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Total elemental analysis in clinical research using the Thermo Scientific iCAP TQ ICP-MS

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Introduction

Trace element analysis of biological samples provides significant information to support clinical research and forensic toxicology. An interesting example of trace elemental analysis for clinical research purposes is exploring the degradation of titanium based orthopedic and dental implants in humans. Following recent research on the possible carcinogenic effects of titanium dioxide the fate of titanium in the human body has become a growing area of clinical research focus. To support this there is a need for the development of robust analytical methods for the identification and quantification of titanium in a range of samples such as human body fluids and organs.

However, the development of such a method is challenging due to the low concentration of titanium in these types of samples and the potential isobaric interferences which single quadrupole ICP-MS cannot remove.

Advancements in ICP-MS technology have led to the development of triple quadrupole (TQ) ICP-MS instruments, which have the required sensitivity as well as the capability to resolve isobaric interferences resulting from polyatomic and isotopic species.

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This technical note focuses on the development of a robust method for the analysis of titanium and other trace elements in human serum reference materials using the Thermo Scientific™ iCAP™ TQ ICP-MS.

Sample preparation

The certified reference materials (Seronorm™ Trace Elements in Serum L-1 and L-2, SERO, Norway) and volunteered human urine were gravimetrically diluted by a factor of ten in pre-cleaned (72 hours in 2% nitric acid, washed in ultra-pure water) polypropylene bottles with nitric acid (0.5% m/m Fisher Scientific) and tetramethylammonium hydroxide (TMAH, 2% m/m SIGMA-ALDRICH) in ultra-pure water (18 MΩ cm).
A calibration blank, a series of standards and a Quality Control (QC) were prepared using the same procedure, replacing the certified reference material with single element standards (SPEX CertiPrep). The elements and final concentrations are shown in Table 1. All samples and standards were spiked with an internal standard mix (10 $\mu g \cdot L^{-1}$ Ge, Y, Rh, Te and Bi).

Instrumentation

The iCAP TQ ICP-MS consists of three quadrupoles to improve interference removal compared to single quadrupole (SQ) ICP-MS. The first quadrupole (Q1) rejects all unwanted ions such as precursor species that may recombine in the collision / reaction cell (CRC) and subsequently interfere with the target analyte.

The second quadrupole (Q2) is used to selectively shift the interference or target analyte with an appropriate reaction gas.

The third quadrupole (Q3) isolates the product ion and removes any remaining interferences through a second stage of mass filtration allowing for interference free analysis of the analyte.

In this analytical method, TQ mass shift mode was used for the target element titanium (Figure 1). Titanium was reacted with ammonia gas (NH_3) to create the cluster ($^{48}Ti(NH_3)_3NH$) at *m/z* 114 in Q2.

Table 1. Elements analyzed and concentration of calibration standards and the QC.

Unit: $mg \cdot L^{-1}$

	Major STD1	Major STD2	Major STD3	Major STD4	QC CCVs
Ca	5	10	25	50	10
Fe	0.1	0.2	0.5	1	0.2
Mg	5	10	25	50	10
P	5	10	25	50	10
K	5	10	25	50	10
S	50	250	500	1000	100
Na	50	100	250	500	100

Unit: $\mu g \cdot L^{-1}$

	Minor STD1	Minor STD2	Minor STD3	Minor STD4	QC CCVs
Sb	0.5	1	2.5	5	1
As	0.1	0.2	0.5	1	0.2
Ba	5	10	25	50	10
Cd	0.1	0.2	0.5	1	0.2
B	5	10	25	50	10
I	5	10	25	50	10
Pb	0.1	0.2	0.5	1	0.2
Li	500	1000	2500	5000	1000
Mo	0.1	0.2	0.5	1	0.2
Rb	0.5	1	2.5	5	1
Sr	5	10	25	50	10
Ti	0.5	1	2.5	5	1
U	0.005	0.01	0.025	0.05	0.01
V	0.1	0.2	0.5	1	0.2
Zn	50	100	250	500	100
Se	0.1	0.2	0.5	1	0.2
Al	50	100	250	500	100
Cr	0.5	1	2.5	5	1
Mn	5	10	25	50	10
Ni	5	10	25	50	10
Co	0.1	0.2	0.5	1	0.2

