Research article

Quantification of cobalt and nickel in urine using inductively coupled plasma atomic emission spectroscopy

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A B S T R A C T

Cobalt and nickel are micronutrients indispensable for the body, therefore, their use with food or as part of vitamin complexes is necessary to maintain health. As a result, trace cobalt and nickel contents are present in human biological fluids – blood and urine. According to the World Anti-Doping Agency prohibited list, they belong to the group of blood doping preparations – erythropoiesis stimulants. Nowadays, methods for their control in biological fluids are being actively developed to establish reasonable allowable contents of these trace elements in human biological fluids. However, in addition to developing highly sensitive methods for the determination of the total content of cobalt and nickel using ICP-MS and ETAAS, the development and comparison of various sample preparation methods that can provide the greatest accuracy, reproducibility and express analysis are also relevant. In the present paper, a comparison of different sample preparation methods – direct analysis, dilution and microwave mineralization of urine samples was shown, the detection and quantification limits were compared, some metrological characteristics that can be achieved using these sample preparation methods were evaluated. The procedure was tested on artificial and real urine samples. Taking the course of vitamin complexes in therapeutic concentrations was shown not to lead to a significant increase in the concentrations of analytes in urine, while taking elevated concentrations (for example, 2-fold) makes it possible to determine them even using ICP-AES. However, even in this case, cobalt and nickel concentrations remain at a relatively low level, not able to lead to a significant increase in erythropoiesis.

1. Introduction

Hypoxia-inducible stabilizers (HIF) are an emerging class of drugs for the treatment of low blood hypoxia-responsive genes. The effect of cobalt and nickel on erythropoiesis, as HIFs, firstly was shown in the late 20s of the last century [1]. Then, experiments on laboratory animals proved that cobalt salts administration led to a phenomenon similar to polycytomy. However, there was a significant difference from polycytomy: when cobalt was consumed, the number of produced reticulocytes and erythrocytes increased, while the death rate of red blood cells did not change [2]. This process led to an increase in intrinsic erythropoietin and the appearance of cobalt-induced tissue hypoxia due to the impact on the degradation processes of 1α- and 2α-hypoxia-induced factors [3].

Similar effects appear in the case of argon, xenon, krypton and a number of organic compounds, e.g. modulastat and rhodulastat, administration [4], which has led to their inclusion in the list of substances prohibited by the World Anti-Doping Agency (WADA) in 2014 [5]. Athletics and equestrian sports require the closest attention in the

control of these drugs, where, despite the known toxicity of cobalt compounds, there have been cases of their abuse.

The most popular cobalt consumption form is its chloride. Cobalt chloride administration leads to an increase in the performance of an athlete under anaerobic exercise due to an increase in the content of erythropoietin in blood plasma. At the same time, it results in the damage and dysfunction of tissues due to oxidative damage caused by excessive concentrations of cobalt in the body and excess oxygen, not stabilized by hypoxia-inducing factor 1α [6].

Today, a number of methods are known for determining cobalt and nickel in biological fluids using inductively coupled plasma mass spectrometry (ICP-MS) [7, 8, 9, 10], electrothermal atomic absorption spectroscopy (ETAAS) [11, 12] and high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) [13, 14]. Cobalt is known to form a complex with albumin in blood, which was previously considered in [13]. Among non-invasive methods for determining cobalt, a special attention should be paid to the work [12], where its determination in the form of a complex with diethyldithiocarbamate using

HPLC-MS/MS has been proposed, however, this procedure is complicated.

ICP-MS and ETAAS possessing high sensitivity and selectivity are without such shortcomings [7, 8, 9, 10, 11, 12]. These methods can be applied to the analysis of both blood and plasma samples [7, 8] as well as urine [15, 16, 17]. According to the literature data, the concentration of cobalt in the body as a rule does not exceed 1 ng/mL [15], which makes the application of methods for its quantification at trace and ultra-trace levels relevant. However, in the case of cobalt or nickel abuse for stimulating erythropoiesis, their concentrations in biological fluids increase rapidly according to the results of previous studies [9, 15].

Another interesting method for the quantification of cobalt, nickel, arsenic and manganese in urine using ETAAS was described in [12]. Low detection limits were achieved using a palladium modifier, two-stage pyrolysis and temperature optimization at each stage of the analysis. The authors used argon with the addition of 5% hydrogen for blowing the furnace, which allowed to increase the accuracy of the procedure according to the presented results. As in the case of the method [1], the sensitivity of the proposed method is excessive, and the method has been tested only on an artificial urine sample, which does not allow to reliably evaluate the matrix effects due to the variability of the samples.

Thus, the determination of cobalt in biological fluids is an urgent task, especially taking into account the prevalence of vitamin B12 (cobalamin) in athletes.

The most convenient fluid for conducting the study is urine, since the procedure for its collection is non-invasive.

To date, WADA has not established the minimum permissible cobalt content in urine for athletes. At the same time, similar limits have been set for equestrian sports: 100 ng/mL in urine and 25 ng/mL in plasma [18].

