Poster Note 64372

The Role of Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) in Clinical Research and Sports Doping Applications

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Overview

Purpose: To demonstrate the power of ICP-MS for the analysis of different elements in biological matrices and demonstrate the use of trace elemental analysis in clinical research and sports doping applications.

Methods: Total elemental determinations of Pb in blood and different trace elements in urine were performed using the Thermo Scientific™ iCAP™ Qc ICP-MS in a single, optimized operation mode for each of the two applications.

Results: Good agreement between the results and certified reference materials was achieved.

Introduction

The role of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in clinical research is relatively unknown in the mass spectrometry community. However, it is a powerful tool for trace elemental analysis thanks to high sensitivity and robustness when faced with high matrix samples. ICP-MS provides a fully quantitative technique for almost all elements in the periodic table, offers isotopic information and isotope ratio determinations and can also provide species-specific information when coupled with a separation device such as Ion Chromatography (IC) or Liquid Chromatography (LC).

Methods

Sample Preparation

Blood samples were diluted 100-fold in 0.5% HNO_3 prior to an additional 2x dilution from the online addition of the internal standard (1 ng/mL Bi in 0.5% HNO_3)[1].

Urine samples were diluted 20-fold in 2% HNO_3, and 50µl of internal standard solution (5 ng/mL ^{45}Sc, ^{73}Ge, 2.5 ng/mL ^{103}Rh and ^{193}Ir) was added to each sample.

Mass Spectrometry

The iCAP Qc ICP-MS (Figure 1) was used for acquisition of all data.

To analyze blood samples, a SC-2DX autosampler equipped with a 7-port FAST valve (Elemental Scientific Omaha, NE, USA) was used. Through the combination of the iCAP Q and the FAST sample introduction system, sample to sample analysis time (including uptake, data acquisition and washout) of <24 seconds per sample were achieved. To analyze lead, the iCAP Q was operated in STD mode (QCell not used).

An SC-4Q autosampler (Elemental Scientific Omaha, NE, USA) was used to introduce urine samples for the total element quantification (TEQ). The iCAP Qc ICP-MS was operated in single He KED mode for all measurements.

All instrumental parameters can be found in table 1.

FIGURE 1. Thermo Scientific iCAP Qc ICP-MS

TABLE 1. Instrumental Conditions

Parameter	Value
Nebulizer	PFA-ST
Nebulizer Gas Flow	1.06 l/min
Sample Uptake Rate	Approx. 400 µl/min
RF Power	1550 W
Interface set-up	Ni Cones, High Matrix Skimmer insert
QCell conditions (Urine Analyis Only)	
Cell Gas Flow	4.8 mL/min 100% He
KED voltage	3 V

Data Analysis

Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ software was used for quantitative assessment of the data. The modular design of the software allows to seamlessly integrate peripheral devices like autosamplers or segmented flow sample introduction systems (like FAST) for different types of applications using dedicated plug-in software.

Results

Analysis of Lead in Whole Blood

Lead is a neurotoxic metal that affects areas of the brain that regulate behaviour and nerve cell develpment[2]. A series of exposure pathways allow Pb to enter the body, including inhalation of particles (dust from contaminated soils, lead in paint etc.) as well as drinking water or (particluarly for children) toys. Young children and fetuses are particularly vulnerable since the blood-brain barrier is not yet fully developed and absorption rates are higher than in adults.Currently, there is no safe concentration of Pb in blood. The Occupational Safety & Health Administration (OSHA) has guidelines for adult BLLs as it relates to work place exposure. These guidelines include removal from work if levels are > 60 µg/dL and follow-up testing before return.

Figure 1 shows a typical calibration curve used during the routine determination of Pb in whole blood, ranging from 0 to 10 ng/mL (10 ng/mL correspond to 1µg/dL). In order to reduce potentially erroneous results due to the variable isotopic composition of Lead, the sum of all major isotopes ($^{206+207+208}Pb$) was used as only signal for Pb.

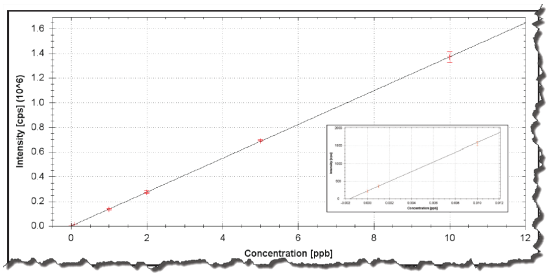


FIGURE 2:Calibration curve for $^{206+207+208}Pb$. The insert shows the same curve between 0 and 0.01 ng/mL

The instrumental detection limit was approximately 1 ng/L (corresponding to 0.0001 µg/dL). Hence, the resulting method detection limit was calculated to be 0.02 µg/dL.

In order to verify the accuracy of the method, appropriate control standards (Lyphochek™ Whole Blood Controls, Bio-Rad Laboratories, Inc.) containing three different levels of Pb in whole blood were analyzed as part of each run. The obtained results for three runs are displayed in Table 2 and show excellent agreement with the reference values.