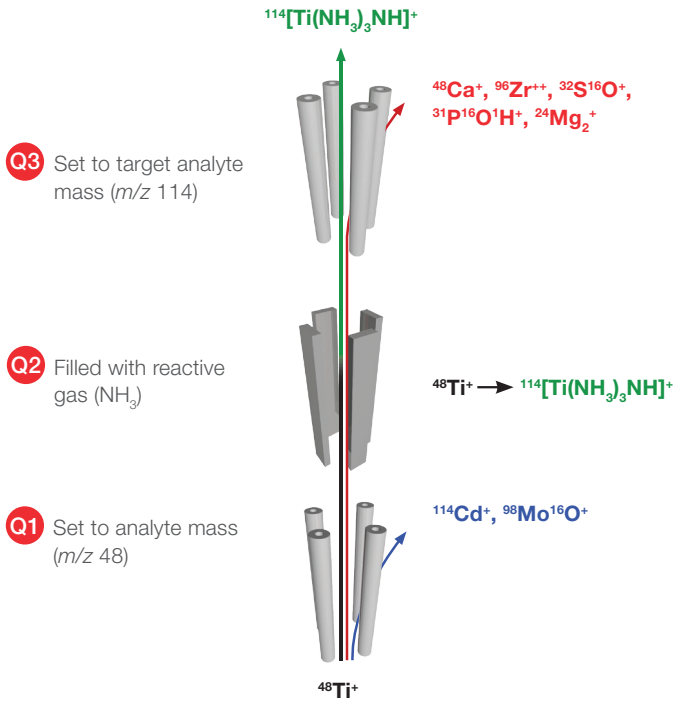

Figure 1. TQ mass shift mode for titanium.

The iCAP TQ ICP-MS also has the ability to operate in single quadrupole mode when advanced interference removal is not required. For many of the analytes in this analytical method, analysis using pure He as a collision gas and Kinetic Energy Discrimination (KED) mode is sufficient.

Method development and analysis

The sample introduction system used is detailed in Table 2. The operating parameters were optimized by the default autotune procedure in the Thermo Scientific™ Qtegra™ Intelligent Scientific Data System™ (ISDS) software that controls the iCAP TQ ICP-MS.

Table 2. Instrument configuration and operating parameters.

Parameter	Value	
Nebulizer	PFA nebulizer 0.2 mL·min^{-1}, pumped at 40 rpm	
Spraychamber	Quartz cyclonic spraychamber cooled at 3 °C	
Injector	2.5 mm Quartz	
Interface	High matrix (3.5 mm), Ni cones	
RF power	1550 W	
Nebulizer gas flow	1.001 L·min^{-1}	
QCell setting	**SQ-KED**	**TQ-NH_3**
Gas flow	4.5 mL·min^{-1}	0.29 mL·min^{-1}
CR Bias	-21 V	-7.9 V
Q3 Bias	-18 V	-11 V
Dwell time	0.2 seconds per analyte, 5 sweeps	

The optimum measurement mode for each analyte was automatically selected by the Reaction Finder method development assistant within Qtegra ISDS Software. Additional measurement modes were selected for Ti to compare the efficiency of the interference removal in TQ mass shift mode:

SQ-KED – single quadrupole mode with CRC pressurized with He, KED applied, no filter on Q1 and Q3 set to mass 48

SQ-NH_3 – single quadrupole mode with CRC pressurized with NH_3, no filter on Q1 and Q3 set to product ion mass of 114

TQ-NH_3 – triple quadrupole mode with CRC pressurized with NH_3, Q1 set to mass 48 and Q3 set to product ion mass of 114

An internal standard was also associated with each analyte on a mass basis. Internal standard association and measurement modes for the final analysis are shown in Table 3.

