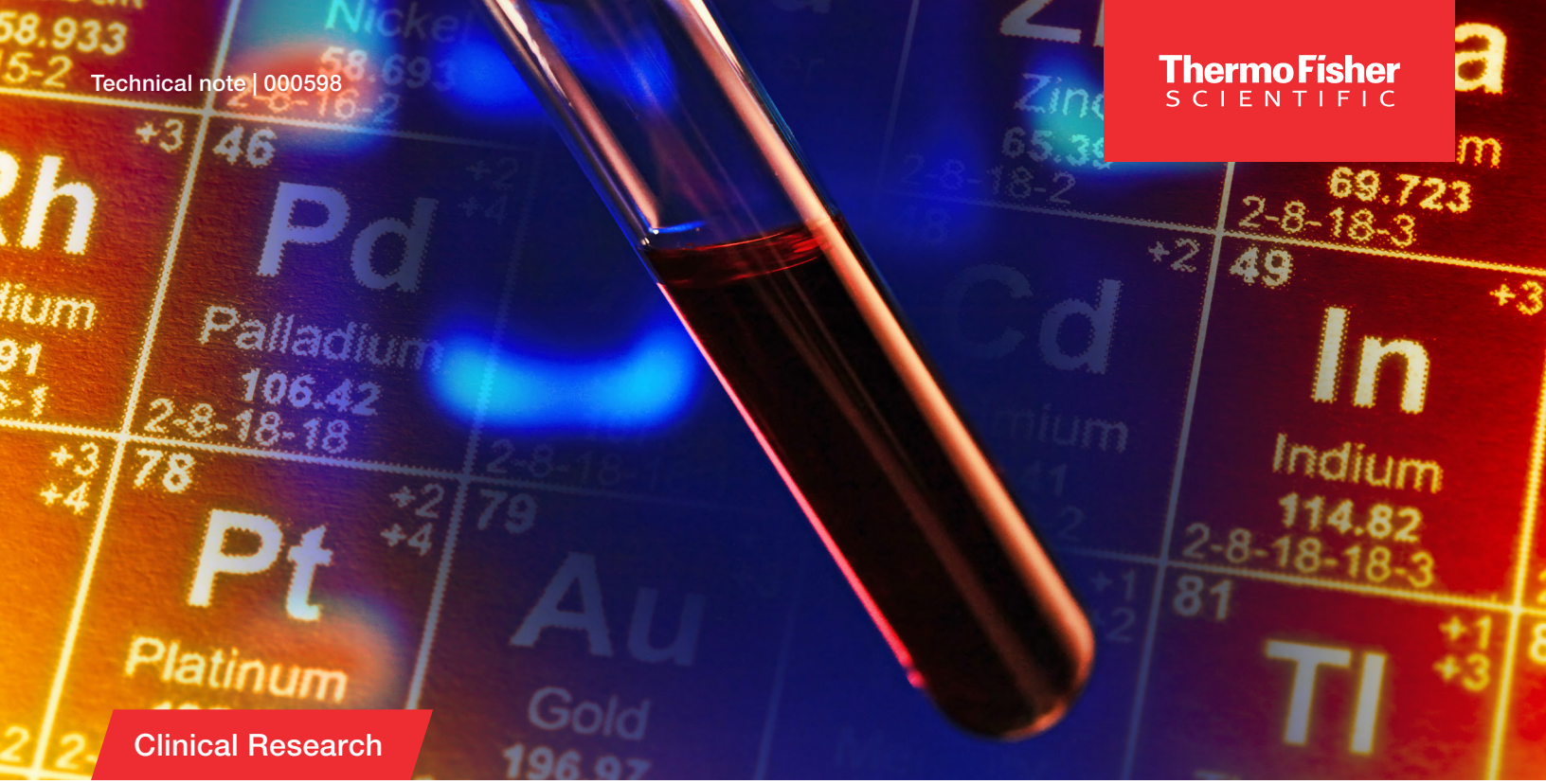

Quantitative analysis of 28 trace elements in human plasma and serum by triple quadrupole inductively coupled plasma mass spectrometry for clinical research

Authors

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Keywords

iCAP TQ ICP-MS, Qtegra Software, clinical research, plasma, serum, trace elemental analysis, triple quadrupole ICP-MS, mass spectrometry

Goal

To develop a robust, reproducible method for the detection of 28 trace elements using inductively coupled plasma mass spectrometry and the ClinMass™ Complete Kit for Trace Elements in Plasma/Serum

Benefits

- Simultaneous measurements of 28 trace elements in a single quantitative method
- Ability to remove polyatomic interferences using triple quadrupole ICP-MS
- Increased method accuracy by implementation of a comprehensive ClinMass Complete Kit for Trace Elements in Plasma/Serum