TABLE 2: Results obtained for the measurement of control standards containing Pb in whole blood

Run #	Level 1 6.28-9.42	RSD	Level 2 20.4-30.6	RSD	Level 3 34.2-51.3	RSD
1	8.19 ± 0.11	1.4	25.4 ± 0.31	1.2	43.0 ± 055	1.3
2	8.42 ± 0.10	1.2	26.1 ± 0.29	1.1	41.2 ± 0.39	0.94
3	8.12 ± 0.12	1.5	25.1 ± 0.50	2.0	42.4 ± 0.50	1.2

Matrix Robustness

The analysis of whole blood is definitely challenging for ICP-MS due to its complex nature. High contents of salt forming components like Na, K, Cl etc. can lead to signal drift over the course of the analysis, other components like proteins can precipitate upon dilution with acids and lead to clogging of parts of the sample introduction system.

Dilution of the sample is typically used to overcome these effects, but requires an elevated detection sensitivity in order to achieve required detection limits.

Typically, the internal standard recovery over the course of running a set of samples is used as a parameter to judge the effect that the sample matrix has on the instrument. For one of the runs this is shown in figure 3 and indicates the robustness of the method.

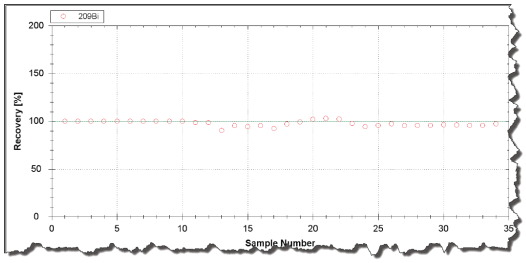
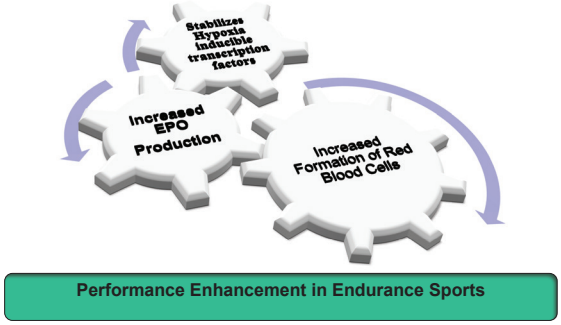


FIGURE 3: Internal Standard Recovery over the course of the analysis

Analysis of Trace Elements in Urine

The analysis of trace metals in urine is a way to address potentially harmful exposure to contaminants. One of the advantages for urine as a sample matrix is that the specimen is easily derived. Apart from screening for potential exposure to toxic elements like As, Cd, Hg and Pb, the determination of Co in urine has gained interest, as Co^{2+} could potentially used as a performing enhancing substance in endurance sports[3]. In a pilot study, approx. 200 samples were analyzed for Co and various other trace elements in order to verify baseline levels of Co in urine. Furthermore, an elimination study was performed to verify that intake of Co^{2+} leads to an increase of the urinary Co concentration. The underlying mechanism is displayed in figure 4.

FIGURE 4. Proposed mechanism for Co^{2+} induced performance enhancement in enduring sports.



UTAK Urine Control Samples (Low and High Range, UTAK Laboratories, Valencia, CA, USA) were regularly interspersed in the sample list (every 10 samples) to verify the accuracy of the method. The obtained results for the repeated analysis of both control samples within a batch (containing 70 samples) are displayed in table 3. All values are given in ng/mL.

TABLE 3. Results for the repeated analysis of urine controls as a quality control within one sample batch.

	As	Cd	Ca	Cr	Co	Cu	Fe	Pb	Mn	Mo	Ni	Se	Zn
Reference	16.5	0.4	96	1.3	1.9	32	30	0.6	3.5	52	2.6	40	269
Recovery	94%	95%	98%	95%	95%	103%	97%	122%	95%	94%	117%	90%	117%
RSD	1.8	6.6	1.6	4.6	3.3	2.9	4.9	4.7	2.9	2.2	3.0	2.7	2.5
Reference	118	4.6	490	6.2	7.6	81	565	137	4.2	71	27	55	859
Recovery	94%	96%	91%	105%	98%	95%	87%	89%	100%	95%	107%	91%	100%
RSD	3.6	2.9	1.7	1.9	1.6	3.2	3.1	1.0	4.1	3.8	1.7	5.2	2.8
IDL	0.007	0.0006	0.3	0.001	0.0006	0.0008	0.002	0.0003	0.002	0.0007	0.002	0.02	0.006

Utak 12111 Normal Range | Utak 12110 High Range

In a second step, the excretion of Co in urine was monitored upon intake of single and multiple dosages of Vitamin B12 (500 µg/day, accounting for 22 µg Co) and Co^{2+} (500µg/day). Results show that the Co concentration in urine is increased to between 40 and 318 ng/ml within 6h after intake of Co^{2+}, whereas the normal concentration was observed to be between 0.1-2.2 ng/mL[3]. Intake of Vitamin B12 does not seem to affect the urinary Co level.

Conclusion

- The iCAP Q ICP-MS allows researchers to determine low levels of lead in whole blood samples using a robust and accurate routine research method with excellent sample turnover times.
- The detection of Co in urine has been shown to be a potential tool to investigate its abuse in sports doping. However, further investigation is required to establish a robust testing method.

Acknowledgment

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References

1. Simple,fast and reliable analysis of lead in whole blood using the Thermo Scientific iCAP Q ICP-MS, Application Note 43137
2. Measuring lead exposure in infants, children and other sensitive populations, National Research Council, National Academies Press, Washington, DC
3. O. Krug et al., Quantifying cobalt in doping control urine samples – a pilot study, Drug Testing Analysis, DOI 10.1002/dta.1694

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