Table 3. Measurement modes and internal standards used for each element.

	Measurement mode	Analyte/Product Ion mass	Internal standard
Na	SQ-KED	23	^{74}Ge
Mg	SQ-KED	24	^{74}Ge
P	SQ-KED	31	^{74}Ge
S	SQ-KED	34	^{74}Ge
K	SQ-KED	39	^{74}Ge
Ca	SQ-KED	44	^{74}Ge
Fe	SQ-KED	56	^{74}Ge
Li	SQ-KED	7	^{74}Ge
B	SQ-KED	11	^{74}Ge
Al	SQ-KED	27	^{74}Ge
V	SQ-KED	51	^{74}Ge
Cr	SQ-KED	52	^{74}Ge
Mn	SQ-KED	55	^{74}Ge
Co	SQ-KED	59	^{74}Ge
Ni	SQ-KED	60	^{89}Y
Zn	SQ-KED	66	^{74}Ge
As	SQ-KED	75	^{89}Y
Se	SQ-KED	78	^{74}Ge
Rb	SQ-KED	85	^{89}Y
Sr	SQ-KED	88	^{89}Y
Mo	SQ-KED	95	^{103}Rh
Cd	SQ-KED	111	^{103}Rh
Ti	TQ-NH_3	114	$^{74}Ge^{14}N^{1}H_2$
Sb	SQ-KED	121	^{125}Te
I	SQ-KED	127	^{125}Te
Ba	SQ-KED	138	^{103}Rh
Pb	SQ-KED	208	^{209}Bi
U	SQ-KED	238	^{209}Bi

The sample analysis consisted of an external calibration curve followed by replicate analyses of the urine and serum samples. Continuous calibration verication (CCV) samples were analyzed every 10 samples and a total of 124 samples were measured during the analysis. All samples were presented for analysis using a Teledyne CETAC Technologies ASX-560 Autosampler. The rinse solution used on the autosampler between samples was the same as the diluent (0.5% HNO_3/2% TMAH).

Results

Titanium in biological samples is particularly challenging due to the isobaric overlap of ^{48}Ca and polyatomic interferences from SO^+ and POH^+. To evaluate the efficiency of interference removal, three different measurement modes (SQ-KED, SQ-NH_3 or TQ-NH_3) were used to measure a certified reference material (CRM). The results for titanium quantification in both Serum L-1 and L-2 for each of the measurement modes are shown in Table 4. The result from the TQ-NH_3 is the most accurate when compared to the reported values for these materials. The Reaction Finder method development assistant automatically selects this mode for analysis.

To demonstrate the improved interference removal, the effect of the presence of cadmium in the sample was investigated. A ten-fold diluted serum sample and a 10 mg·L^{-1} cadmium standard were analyzed with TQ-NH_3 mode and spectra recorded. The ten-fold diluted serum sample shows a typical spectral fingerprint associated with the creation of $Ti(NH_3)_3X^+$ clusters (Figure 2). The 10 mg·L^{-1} cadmium standard (Figure 3.) measured with the same conditions and measurement mode, shows no presence of Cd in the spectra (only residual counts from the analysis of the serum), the Cd having been eliminated by Q1. This prevents any trace Cd in the sample from interfering with the analysis of Ti at *m/z* 114.

Table 4. Comparison of titanium results in the serum CRMs with different measurement modes.