Introduction

Trace element analysis of biological samples is very important for clinical research as it provides significant information on cell functions at biological, chemical, and molecular levels. Certain trace elements like zinc (Zn), copper (Cu), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), and molybdenum (Mo) are essential as they mediate vital biochemical reactions by acting as cofactors for many enzymes as well as centers for stabilizing structures of enzymes and proteins. On the other hand, anthropogenic activities have contributed to increased exposure to toxic heavy metals

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like arsenic (As), cadmium (Cd), and mercury (Hg) that can cause adverse health outcomes. To support the detection of these substances, an analytical method for the quantification of 28 trace elements in human plasma and serum (Table 1) is reported here. The method is based on the ClinMass Complete Kit for Trace Elements in Plasma/Serum from RECIPE Chemicals + Instruments GmbH (in preparation). A Thermo Scientific™ iCAP™ TQ ICP-MS (triple quadrupole inductively coupled plasma mass spectrometer) coupled with a SC 4DX autosampler (Elemental Scientific, Omaha, NE) was used for detection of these trace elements using ^{45}Sc, ^{74}Ge, ^{103}Rh, and ^{187}Re as internal standards. This technical note provides details about the limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precision for each element.

Experimental

Sample preparation

Reagents included four serum calibrators and two controls each for plasma and serum from RECIPE, which were within the concentration ranges specified in Table 3, as well as internal standard (IS) for correction of potential matrix effects. Sample preparation was carried out by mixing 0.5 mL of the sample with 4.5 mL of diluting solution D provided with the kit. IS was added continuously by the peristaltic pump of the instrument.

Mass spectrometry

The iCAP TQ ICP-MS consists of three quadrupoles to enhance interference removal compared to a single quadrupole (SQ) ICP-MS. The first quadrupole (Q1) rejects all unwanted ions that may recombine in the collision/reaction cell (CRC) and subsequently interfere with the target analyte. The second quadrupole (Q2) is a collision/reaction cell (CRC) used to induce chemical reactions of either the analyte or the interference with an appropriate reaction gas. Finally, the third quadrupole (Q3) isolates the analyte ion from all potential interferences. The iCAP TQ ICP-MS can operate in SQ mode when advanced interference removal is not required. In the proposed method, a combination of appropriate modes was used, including the use of pure helium (He) as the collision gas and Kinetic Energy Discrimination (KED), as well as modes using TQ technology with reactive gases, such as oxygen (O_2) or ammonia (NH_3), for elements requiring removal of polyatomic interferences (Table 1 and Figure 1). Method verification was performed with the analytical system specified in Table 2.

Table 1. List of isotopes and corresponding instrument conditions (^{45}Sc, ^{74}Ge, ^{103}Rh, ^{187}Re were used as internal standards)

Q1 analyte	Q3 analyte	SQ/TQ	CR gas
Lithium (^{7}Li)		SQ	None
Beryllium (^{9}Be)		SQ	None
Magnesium (^{25}Mg)		SQ	None
Magnesium (^{25}Mg)		SQ	He (KED)
Aluminum (^{27}Al)	^{27}Al	TQ	O_2
Aluminum (^{27}Al)	^{27}Al	TQ	NH_3
Scandium (^{45}Sc)		**SQ**	**He (KED)**
Scandium (^{45}Sc)	**^{45}Sc**	**TQ**	**NH_3**
Scandium (^{45}Sc)	**^{45}Sc, ^{16}O**	**TQ**	**O_2**
Titanium (^{48}Ti)	^{48}Ti, $^{14}N_4$, $^{1}H_{10}$	TQ	NH_3
Vanadium (^{51}V)		SQ	He (KED)
Vanadium (^{51}V)	^{51}V, ^{16}O	TQ	O_2
Chromium (^{52}Cr)	^{52}Cr, ^{16}O	TQ	O_2
Manganese (^{55}Mn)		SQ	He (KED)
Iron (^{56}Fe)	^{56}Fe, ^{16}O	TQ	O_2
Iron (^{56}Fe)		SQ	He (KED)
Cobalt (^{59}Co)		SQ	He (KED)
Cobalt (^{59}Co)	^{59}Co, ^{16}O	TQ	O_2
Nickel (^{60}Ni)		SQ	He (KED)
Copper (^{63}Cu)		SQ	He (KED)
Zinc (^{66}Zn)		SQ	He (KED)
Zinc (^{66}Zn)	^{66}Zn, ^{16}O	TQ	O_2
Germanium (^{74}Ge)		**SQ**	**He (KED)**
Germanium (^{74}Ge)		**SQ**	**None**
Germanium (^{74}Ge)	**^{74}Ge, ^{16}O**	**TQ**	**O_2**
Arsenic (^{75}As)		SQ	He (KED)
Arsenic (^{75}As)	^{75}As, ^{16}O	TQ	O_2
Selenium (^{78}Se)		SQ	He (KED)
Selenium (^{80}Se)	^{80}Se, ^{16}O	TQ	O_2
Molybdenum (^{95}Mo)		SQ	He (KED)
Rhodium (^{103}Rh)		**SQ**	**He (KED)**
Palladium (^{105}Pd)		SQ	He (KED)
Silver (^{107}Ag)		SQ	He (KED)
Cadmium (^{111}Cd)		SQ	He (KED)
Tin (^{120}Sn)		SQ	He (KED)
Antimony (^{121}Sb)		SQ	He (KED)
Iodine (^{127}I)		SQ	He (KED)
Barium (^{137}Ba)		SQ	He (KED)
Rhenium (^{187}Re)		**SQ**	**He (KED)**
Platinum (^{195}Pt)		SQ	He (KED)
Gold (^{197}Au)		SQ	He (KED)
Mercury (^{202}Hg)		SQ	He (KED)
Thallium (^{205}Tl)		SQ	He (KED)
Bismuth (^{209}Bi)		SQ	He (KED)

