Quantifying Co in Doping Control Urine Samples – A Pilot Study

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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 applications.

Methods: Total elemental determinations of Co and other trace elements in urine were performed using the Thermo Scientific™ iCAP™ Qc ICP-MS in a single, optimized operation mode. The excretion of Co in urine was measured in an elimination study after intake of Co^{2+} and vitamin B12 respectively.

Results: Good agreement between the results determined using a certified reference material was achieved. The elimination study showed that the determination of Co in urine can be a potential tool to screen for its misuse as a performance enhancing substance.

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).

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 be used as a performing enhancing substance in endurance sports[1].

Methods

Sample Preparation

Urine samples were diluted 10-fold in 2% HNO_3, and 50 µl of an internal standard solution (5 $ng \cdot mL^{-1}$ Sc, Ge, 2.5 $ng \cdot mL^{-1}$ Rh and Ir) was added to each sample.

Mass Spectrometry

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

An SC-4Q autosampler (Elemental Scientific, Omaha, NE, USA) was used for all measurements. The iCAP Qc ICP-MS was operated in single He KED mode for all analyte ions.

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^{-1}
RF Power	1550 W
Interface Set-Up	Ni Cones, High Matrix Skimmer Insert
QCell conditions	
Cell Gas Flow	4.8 mL·min^{-1} 100% He
KED Voltage	3 V

Data Analysis

Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ software was used for quantitative assessment of the data.

Results

The underlying mechanism in which Co^{2+} may lead to improved performance of especially endurance athletes is displayed in figure 2. In 2014, Co^{2+} was not listed on the World Anti-Doping Agency´s list of prohibited substances[2], so that dishonest sportsmen could potentially use this option to increase their oxygen transport capacity. In contrast, testing for elevated levels of Co is already done in e.g. horse racing[3].

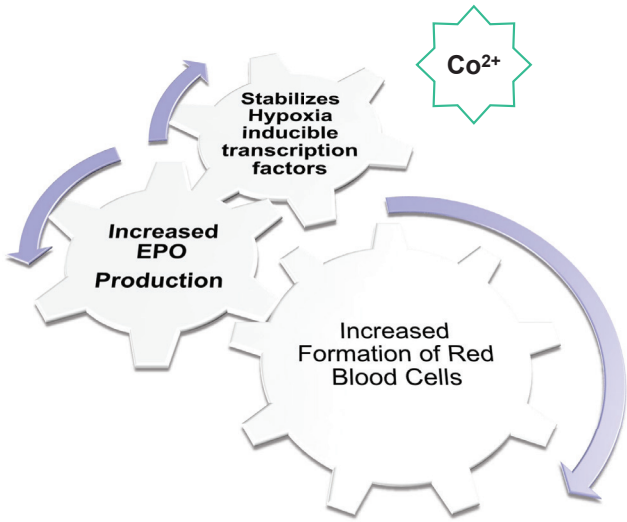

FIGURE 2. Proposed mechanism for Co^{2+} induced performance enhancement in enduring sports.

- The effect of 5 µmol Co is equivalent to stimulation of Erythropoiesis as 1 IU rhEPO.
- Typically, 6-25 $IU \cdot L^{-1}$ of EPO are circulating in humans.
- 150 mg $CoCl_2$ leads to an increase of RBC from 0.5 to 1.19 Mio $cells \cdot mm^{-2}$ in 7-22 days.

Analysis of Trace Elements in Urine

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.

✓ Verify baseline Co levels in urine
-100 healthy non-elite athletes
- 96 endurance sports athletes

- Co concentrations in urine were found to be in the typically observed range [0.1 to 2 $ng \cdot mL^{-1}$]

All calibration standards were prepared in diluted nitric acid and a six point calibration curve was generated between 0.025 to 25 ng·L^{-1} (verification of reference levels) and 0.25 to 100 ng·mL^{-1} (elimination study). The attainable instrumental limit of detection for ^{59}Co was found to be 0.6 ng L^{-1}. 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 2.