	Ti SQ-KED, µg·L^{-1}	Ti SQ-NH_3, µg·L^{-1}	Ti TQ-NH_3, µg·L^{-1}	Ti Reported Value, µg·L^{-1}
Serum L-1	167	1800	6.64	6.8
Serum L-2	262	1850	6.38	6.8

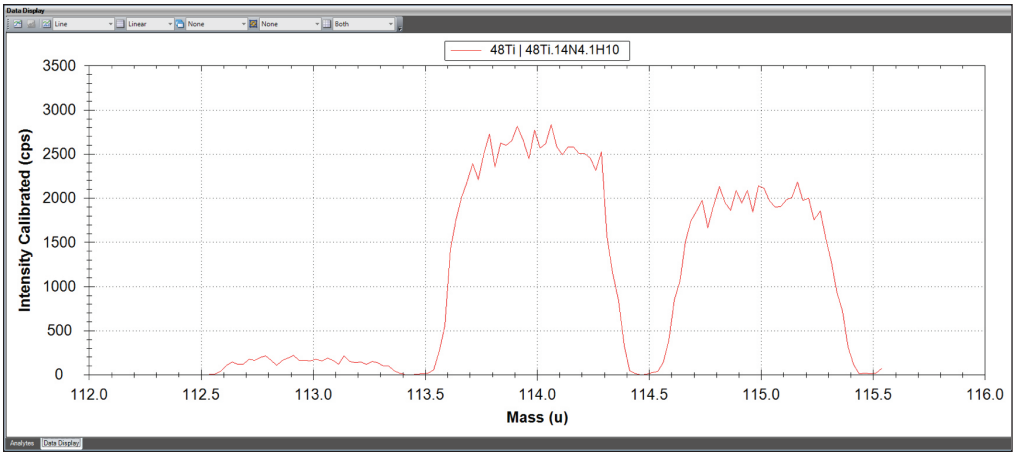

Figure 2. Spectra of serum sample (diluted 10-fold).

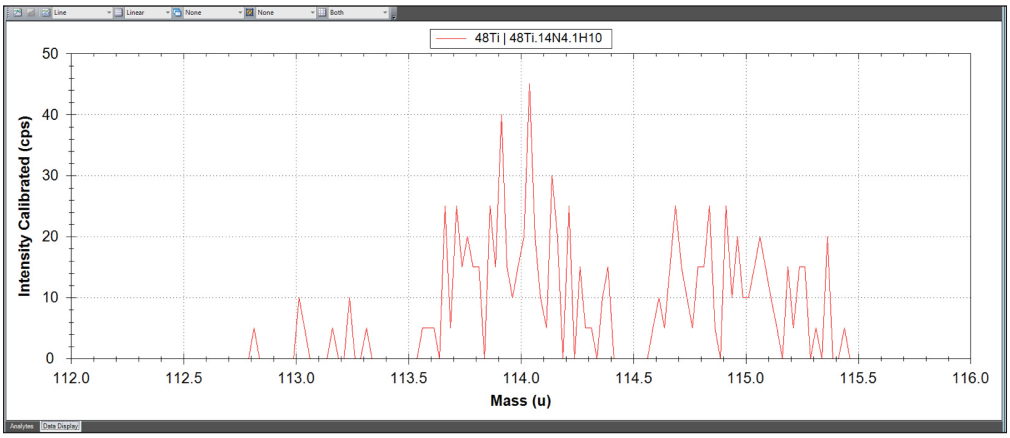

Figure 3. Spectra of 10 mg·L^{-1} cadmium.

Selected calibrations for the multi-elemental analysis are shown in Figures 4 to 7. The calibration curve for the titanium with TQ mass shift mode (Figure 4) shows high sensitivity at 3903 cps/µg·L^{-1} and excellent linearity with an R^2 value of 0.9998 for the calibration consisting of a blank and four standards (0, 0.5, 1, 2.5 and 5 µg·L^{-1}).

All other elements analytes apart from Ti were analyzed using SQ-KED. When analyzing in this mode the first quadrupole simply acts as an ion guide. Calibration curves for arsenic and selenium using the SQ-KED mode are shown in Figures 5 and 6 respectively with the concentration range of 0.1 to 1 µg·L^{-1}. The calibration curve for sulfur (Figure 7) is performed with the concentration range of 50 to 1000 mg·L^{-1}. These are typical elements and typical concentration ranges expected in clinical research.

