Rapid and Reliable Routine Analysis of Urine by Octopole Reaction Cell ICP-MS

Application

Clinical

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Abstract

The Agilent 7500c Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was used for the fast, routine, and reliable analysis of 23 trace elements in urine. The complete method validation for all elements is described, including the evaluation of short-term and long-term stability, the analysis of different reference materials and the discussion of precision and accuracy in internal and external quality assurance. The urine samples were analyzed directly after a low dilution of 1/5 (v/v) with 1% (v/v) nitric acid. Using this simple sample preparation method, one operator can prepare more than 100 samples in less than 1 hour. An ICP-MS fitted with an octopole-based collision/reaction cell enables the simultaneous determination of all 23 elements either in the normal concentration range for the essential elements or at concentration levels relevant for occupational and environmental health.

Introduction

Assessment of the concentration of bioactive elements present in patients is typically achieved by analyzing easily extractable fluids (urine, blood, serum, and plasma). The concentrations of essential (for example, Se, Mo, Co, Cu, and Zn) and nonessential elements including toxic or carcinogenic substances (for example, Be, Pb, Pt, Cd, U) can provide valuable information regarding exposure and can suggest possible treatments for many complaints.

Many laboratories involved in trace metal analysis of these sample types are replacing single-element techniques such as Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) with multi-element techniques, such as collision/reaction cell Inductively Coupled Plasma Mass Spectrometry (ICP-MS). While ICP-MS was previously used for the analysis of clinical samples, new octopole reaction cell technology has significantly improved the measurement of some clinically important analytes that suffer overlaps from matrix-based interferences.

Instrumentation

Method development and routine analysis was carried out using an Agilent 7500c ICP-MS, which features an Octopole Reaction System (ORS) collision/reaction cell. The cell is pressurized with a gas, such as helium or hydrogen, to remove the plasma and matrix-based interferences that inhibit the trace analysis of elements such as Cr, As, and Se by conventional ICP-MS. The design of the ORS

enables the routine, high throughput, multi-element determination of trace level analytes in complex and varying matrices.

The 7500c was used with a Babington nebulizer and standard quartz Scott-type double-pass spray chamber. The instrument was optimized using a solution containing ^{7}Li, ^{59}Co, ^{89}Y, and ^{205}Tl (10 µg/L) for sensitivity (high signal/background ratio) across the mass range and low interference levels (CeO^{+}/Ce^{+} <1% and Ce^{2+}/Ce^{+} <3%). The collision/reaction gases were optimized using the in-built software routines.

All instrumental conditions (see Table 1) are controlled from the 7500 system's ChemStation PC, including all gases (four plasma gases, plus helium and hydrogen reaction gases), torch position and sample introduction parameters such as sample flow rate and plasma power. A major benefit of fully automated and autotuneable system optimization is that "target-tuning" conditions can be selected, which are then achieved independently of operator expertise. This greatly simplifies the validation of the system performance, for both internal and external audit purposes, as well as ensuring consistent operation in laboratories where multiple users may operate the instrument on a routine basis.

Further details of the instrument have been described in previous publications [1, 2, 3].

Table 1. ICP-MS Operating Conditions

Power/W	1500
Outer gas flow (L/min)	14
Intermediate gas flow (L/min)	1.0
Nebulizer gas flow (L/min)	1.14
Torch injector tube diameter (mm)	2.5
Hydrogen cell gas flow (mL/min)	3.4
Helium cell gas flow (mL/min)	3.2
Sample uptake rate (mL/min)	0.4
Integration time per mass (s)	1.0
Extract lens voltage (V)	2
Lens 1, 3 voltage (V)	–160
Lens 2 voltage (V)	40
Cell entrance voltage (V)	–36
Cell exit voltage (V)	–27
Plate bias voltage (V)	–43

Sample Preparation and Calibration

Urine samples were collected in polyethylene containers over a period of 24 hours. Prior to collection the containers were cleaned with 5% (v/v) ultrapure HNO_3. The samples were acidified with 100 µL of 65 % (v/v) HNO_3 per 20 mL and stored in a refrigerator at 5 °C. The instrument was calibrated using the method of standard additions (MSA). The dilution factor was 1 mL of urine in 5 mL of total volume. In seven calibration solutions the concentrations of the elements Ba, Be, Co, Cd, Cr, In, Li, Mn, Mo, Pb, Pt, Rh, Sb, Sn, Tl, U, V, and W were 0, 0.01, 0.05, 0.1, 0.2, 0.5, and 2 µg/L, respectively. The concentrations for the elements As, Se, Li, Cu, and Zn were 0, 0.1, 0.5, 1, 2, 5, and 20 µg/L respectively, and two higher concentration Zn standards were included at 100 and 200 µg/L. The concentration of the internal standard was 5 µg/L Tb in all sample and calibration solutions. Tb was chosen because it is monoisotopic and shows no relevant interferences from oxide or doubly ionized species.

