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Optimizing the determination of mercury in human urine by ICP-MS with a collision cell mode

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The applicability of ICP-MS (Thermo Scientific iCAP Qc) and possible interferences from Tungsten oxide were evaluated for mercury quantification in human urine. The mass spectrometer is equipped with a collision cell with helium gas, working with Kinetic Energy Discrimination (KED) mode. This mode is widely used to suppress polyatomic interferences, which are inherent to the ICP-MS. A demerit of the KED mode is the worsening of the sensitivity and lowering of the signal to noise ratio, especially for the lower mass region. Therefore instead of KED, STD mode (not pressurized cell) is preferable, where low limit of detections are desirable.

The present work shows that after optimizing, the mercury determinations by He KED mode, more than double improved signal to noise ratio could be obtained, compared to STD mode. Instead of decreased sensitivity, the signals for all Hg isotopes were higher and precise, when working in KED. The ratios of recorded signals were compared with the natural abundance of Hg isotopes and a very good match was estimated. This is an evidence of the absence of spectral interferences. By the optimized method instrumental detection limits of 2 ng/L (^{202}Hg) were achieved. A certified reference material - human urine was analyzed with an excellent recovery of Hg, in the range of 99 and 101 %. A very good recovery (90-110%) was found also in human urine samples, spiked with 5 ppb Hg and diluted with DF=20.

Key words: *ICP-MS, mercury determination, Hg, collision cell, human urine*

INTRODUCTION

Mercury (Hg) is considered as one of the most toxic and dangerous to the human organism heavy metals [1]. At levels above the permissible concentrations Hg has toxic and carcinogenic effects on the kidneys, immune and reproductive systems, the myocardium and nervous system [2, 3, 4]. It is easily concentrated in inner organs, due to its affinity for S-H groups [3]. Hg is mainly absorbed through the respiratory system, the gastrointestinal tract and the skin. It is excreted from the organism through urine and feces, with about two months' half-life [5].

Quantification of mercury in urine is a noninvasive method widely used for assessment of the risk of human exposure.

The substances suitable for use as biomarkers are defined through experimental and epidemiological toxicological studies among workers [6]. Mercury is also used as a biomarker in the international occupational medicine practice. Table 1 presents data on the permissible concentrations of mercury in urine [8].

Table 1. Reference levels for monitoring of the exposure to Hg (Reymond Meister, 2004)

Biological material	Without professional exposition	NOAEL*	Manifestation of clinical effect	Half-life period	Comment
Urine (μg/L)	< 5	35-50	100	60 days	Reflects the exposition of the past 2-4 months

*NOAEL - No-observed-adverse-effect level

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In Bulgarian legislation mercury is listed along with 14 substances, which are monitored for protection of employees from the risks related to occupational exposure to chemicals. Biological environment, appropriate biomarker, biological limit and time for sampling are specified [7]. According to WHO a biological limit up to 100 μg/L is given for mercury in urine (2003) [9]. Patients, who do not consume contaminated with Hg foods, have a level of Hg in urine ≤ 0.5 μg/L [1].

Quantification of Mercury is a complicated analytical task, especially in biological materials, where the maximum permissible concentration is very low. ICP-MS is a powerful tool for trace elements determinations. Aim of this study is to optimize an ICP-MS method for determination of Hg in human urine, capable to achieve limits of quantification < 1 μg/L.

MATERIALS AND METHODS

The analysis was performed on a Thermo Scientific ICAP Qc ICP-MS (Thermo Scientific, Germany), equipped with a collision cell with helium (He) and kit for online introduction of internal standard. Running conditions for ICP-MS are summarized in Table 2.

Stock solutions of mercury (Hg) with a concentration of 1000 mg/L (Honeywell Fluka™) was used. Rhenium (CPAchem Ltd) was used as internal standard with a final working concentration of 15 μg/L. The isotope ^{185}Re was preferred because it is free of isobaric interferences. For the preparation of all solutions and reagents, ultra-pure water (18.2 M Ω.cm) from VWR Puranity TU and nitric acid (HNO_3, 2 v/v) from Fisher Chemical were used. Reference material was purchased from Seronorm (Trace Elements urine, Seronorm, Nycomed AS, Oslo, Norway).

Urine samples were collected in plastic bottles. Total volume excreted was measured and recorded for each 24-h urine specimen. Specimens were stored at 4°C, and analysis was performed within 7 days. The samples were thoroughly mixed before dilution with 2% HNO_3. Two dilution factors were used (DF = 20 and DF = 100).

The certified reference material Seronorm was prepared according to the supplier instruction and diluted with 2% HNO_3 (DF = 200) before measuring.