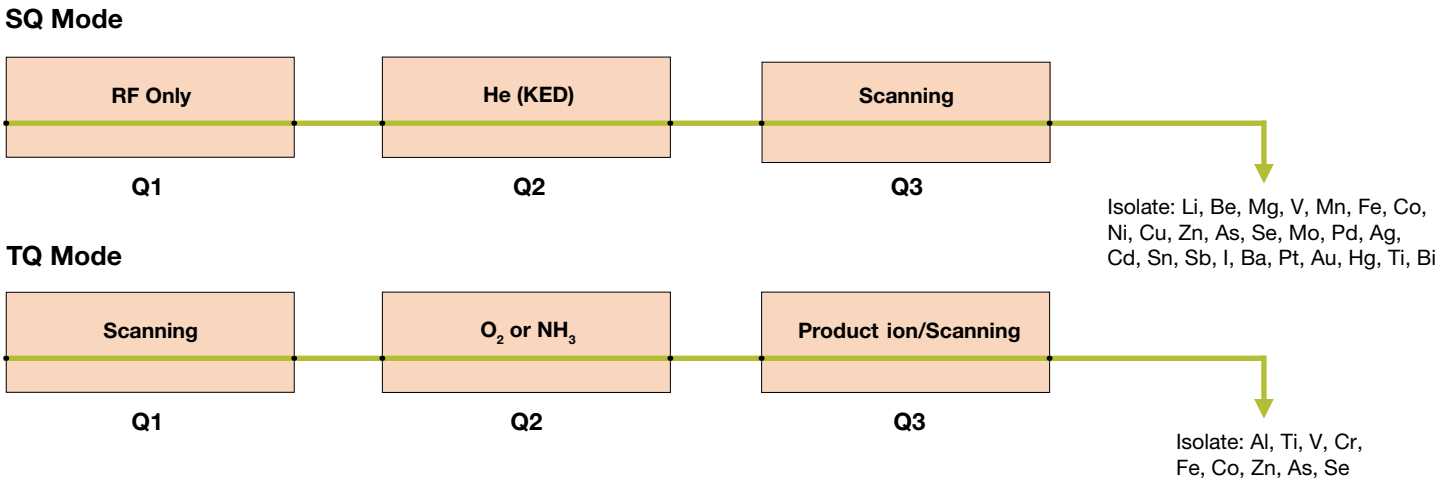

Figure 1. Schematics of iCAP TQ ICP-MS operation in the Single Quadrupole (SQ) mode and Triple Quadrupole (TQ) mode

Table 2. Instrumentation for method verification

Thermo Scientific iCAP TQ ICP-MS	
Components	
Autosampler	Elemental Scientific™ ESI SC-4 DX
Switching valve	Elemental Scientific™ ESI FAST DX
Nebulizer	PFA-ST nebulizer
Spray chamber	Quartz cyclonic spray chamber
Injector	2.5 mm ID Quartz
Torch	iCAP Q/Qnova Quartz Torch Organics
Cones	Ni sample cone. Ni skimmer cone with insert 3.5, high matrix

Method evaluation

The method was optimized using 15 different levels of calibration standards prepared by spiking human serum with each respective element. Method performance was evaluated in terms of limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precision for each element. Analytical accuracy was evaluated by using the INSTAND e.V. Proficiency Test Samples #206 and #207 prepared and analyzed in triplicate and analyzed in three individual runs. Intra-assay precision was evaluated in terms of percentage coefficient of variation (%CV) using the controls at three levels in replicates of twenty (n = 20) prepared and analyzed in one batch. Inter-assay precision was evaluated on the same controls in duplicate, prepared and analyzed on twenty (20) different days. The acceptance criteria for percentage coefficient of variation (%CV) was set at < 15%.