Following vigorous shaking of the actual urine samples, 1 mL of the sample was acidified with 100 µL of concentrated HNO_3 in a 10-mL autosampler polypropylene tube and 500 µL of the internal standard solution was added. This solution was made up to 5 mL with deionized water using a 5-mL bottle-top dispenser and the samples were homogenized using a magnetic stirrer. This is a simple and accurate procedure that enables the preparation of more than 100 samples in less than 1 hour by a single analyst.

Control samples were used for internal quality assurance purposes. One mL of the freshly prepared control material was made up with nitric acid, internal standard solution and deionized water in the same way as described for the actual samples.

Results and Discussion

It was found that the urine samples could be analyzed directly in a 1/5 (v/v) dilution with deionized water and nitric acid. No clogging of the nebulizer and no particle deposition in the injector tube was observed during 12 hours of analyses. It should be noted that the standard torch of the 7500c has an injector internal diameter of 2.5 mm, which is wider and therefore more matrix tolerant than the "high matrix" torches available as an option on other ICP-MS instruments.

LODs (limits of detection) were determined from LOD=3 RSD_b *c*/SBR as proposed by Boumans [4], where RSD_b is the relative standard deviation of the background intensity of 10 measurements, c is the concentration of the element in solution, and SBR is the signal-to-background ratio.

The LODs in the undiluted urine are in the range 0.4 ng/L (for ^{238}U) to 143 ng/L (for ^{78}Se). See Table 2. The LODs of the essential trace elements Co, Cu, Zn, Se, and Mo are two orders of magnitude below the normal range of these elements in the urine of healthy humans. For many elements, the LODs are mainly limited by contamination. Therefore, careful control of impurities in all reagents is important.

Table 2. Analytical Figures or Merit: LODs and RSDs of Measured Intensities for the Evaluation of Repeatability, Short-Term Stability and Long-Term Stability

	LOD (ng/L)	% RSD		
Isotope	Undiluted urine	2 min, *n*=10	20 min, *n*=20	5 hours, *n*=60
^{7}Li	6	0.8	2.7	4.5
^{9}Be	4	1.5	4.2	3.9
^{52}Cr	16	1.7	3.1	4.6
^{51}V	16	2.4	3.4	4.1
^{55}Mn	21	3.0	3.1	3.6
^{59}Co	5	1.8	2.6	4.9
^{60}Ni	9	2.9	3.4	5.1
^{63}Cu	121	0.9	3.1	4.4
^{66}Zn	92	0.8	3.0	5.1
^{75}As	73	2.1	2.8	5.6
^{78}Se	143	2.4	3.7	6.2
^{98}Mo	27	1.8	2.8	5.4
^{103}Rh	0.6	1.2	3.3	4.8
^{111}Cd	8	2.4	3.2	3.9
^{115}In	4	0.5	3.0	3.7
^{118}Sn	19	1.6	3.2	5.8
^{121}Sb	6	1.1	2.6	5.3
^{138}Ba	8	1.5	2.8	4.1
^{184}W	7	0.9	2.2	4.6
^{195}Pt	3	0.7	1.9	3.8
^{205}Tl	3	0.6	2.1	2.3
^{208}Pb	2	0.7	2.3	2.5
^{238}U	0.4	1.2	2.2	3.2

Repeatability and short-term stability were investigated by measuring the RSDs of the mass intensities for all elements (c=5 µg/L) in two periods of 2 min (with 10 measurements) and 20 min (with 20 measurements), respectively. The RSDs are in the range 0.6%–3.0% for reproducibility and 1.9%–4.2% for short-term stability (Table 2), which are completely satisfactory figures of merit for urine analysis. Long-term stability was investigated by measurement of the intensities every 5 minutes over a period of 5 hours (60 measurements). RSDs are in the range 2.5%–6.2% with some excellent values, for example, for ^{208}Pb and ^{205}Tl (Table 2). All results for long-term stability investigations were obtained without internal standardization.

Interference Removal

In order to avoid the possible interferences described in Table 3, the elements As, Ba, Co, Cd, Cu, V, Cr, Mn, Mo, Rh, and Zn were determined using helium as a collision gas. Se was determined using hydrogen as a reaction gas. Hydrogen was found to be better in overcoming interferences from argon molecular ions $^{40}Ar^{40}Ar^{+}$ or $^{38}Ar^{40}Ar^{+}$. Hydrogen (3.4 mL/min) was added to achieve the best compromise for the lowest background equivalent concentration (BEC) and reduced background intensity for ^{80}Se and ^{78}Se. A benefit of the ORS system is its simple optimization. After initial optimization, cell gas flow rates do not need to be adjusted, and the same He flow rate was used for all analytes measured in this mode. The same cell voltages were used for all analytes. In addition, the ORS eliminates the need for interference correction equations, which improves accuracy in variable matrices such as urine.