Aqueous standards for Hg external calibration were prepared in 2% HNO_3 at concentrations of 25, 50, 100, 250 and 500 ng/L from a stock solution of 1000 mg/L Hg.

The laboratory analysis was performed by external calibration, with online introduction of internal standard rhenium.

Table 2. Optimized operating conditions of the ICP-MS ICAP Qc

Plasma conditions	
RF-power	1550 W
Nebulizer Gas Flow	0.96 L min^{-1}
Auxilliary Gas Flow	0.80 L min^{-1}
Plasma Gas Flow	14.00 L min^{-1}
He gas flow	3.2 mL min^{-1}
Mass Spectrometer Settings	
Dwell time	0.3 sec
Sweeps	15
Replicates	4
Survey run	180-220 amu

RESULTS AND DISCUSSION

Determination of mercury by ICP-MS may be performed by registration of the mercury isotopes' signals, shown in Table 3. In the same table are given the potential polyatomic interferences of Tungsten oxides. The signals of five from seven isotopes of mercury were measured - ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg. Two isotopes - ^{196}Hg and ^{204}Hg were excluded from data processing, due to their low natural abundance percentage. Moreover the latest is overlapped by ^{204}Pb and if Lead is present in the urine samples, it could cause isobaric interference. Rhenium (^{185}Re) was used as internal standard for nonspectral interferences and sensitivity drift correction. There are five isotopes of tungsten - ^{180}W, ^{182}W, ^{183}W, ^{184}W and ^{186}W, which are able to generate oxide radicals with mass range from 196 to 202 amu. Table 3 presents the isotopes and possible interferents.

Optimization of the experimental determination was performed to achieve the best signal to noise ratio. The mass spectrometer is equipped with a collision cell with helium gas, working in Kinetic Energy Discrimination (KED) mode. This mode is widely used to suppress polyatomic interferences in ICP-MS. A demerit of the KED mode is the worsening of the sensitivity and lowering the signal to noise ratio, especially for the lower mass region. Therefore, a standard mode (not pressurized cell - STD) is preferable instead of KED, where the desirable limits of detections are low. Both KED and STD modes were tested for mercury measurements of the lowest calibrator – 25 ng/l Hg. Higher sensitivity and precision were expected for

the signals measured in STD mode. The results of the experiment, however, show that when working with a collision cell with helium gas in KED mode, RSD for all isotopes of Hg observed are almost 2 times lower than in STD mode (Figure 1).

Table 3. Mercury and tungsten isotopes with natural abundance (NA) and possible mass spectra overlaps from WO interferences

Isotop	NA %	Isotop	NA %	Interference
^{196}Hg	0.146	^{180}W	0.126	^{180}W^{16}O
^{198}Hg	10.02	^{182}W	26.31	^{182}W^{16}O
^{199}Hg,	16.84	^{183}W	14.28	^{183}W^{16}O
^{200}Hg	23.13	^{184}W	30.64	^{184}W^{16}O
^{201}Hg	13.23	^{186}W	28.64	^{184}W^{16}OH
^{202}Hg	29.8			^{186}W^{16}OH
^{204}Hg	6.85			^{204}Pb

This effect could be explained with better focusing of the ion beam, when the cell is working in KED mode. Probably this tolerates the passage of Hg^+ ions, with negligible effect of their kinetic discrimination. Because the mercury isotopes are heavy enough, they are barely discriminated, when hit with Helium. Another test was performed, measuring the signals in KED with variable Helium collision gas flow. It was observed that the maximum signal to noise ratio (S/N) for all Hg isotopes measured could be recorded at 3.2 ml/min He gas flow. When a lower He flow is used the S/N ratio is dropping with up to 40%. Human urine samples were measured in KED mode at 3.2 ml/min He. In this case spectral interferences were found, due to the variable concentrations of tungsten (see Table 4). Therefore, after tune optimization, 4.925 ml/min was chosen as optimal collision gas flow. The efficiency of the interference correction was checked by scans in a mass range of 180-210 amu (atomic mass unit). The experimentally recorded signals of the five selected mercury isotopes were compared and normalized to ^{200}Hg (Figure2).

A very good match with the natural abundance of the results, obtained from the analysis of 25 ng/l Hg standard and the Seronorm reference material (DF=200), was observed. This is an evidence of the analytical selectivity of the proposed method.