Thus, it seems appropriate not only to study the effect of cobalt and nickel administration in the form of vitamin complexes on their contents in urine, but also to assess the impact of the sample preparation procedure on the results obtained.

2. Experimental

2.1. Materials and methods

To quantify target elements, 1 g/L stock standard Ni and Co solutions were used. For the preparation of test samples, nitric acid (Component-Reagent, Russia) and hydrogen peroxide (ECOS-1, Russia) were used. Vitamin complex Solgar B-complex "100" (Solgar, USA) was used for the administration studies.

2.2. Samples collection and preparation

Fasting urine samples of 20 volunteers (men and women aged between 19–25 years) who gave informed consent for the experiment were stored at –20 °C until analysis to prevent any sample degradation processes.

To study the effect of urine sample preparation on sensitivity and accuracy, several sample preparation schemes were compared: direct analysis, 5- and 10-fold sample dilution and the use of microwave mineralization to eliminate organic matrix. Microwave mineralization was carried out using a MARS6 system as follows: 5.0 mL of urine was placed in an autoclave, then, 5 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added with following autoclaves sealing for further digestion. The sample opening program included gradual heating to 200 °C for 10 min (stage 1) and maintaining the reaction chamber at 200 °C for 10 min (stage 2) to achieve full sample digestion. In order to avoid the possibility of analyte losses, autoclaves were opened at a temperature below 40°C. The resulting digestion solution was transferred to a 25 mL volumetric flask and made up to the mark with double distilled water. The volumes of added reagents and the mineralization

program were selected according to the recommendations of the system manufacturer.

Metals were determined in the studied samples using an iCAP-7000 series inductively coupled plasma atomic emission spectrometer (Thermo, USA). The most sensitive analytical lines for determining metals in urine samples were used (Table 1). The operating parameters of the device are presented in Table 2.

3. Results and discussion

To ensure better accuracy in the study, evaluation of the sample preparation effectiveness was conducted using artificial urine (the composition is given in Table 3), which allowed to assess some metrological parameters more objectively and partially consider the influence of salt and organic compounds present in real samples.

Owing to the possibility of a significant effect of matrix components on the results, not only microwave mineralization application was of interest, which minimizes organic matrix effects, but also the possibility of sample dilution to decrease analysis time. For this purpose, a series of 5- and 10-fold diluted solutions were prepared.

Assessing the interferences and accuracy of the results obtained was carried out by sequential analysis of blank urine samples, as well as urine samples spiked with target elements; the obtained data were also compared with the results achieved using artificial urine.

It should be noted that cobalt and nickel contents in all the studied blank urine samples did not exceed the quantification limit. Thus, they were used to prepare quality control solutions with low, medium and high concentrations (Table 4). 10 μg/L scandium solution was used as an internal standard in this study. However, in the course of the research, it was established that it was possible not to use an internal standard; nevertheless, in this case, it is advisable to check the stability of the obtained results using control calibration points at least every 10–15 measurements.

Table 1. Spectral lines of target elements.

Element	Wavelength, nm
Co	228.616
Co*	238.892
Ni*	231.604
Ni	221.647

* Alternative wavelength.

Table 2. Instrument operating parameters.

Parameter	Value
Nebulizer gas flow rate, L/min	0.5
Plasma gas flow rate, L/min	0.5
Cooling gas flow rate, L/min	12
RF-generator power, W	1150

Table 3. Artificial urine composition (pH = 7.0 ± 1.0).

Compound	Concentration, g/L
Urea	17.3
NaCl	14.1
KCl	2.8
CaCl$_2$	0.60
MgSO$_4$	0.43
NH$_4$OH	0.50
HCl	0.73

Table 4. Determination of nickel and cobalt in urine with the use of internal standard (n = 6).

Element	QC, ng/mL	Concentration, ng/mL			
		10-fold dilution	5-fold dilution	Direct analysis	Microwave mineralization
Co (228.616)	5	5.0 ± 0.5	5.0 ± 0.3	5.0 ± 0.6	<LOD
	40	43 ± 3	42 ± 4	40 ± 5	36 ± 4
	90	99 ± 6	99 ± 7	92 ± 10	78 ± 8
Co (238.892)	5	5.0 ± 0.5	4.0 ± 0.6	6.0 ± 1.0	<LOD
	40	44 ± 4	42 ± 3	45 ± 5	34 ± 4
	90	99 ± 7	98 ± 5	101 ± 11	75 ± 8
Ni (221.647)	5	5.0 ± 0.6	5.0 ± 1.0	5 ± 0.8	<LOD
	40	43 ± 3	41 ± 3	40 ± 5	35 ± 4
	90	98 ± 6	98 ± 5	92 ± 8	77 ± 8
Ni (231.604)	5	6.0 ± 0.5	5.0 ± 0.6	5.0 ± 0.6	<LOD
	40	45 ± 4	45 ± 4	41 ± 4	34 ± 4
	90	101 ± 10	103 ± 6	95 ± 6	74 ± 8

Table 5. Comparison of detection and quantification limits for cobalt and nickel in urine using different sample preparation procedures (n = 6).

