and quasi-simultaneous capability of the proposed method ensure non-affected isotopic ratios serving as a tool for statistical evaluation of different breeding approaches. Moreover, the rapidity is also important to treat small samples where the sample consumption is crucial. The way for sample preparation was developed and a sufficient washing process was proposed. We designed a multielemental analysis with high throughput of 100 (50 duplicates) digested horse hair samples per 6 h independently on a number of isotopes. Accuracy of the method was verified by analysis of the reference material Human Hair NCS ZC 8100 and by the standard addition technique. Detection limits for the ICP-MS method ranged from 0.13 µg kg^{-1} (Eu, Gd, Tm) to 27.9 µg kg^{-1} (Au) except for Ni (48.5 µg kg^{-1}) affected by contamination. Repeatabilities given as relative standard deviations were lower 10%. oaTOF-ICP-MS was confirmed as a very fast and powerful quasi-simultaneous technique that allows a very precise measurement of isotopic

ratios across the whole mass spectra. These features are highly valuable for rapid screening methods, for provenance studies or for multivariate statistical analysis.

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AQ3

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