The five human urine samples were measured after dilution as follows: (i) dilution factor DF=100 and (ii) dilution factor DF=20. The estimation of the matrix effect was monitored by the Internal standard shift. The drop of the signals of ^{185}Re, used as Internal standard, was insignificant for both dilution factors. Hence working with less diluted urine samples (DF=20) is preferable, related to lower methodological limit of quantification (LoQ). The use of internal standard is necessary for correction of the typical for ICP-MS sensitivity drift (see Table 4).

For better precision relatively long dwell times were selected 0.3 s per mass. A forced to Blank calibration was preferred. The calculated instrumental detection limits are as follows: 12 ng/L for ^{198}Hg; 4 ng/L for ^{199}Hg; 4 ng/L for ^{200}Hg; 15 ng/L for ^{201}Hg; 2 ng/L for ^{202}Hg. A very high value for BEC (background equivalent concentration) was recorded for ^{201}Hg (BEC=79 ng/L). For this reason only three of the isotopes (^{199}Hg ^{200}Hg ^{202}Hg) were chosen for quantitative measurements of Hg in urine. The concentrations of Hg measured in the five samples human urine are given in Table 4. The difference between the results from the three Hg isotopes is acceptable. Taking into consideration the used dilution factor DF=20, mercury could be measured in urine samples with limit of quantification 0.26 μg/L for ^{199}Hg ^{200}Hg and 0.15 μg/L for ^{202}Hg (LoQ was calculated using 10σ criteria). All patient samples tested showed lower than 5 μg/L content of Hg, from 1 to 2.7 μg/L with acceptable precision. Regardless of the variable content of Tungsten in the urine (see the signals for ^{182}W given in Table 4) no spectral interferences were registered.

In order to validate the method all patient urine samples were spiked with 5 μg/L Hg before dilution. The recovery of the spikes was found to be in the range from 90% to 108%, which refers the method as “fit for purpose” for quantification of mercury in human urine

A certified reference material - human urine (Seronorm Trace Elements Urine, Nycomed AS, Oslo, Norway) was analyzed with an excellent recovery of the referent concentration of Hg. The results are shown in Table 4.

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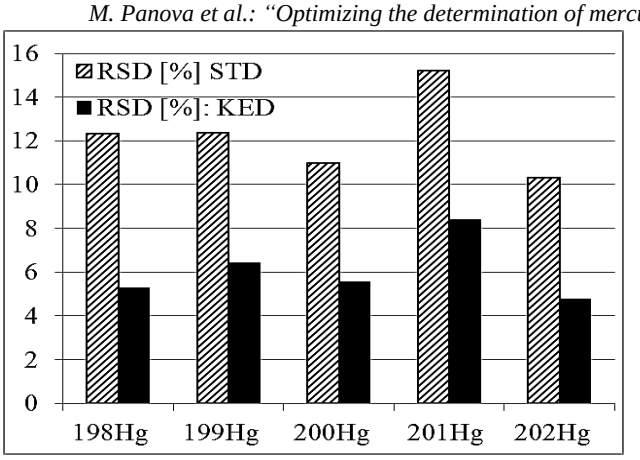

Fig. 1. RSD % obtained by STD and KED mode for 25 ng/L Hg (n=4).

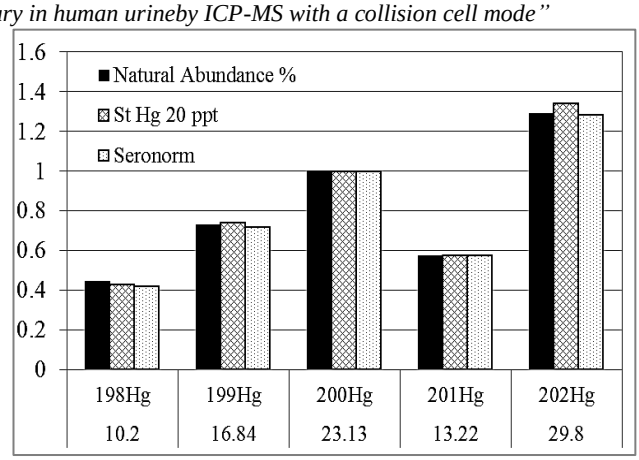

Fig. 2. Hg isotope ratio normalized to ^{200}Hg. The numbers on the bottom are natural abundance in %

Table 4. Results of ICP-MS determination of Hg (μg/L) in human urine, urine spiked with 5 μg/L Hg and CRM – Seronorm, obtained from different isotopes. An interval is given as ± SD, n=4. Recovery is given in brackets (R %).