Table 3. Some Common Spectral Interferences for Urine Analysis by ICP-MS

Ion	Interfering ions
$^{51}V^{+}$	$^{35}Cl^{16}O^{+}$
$^{52}Cr^{+}$	$^{40}Ar^{12}C^{+}$, $^{35}Cl^{16}OH^{+}$
$^{53}Cr^{+}$	$^{37}Cl^{16}O^{+}$
$^{59}Co^{+}$	$^{43}Ca^{16}O^{+}$
$^{60}Ni^{+}$	$^{44}Ca^{16}O^{+}$, $^{23}Na^{37}Cl^{+}$
$^{63}Cu^{+}$	$^{40}Ar^{23}Na^{+}$
$^{66}Zn^{+}$	$^{32}S^{16}O^{18}O^{+}$
$^{75}As^{+}$	$^{40}Ar^{35}Cl^{+}$, $^{40}Ca^{35}Cl^{+}$
$^{78}Se^{+}$	$^{38}Ar^{40}Ar^{+}$
$^{80}Se^{+}$	$^{40}Ar^{40}Ar^{+}$, $^{79}BrH^{+}$
$^{82}Se^{+}$	$^{32}S^{16}O_3^{+}$
$^{95}Mo^{+}$	$^{79}Br^{16}O^{+}$
$^{98}Mo^{+}$	$^{81}Br^{16}OH^{+}$
$^{103}Rh^{+}$	$^{40}Ar^{63}Cu^{+}$
$^{111}Cd^{+}$	$^{95}Mo^{16}O^{+}$

Quality Assurance

Several control materials from different suppliers and pooled urines were analyzed to investigate the precision and accuracy of our method for internal quality assurance purposes. The variation coefficients (VCs) for the analyses of two control samples for each element were measured. Intra-day VCs (one sample preparation, 10 measurements of the same sample) are in the range 2.2%–5.4%, whereas inter-day VCs (10 different sample preparations, 10 measurements at different days by different analysts) are in the range 6.0%–14.2%. All of these values are normally acceptable for essential trace element and toxic metal analysis of clinical samples.

The data in Tables 4, 5, 6 are the measured concentrations compared with the certified concentrations of the control materials. The measured concentrations are the average values of 10 acquisitions of the control sample by different analysts taken on different days. Plus/Minus values are calculated from the standard deviation of the 10 measurements. The limits of the target values were set by the supplier of each control material. The comparison of the target values with the measured values shows sufficient agreement for most of the elements, without significant outliers for samples with lower concentrations. We found some disagreement for a few elements, for example, the measured Sb concentration is low in Lyphochek® level 1 and the Cr concentration is high in the same control sample. The As value for Clinchek® level 2 was found to be low. Because good recoveries for those elements were achieved in the other control materials, it is possible that the recommended values are not accurate, possibly as a result of contamination.

The analysis results for 63 urine samples of the general population are listed in Table 7. These results are very helpful to demonstrate the performance and applicability of the method. The concentrations in Table 7 are not creatinine-adjusted, but creatinine was measured in the range 0.6–1.7 g/L urine for all subjects with a geometric mean of 1.1 g/L which is typical for the general population. The geometric mean values of Pb, Co, Ba, and Tl are in good agreement with results published in the American Second National Report on Human Exposure to Environmental Chemicals published in 2003 [5]. Essential trace element concentrations of Cu, Zn, and Se are in the typical concentration ranges described for healthy humans in the European Community [6]. Our mean value for Mo of 45 µg/L is close to 43 µg/L determined for the Danish population [7]. The geometric mean value for Li (18 µg/L) is in good agreement with a study in Japan [8]. Table 7 provides additional information about the elemental concentration range in urine for other elements including Be, Cr, Ni, and Cd.