The results of the multi-elemental analysis of the serum CRMs are shown in Table 5. Measured values for the analytes in the reference materials are in good agreement with the reference or reported values. These values cover a wide concentration range from sub ppb to low % levels, demonstrating the importance of the dynamic range of the iCAP TQ ICP-MS. A urine sample, analyzed in the same analytical run, was found to contain typical elemental concentrations.

The detection limit (LOD) was determined based on three times the standard deviation of a ten replicate measurement of the calibration blank. The method detection limits (MDL) for all of the elements analyzed were calculated by multiplying the LOD by the dilution factor (1:10) (Table 5). The LODs for all the elements of interest are well below the target levels required for clinical research sample analysis.

Table 5. Results for the serum CRMs and urine sample. The analyte labeled with a * are reported at mg·L^{-1}, all other results are reported in µg·L^{-1}.

	LOD	MDL	*Serum L-1*		*Serum L-2*		*Urine*
			Measured	Reference or reported value	Measured	Reference or reported value	Measured
Na*	0.0027	0.027	2743	2330-3504	3255	2820-4241	2977
Mg*	0.0001	0.0010	21.0	13.4-20.1	39.7	27.1-40.7	85.6
P*	0.0008	0.08	52.3	43.3-65.1	120	88-132	710
S*	0.145	1.3800	1100	1008	1495	1335	476
K*	0.0021	0.02	150	101-153	260	176-265	1946
Ca*	0.002	0.0200	90.1	69-104	124	95-143	99.8
Fe*	0.00002	0.00023	1.64	1.17-1.77	2.18	1.72-2.58	0.005
Li	1.13	11.2920	5778	4202-6320	10806	7739-11639	22.4
B	0.67	6.746	70.1	79.4	87	82.1	1548
Al	0.20	1.9670	54.2	25.2-75.7	122	96-144	13.7
V	0.002	0.022	1.04	1.10	1.26	1.10	0.229
Cr	0.008	0.0800	1.70	1.30-3.05	5.20	4.00-7.50	0.838
Mn	0.008	0.084	10.7	7.9-11.9	14.2	11.6-17.4	0.914
Co	0.0001	0.0010	1.38	0.67-1.57	2.16	2.13-3.97	0.027
Ni	0.006	0.055	6.26	3.38-7.9	9.41	7.9-11.9	1.45
Zn	0.051	0.5130	1052	844-1269	1527	1404-1831	359
As	0.002	0.018	0.383	0.400	0.374	0.380	1.31
Se	0.010	0.1000	80.8	51-120	124	95-176	7.31
Rb	0.004	0.035	4.20	4.40	8.70	8.70	812
Sr	0.006	0.0570	95.7	95.0	106	110	89.2
Mo	0.005	0.048	0.710	0.760	1.20	1.21	7.62
Cd	0.001	0.0100	0.130	0.130	0.140	0.140	0.229
Ti	0.002	0.02	6.64	6.80	6.38	6.80	0.151
Sb	0.006	0.0600	11.6	10.4	16.1	15.0	0.040
I	0.022	0.219	75.5	71.8	69.9	60.9	82.8
Ba	0.003	0.0300	172	190	133	139	2.09
Pb	0.0007	0.007	0.370	0.400	0.666	0.660	0.446
U	0.0001	0.0010	0.288	0.302	0.357	0.359	0.020