	IS drift(1)	^{182}W (cps) (2)	^{199} Hg	^{200} Hg	^{202} Hg
Urine 1	97.3%	16,454	1.28 ± 0.08	1.31 ± 0.05	1.19 ± 0.06
Urine 2	98.5%	7,472	1.85 ± 0.05	1.85 ± 0.07	1..97 ± 0.15
Urine 3	99.4%	1,415	2.49 ± 0.12	2.69 ± 0.13	2.63 ± 0.013
Urine 4	98.8%	36,612	1.1 ± 0.16	1.37 ± 0.18	1.17 ± 0.17
Urine 5	99.8%	35,844	0.82 ± 0.003	0.93 ± 0.05	0.76 ± 0.04
Urine 1 + Hg	95.3%	16,986	5.2 (105 %)	5.2 (105 %)	5.2 (104 %)
Urine 2 + Hg	95.5%	7,320	4.5 (90 %)	4.5 (91 %)	4.5 (90 %)
Urine 3 + Hg	96.2%	1,441	5.4 (108 %)	5.4 (107 %)	5.3 (106 %)
Urine 4 + Hg	95.8%	34,646	4.7 (93 %)	4.6 (92 %)	4.6 (92 %)
Urine 5 + Hg	95.6%	33,301	4.6 (91 %)	4.4 (92 %)	4.5 (90 %)
Seronorm 40.7 (36.1 – 45.3) (3)	95.9%	3,433	41.2 (101.2 %)	40.5 (99.5 %)	40.4 (99.3 %)

Human urine was diluted with DF=20, Seronorm was diluted with DF=200. Urine was spiked (+ Hg) with Hg 5 μg/L before dilution. (1) ^{185}Re Internal Standard drift in %; (2) Signals in counts per second measured for ^{182}W; (3) Hg acceptable range from the Seronorm certificate

CONCUSION

Even if Tungsten is not present in the urine of patients and the risk for a potential spectral interference for ICP-MS mercury determination is low, we recommend the collision cell with KED mode to be used. The KED mode improves the signal to noise ratio and allows reaching lower instrumental limits of quantification more than twice comparing with the STD mode. Maximum signal to noise ratio was obtained with 3.2 mL/min He collision gas flow, but to guaranty the effective interference suppression from the variety of the urine constituents it is preferable to work with 4.925 mL/min He flow. A simple dilution of collected urine with dilution factors DF=20 and use of Re as Internal standard is enough to avoid the multiplicative matrix effects. The optimized ICP-MS method is valid for determination of mercury in human urine for concentrations higher than 0.15 μg/L with 90-110% recovery. It is applicable for medical studies in cardiovascular toxicity, reproductive toxicity, neurotoxicity, nephrotoxicity, immunotoxicity and carcinogenicity, where ultra-trace amounts of Hg in human urine have to be measured.

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ОПТИМИЗИРАНЕ ОПРЕДЕЛЯНЕТО НА ЖИВАК В ЧОВЕШКА УРИНА ЧРЕЗ ICP-MS В РЕЖИМ С КОЛИЗИОННА КЛЕТКА

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(Резюме)

Оценена е приложимостта на ICP-MS (Thermo Scientific ICAP Qc) и възможните интерференции от волфрамов оксид при количествено определяне на Hg в човешка урина. Масспектрометърът е оборудван с колизионна клетка с газ хелий, работеща в режим KED (Kinetic Energy Discrimination). Този режим се използва широко за подтискане на полиатомни пречения, които са присъщи на ICP-MS. Недостатък на режима KED е влошаването на чувствителността и намаляването на съотношението сигнал/шум, особено за по-ниския масов диапазон. По тази причина в случаите, изискващи ниски граници на откриване, за предпочитане е режим STD (клетката не е под налягане), вместо KED.

Настоящата работа показва, че след оптимизиране на условията за определяне на Hg в режим KED, отношението сигнал/ шум може да се подобри до два пъти, в сравнение с режим STD. Вместо намаляване на чувствителността, сигналите за всички измерени изотопи на Hg, са по-високи и с по-добра повторяемост използване на KED режим. Отношенията на регистрираните сигнали на изотопите на Hg бяха сравнени с табличните стойности на тяхното природно разпространение, при което се установи много добро съвпадение, което е доказателство за липсата на спектрални пречения върху сигналите на Hg. Чрез оптимизирания метод са постигнати инструментални граници на откриване от 2 ng/L (за ^{202}Hg). Отличен аналитичен добив (в границите от 99 до 101 %) е постигнат спрямо сертифицираната стойност на референтен материал урина. ICP-MS анализът на дотирани с 5 μg/L Hg и разредени с фактор 20 проби урина показва аналитичен добив в интервала от 90 – 110 %.

Ключови думи: *ICP-MS, определяне на живак, Hg, колизионна клетка, урина*