Table 4. Comparison of Measured and Certified Concentrations in the Urine Reference Material Lyphochek®

	Concentration (µg/L)			
	Lyphochek, level 1		Lyphochek, level 2	
Element	Measured	Certified	Measured	Certified
Cr	1.7±0.2	1.2±0.2	18.6±2.6	20.2±4.1
Co	6.6±0.7	6.9±1.4	18.9±1.4	19.1±4.2
Cu	24±2.1	26.7±5.4	45±5.5	50±10
Se	56±5.3	49±10	192±17	187±37
As	65±6	67±14	162±15	163±33
Cd	8.4±1.1	8.6±1.7	14.9±1.9	15.6±3.1
Sb	6.9±1.1	9±1.8	34.8±4.4	36.9±7
Tl	9.6±0.8	9.7±2.0	185±17	198±40
Pb	13.5±1.1	14.3±2.9	68±5	69±14

Table 5. Comparison of Measured and Certified Concentrations in the Urine Control Materials Clinchek® and Medisafe™

Element	Concentration (µg/L)							
	Clinchek, level 1		Clinchek, level 2		Medisafe, level 1		Medisafe, level 2	
	Measured	Certified	Measured	Certified	Measured	Certified	Measured	Certified
Be	0.02±0.01	0.03±0.01	0.12±0.02	0.13±0.04				
V	17.2±1.5	17±5	41±3.6	45±9				
Cr	9.3±0.9	10±1.8	31±2.9	33±5	10±1.1	10±3	1.8±0.3	2±0.6
Mn	5.8±0.6	5.6±1.4	16±1.7	17±4				
Ni	10.2±1	9.8±2	45.7±3.2	44±8				
Co	5.0±0.3	5.4±1.1	33±2.1	33±6				
Cu	56±5	62±10	121±9	120±20				
Zn	225±31	250±40	567±65	620±75				
Se					23±3	20±6	205±28	200±50
As	30±3.3	31±8	61±7	72±16	57±7	50±11	248±30	250±60
Cd	8.3±1.2	8.4±1.4	15.1±1.2	15±3	11.6±1.4	13±3	7.2±0.9	8±1.8
Sn					4.5±0.6	5±1.2	45±6	50±12
Ba					5.4±0.6	5±1.7	56±7	50±18
Pb	29±2.5	28±6	72±9	64±11	133±11	130±32	85±7	80±18
Tl	3.1±0.3	3.2±0.9	15.9±1.3	17±5				

Table 6. Comparison of Measured and Certified Concentrations in Pooled Urine Control Samples

	Concentration (µg/L)			
	Urine pool, level 1		Urine pool, level 2	
Element	Measured	Certified	Measured	Certified
Li	5.4±0.7	5±0.8	11±0.9	10±1.2
Be	0.5±0.07	0.5±0.08	0.9±0.1	1±0.1
In	0.5±0.1	0.5±0.07	1.1±0.13	1±0.07
Mo	1.1±0.13	1±0.13	5.3±0.4	5±0.26
Rh	0.5±0.11	0.5±0.1	0.9±0.08	1±0.08
W	0.6±0.1	0.5±0.12	0.9±0.14	1±0.1
Pt	0.4±0.11	0.5±0.1	0.9±0.1	1±0.08
U	0.5±0.1	0.5±0.07	1.1±0.06	1±0.05

Table 7. Analytical Results for 63 Human Subjects

Element	Range (µg/L)	*Geometric mean (µg/L)	95 % Percentile (µg/L)
Li	3–86	14	47
Be	LOQ–0.028	0.007	0.02
V	LOQ–0.19	0.037	0.13
Cr	LOQ–0.47	0.11	0.44
Mn	LOQ–0.52	0.049	0.14
Co	0.03–2.1	0.11	0.98
Ni	LOQ–3.5	0.37	1.5
Cu	1.3–10.8	4.7	8.3
Zn	19–665	139	305
As	0.5–197	14	149
Se	1–140	15	90
Mo	10–174	38	91
Rh	LOQ–0.004	0.002	0.003
Cd	LOQ–0.35	0.075	0.43
In	LOQ–0.8	0.039	0.43
Sn	0.06–12.6	0.8	5.5
Sb	LOQ–1.3	0.037	0.18
Ba	0.4–5.1	1.3	3.3
W	LOQ–0.19	0.012	0.08
Pt	LOQ–0.026	0.005	0.008
Tl	0.005–0.11	0.1	0.19
Pb	0.1–0.24	0.52	0.76
U	LOQ–0.024	0.0014	0.01

* Concentrations below the LOQ were calculated as LOQ/2

Conclusions

Methodology based on the Agilent 7500c ORS ICP-MS was used successfully for the routine analysis of urine. The system is especially applicable to laboratories with high sample throughput requirements for multi-element determinations and elemental screening. Because spectral interferences are removed with the ORS and LODs are completely satisfactory, the instrumental long-term stability becomes one of the most important criteria for reliable routine analysis. Long-term stability is determined by the robustness of the ICP-MS and its tolerance to continuous exposure to high matrix samples. A combination of system design (low flow nebulizer, exceptionally wide diameter torch injector and high plasma temperature) and optimization (tuning for minimal matrix oxides) provides the 7500c with the required matrix tolerance. In combination with a fast and simple sample preparation method, this is the key to fast and accurate analysis of urine samples in a routine clinical laboratory.

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