Element	LOD, ng/mL			LOQ, ng/mL		
	Microwave mineralization	5-fold dilution	Direct analysis	Microwave mineralization	5-fold dilution	Direct analysis
Co (228.616)	0.9	1.6	2.6	3.0	5.3	7.4
Ni (231.604)	0.8	1.5	2.3	2.7	5.0	7.0

Table 6. Comparison of different sample preparation techniques for the quantification of cobalt and nickel in urine (n = 6).

Sample	Sample preparation procedure					
	$C_{Co (228.616)}$, ng/mL			$C_{Ni (231.604)}$, ng/mL		
	Direct analysis	5-fold dilution	Microwave mineralization	Direct analysis	5-fold dilution	Microwave mineralization
5 ng/mL spiked distilled water	5.0 ± 0.5	5.0 ± 0.4	4.0 ± 0.5	6.0 ± 1.0	5.0 ± 0.4	5.0 ± 0.6
Blank	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1 ng/mL spiked blank	n.d.	n.d.	n.d.	n.d.	n.d.	1.0 ± 0.1
5 ng/mL spiked blank	8.0 ± 1.8	4.0 ± 0.3	4.0 ± 0.5	9.0 ± 2.0	5.0 ± 0.4	4.0 ± 0.5
10 ng/mL spiked blank	14 ± 3	8.0 ± 0.6	7.0 ± 0.8	13 ± 2	9.5 ± 0.8	8.5 ± 1.0
Sample after taking single dose of vitamins	8.5 ± 2.0	5.7 ± 0.4	5.8 ± 0.7	11 ± 2	7.8 ± 0.6	8.2 ± 1.0
Sample after taking double dose of vitamins	13 ± 2	11 ± 1	11 ± 1	21 ± 4	15 ± 1	17 ± 2
Sample after taking double dose of vitamins and 10 ng/mL spike	34 ± 6	16 ± 1	20 ± 2	27 ± 5	20 ± 2	21 ± 2

*n.d. – not detected.

From the data presented (Table 4), it can be seen that 10-fold sample dilution does not lead to better accuracy of the results comparing to 5-fold dilution, however, a larger dilution can significantly affect the sensitivity of the determination, therefore, 5-fold dilution is more preferable. At the same time, the lack of sample preparation negatively affects the accuracy of the results. Unexpectedly, microwave mineralization provided low efficiency toward the studied object and the worst sensitivity, despite the fact that these samples were expected to be the least susceptible to matrix influences. At the same time, it is seen that the optimal analytical wavelengths for cobalt and nickel are 228.616 and 231.604 nm, respectively.

The data obtained were used to establish detection and quantification limits for various sample preparation techniques (Table 5). The quantification limit was set as the lowest analyte concentration which could be determined with acceptable accuracy and precision. The detection limit was calculated as the concentration at which the useful signal exceeded the background signal by at least 3 times. In the course of the research, the slope of the calibration curve obtained with the addition of the internal standard was established to be approximately two times less

(depending on the element) than that obtained without the internal standard.

Based on the data obtained, a number of urine samples of volunteers who consumed vitamin complexes containing cobalamin and nickel for a month were analyzed. Also, the volunteers took a double dose of vitamins to establish whether the excess of the recommended dose of vitamins influenced the background content of the determined elements or not (Table 6). From the results obtained, endogenous concentrations of cobalt and nickel are lower than LOD, as it can been seen from analysis of blank samples. At the same time, administration of the recommended daily dose of vitamins provides an increase in their urinary concentrations up to 5.5–5.8 ng/mL for cobalt and 7.8–8.2 ng/mL for nickel in three days after the course beginning. In the first three days, analyte concentrations cannot be established using ICP-AES. After the third day, no significant changes in their urinary concentrations were observed. After taking a double dose of vitamins, cobalt and nickel concentrations exceed 10 ng/mL and can be easily detected using ICP-AES. However, even in this case, these concentrations are insignificant, and the athlete cannot be accused of HIF abuse.

4. Conclusions

The possibility of ICP-AES application to determine cobalt and nickel in urine was studied using various sample preparation methods: direct analysis, preliminary dilution and microwave mineralization. Detection and quantification limits for cobalt and nickel were estimated, the analysis of real urine samples of volunteers who consumed vitamin complexes was carried out. The administration of vitamins has been shown not to cause a sharp increase in the concentrations of cobalt and nickel in urine, and, consequently, cannot result in false positives during the doping test. Sample dilution was shown to provide not only fast analysis time, but also sufficient accuracy and reproducibility of the results.

Declarations

Author contribution statement

Evgeniy Ph. Galay: Performed the experiments; Analyzed and interpreted the data.

Ruslan V. Dorogin: Performed the experiments.

Azamat Temerdashev: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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