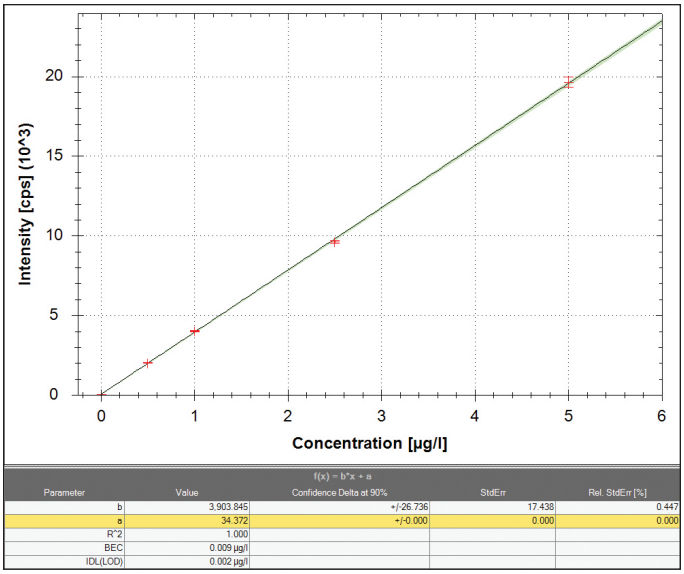

Figure 4. Calibration curve for titanium.

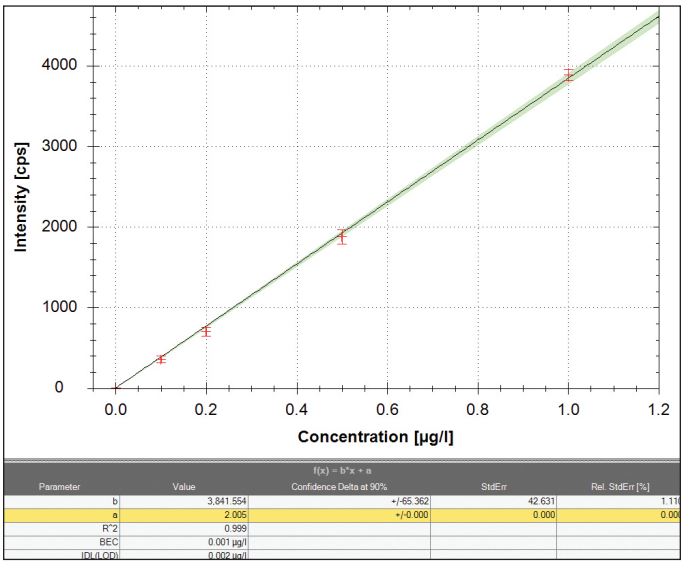

Figure 5. Calibration curve for arsenic.

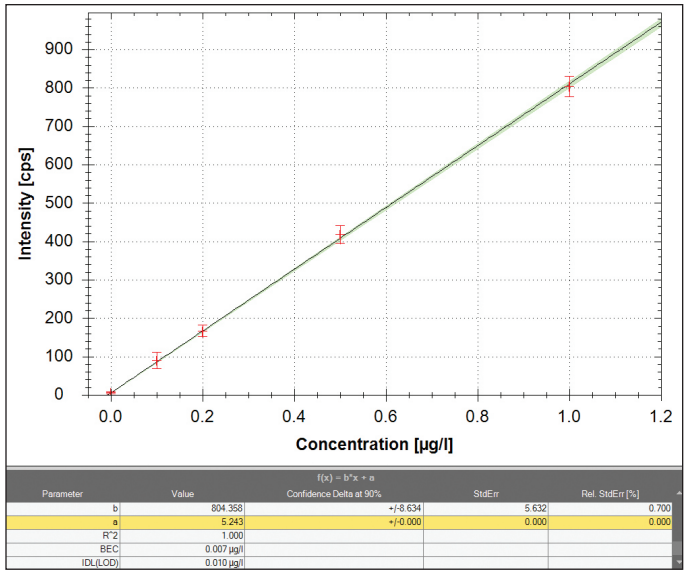

Figure 6. Calibration curve for selenium.