TABLE 2. Results for the repeated analysis of urine controls as a quality control within one sample batch, all values given in µg·L^{-1}.

	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
Minimum	14	0.34	82	1.1	**1.6**	27	26	0.5	3.0	44	2.2	34	229
Maximum	19	0.46	110	1.5	**2.2**	37	35	0.7	4.0	60	3.0	46	309
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
Minimum	100	3.9	417	5.3	**6.5**	69	480	116	3.6	60	23	47	730
Maximum	136	5.3	564	7.1	**8.7**	93	650	158	4.8	82	31	63	988
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**

Good agreement between the certified and the obtained values was achieved. The low relative standard deviation between the different replicates highlights the robustness of the method.

The obtained results for both groups (mean values 0.28 ng·mL^{-1} for non elite athletes and 0.36 ng·mL^{-1} for listed endurance athletes) were found to be within the expected range, but showing a modest but statistically significant increase for professional endurance athletes (Wilcoxon rank sum test, $p<0.01$). However, strongly increased urinary Co concentrations were not observed.

Feasibility of Co Urinary Concentration as a Tool for Anti Doping Testing

In a second step, an elimination study was performed to verify that intake of Co^{2+} leads to an increase of the urinary Co concentration. The results obtained for both groups are displayed in figure 3.

Investigate excretion after intake of Co^{2+}
- 3 participants. single dose; 500 µg
- 3 participants. multiple doses of 500 µg

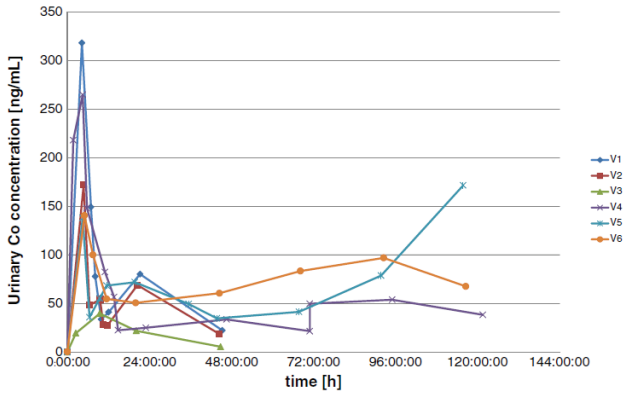

FIGURE 3. Proposed mechanism for Co^{2+} induced performance enhancement in enduring sports.

The results show that the Co concentration in urine is increased in comparison to the reference values found between 0.1-2.0 ng·mL^{-1}. A similar picture was found after normalization of the determined Co concentration in order to compensate for the specific gravity of the samples. There are two conclusions to be drawn from the results:

- A strong increase of the urinary Co concentration is observed within 6 h (between 40 and 318 ng·mL^{-1}) after intake of Co^{2+}.
- Multiple doses of Co^{2+} lead to elevated levels of Co^{2+} observed even several days after the intake was stopped.

Furthermore, the excretion of Co in urine was monitored upon regular (daily) intake of Vitamin B12 (500 µg·day^{-1}, accounting for 22 µg Co). However, Vitamin B12 does not seem to affect the urinary Co level.

Conclusion

- The iCAP Q ICP-MS is a perfect tool to determine trace elements in a challenging matrix such as urine. The combination of a unique robust interface design and a single analysis mode for all analytes greatly simplifies method development and helps to improve throughput when it comes to routine analysis of a large number of samples.
- The detection of Co in urine has been shown to be a potential tool to investigate its abuse in sports doping.
- Further investigation is required to elucidate the role of additionally influencing factors and to establish tests for complementary sample matrices like erythrocytes.

References

1. O. Krug et al., Quantifying cobalt in doping control urine samples – a pilot study, Drug Testing Analysis **6** (2014), 1186-1190
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