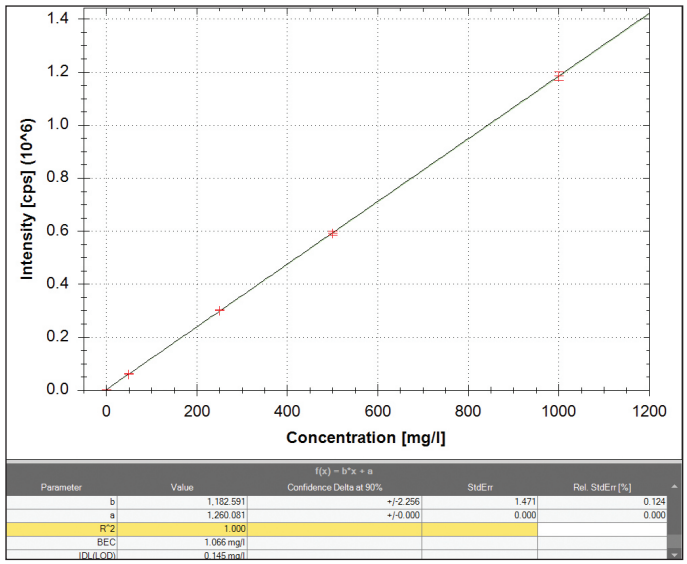

Figure 7. Calibration curve for sulfur.

The average results of the ongoing QC test over a period of eight hours (with a total of nine QC samples being measured) are shown in Figure 8. Average recoveries lie between 95 and 110% with standard deviations typically less than 2% (apart from B, As and Se where the SD was < 4% due to lower sensitivity). These results demonstrate the long term stability of the instrument when analyzing high matrix biological samples.

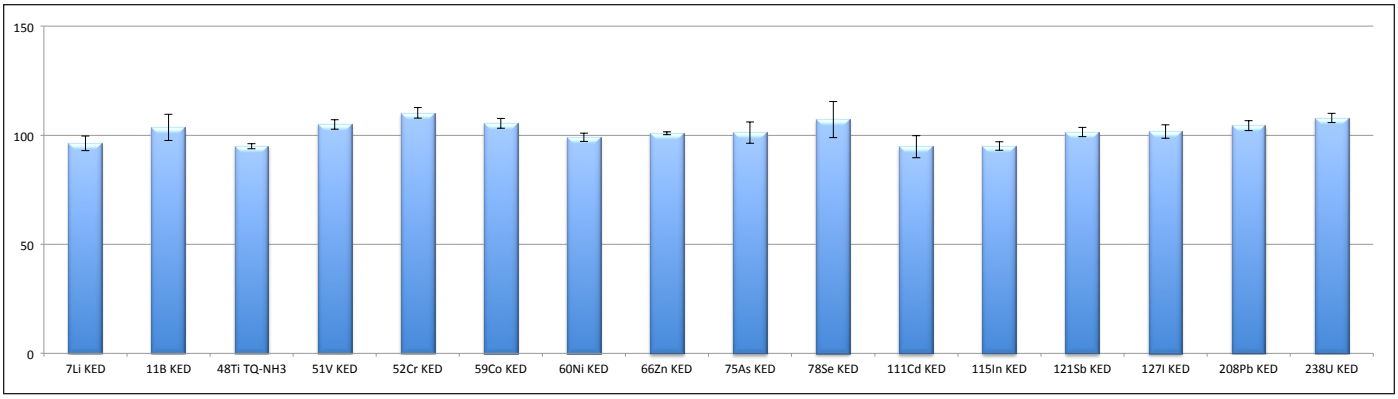

Figure 8. Calibration check verification standards (CCVs) measured during the analysis.

Conclusion

The Thermo Scientific iCAP TQ ICP-MS provides excellent performance for the determination of trace element analysis in biological samples making it ideal for clinical research. One key investigation is the degradation of metal-on-metal hip replacement implants, where Ti is often a component and where accurate analysis is problematic using SQ-ICP-MS.

With the iCAP TQ ICP-MS, powerful triple quadrupole technology provides the advanced performance required for the sensitive and accurate determination of Ti and other trace elements in complex samples, whilst the Reaction Finder tool allows for simple method setup by automatically selecting analytes of interest.

Find out more at **thermofisher.com/TQ-ICP-MS**

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