Data acquisition and analysis

Data were acquired and processed using Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution software.

Results and discussion

The method proved to be linear across the calibration ranges. Representative calibration curves for ^{7}Li, trace element having lowest atomic mass, and ^{209}Bi, trace element having the highest atomic mass, are reported in Figure 2. The obtained linear ranges, correlation factor (R^2), lower limits of quantitation (LLoQ), and limits of detection (LoD) of all the trace elements using the 15 calibration standards are reported in Table 3.

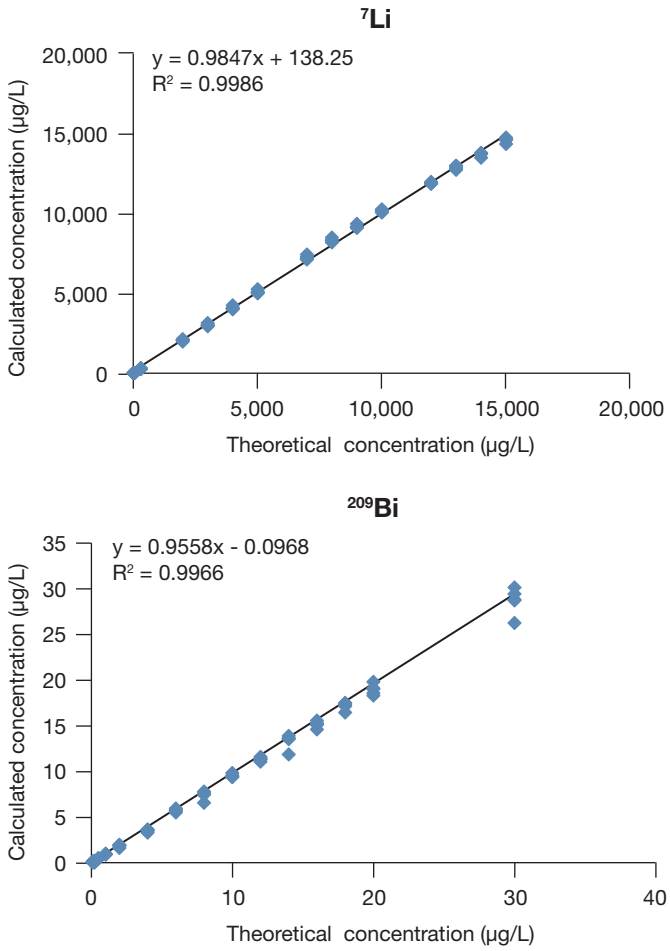

Figure 2. Representative calibration curves for ^{7}Li and ^{209}Bi

Table 3. Linear range, R^2, LLoQ, and LoD evaluation for all trace elements

Substance	R^2	Linear range [µg/L]	LLoQ [µg/L]	LoD [µg/L]
^{7}Li	0.9986	6–15,000	6	2
^{9}Be	0.9978	0.1–40	0.1	0.033
^{25}Mg	0.9993	500–500,000	500	167
^{25}Mg	0.9992	500–500,000	500	167
^{27}Al	0.9995	2–500	2	0.667
^{27}Al	0.9997	4–500	4	1.33
^{48}Ti	0.9968	1–70	1	0.333
^{51}V	0.9982	0.1–40	0.1	0.033
^{51}V	0.9976	1–40	1	0.333
^{52}Cr	0.9982	0.1–100	0.1	0.033
^{55}Mn	0.9999	0.5–500	0.5	0.167
^{56}Fe	0.9987	300–3,000	300	100
^{56}Fe	0.9988	300–3,000	300	100
^{59}Co	0.9970	0.1–30	0.1	0.033
^{59}Co	0.9970	0.2–40	0.2	0.067
^{60}Ni	0.9970	1–60	1	0.333
^{63}Cu	0.9978	200–3,000	200	66.7
^{66}Zn	0.9990	10–3,000	10	3.33
^{66}Zn	0.9985	300–3,000	300	100
^{75}As	0.9981	0.2–40	0.2	0.067
^{75}As	0.9981	0.5–50	0.5	0.167
^{78}Se	0.9992	1–300	1	0.333
^{80}Se	0.9991	1–300	1	0.333
^{95}Mo	0.9966	0.5–40	0.5	0.167
^{105}Pd	0.9978	0.1–30	0.1	0.033
^{107}Ag	0.9991	0.05–50	0.05	0.017
^{111}Cd	0.9965	0.1–30	0.1	0.033
^{120}Sn	0.9997	0.05–50	0.05	0.017
^{121}Sb	0.9971	0.5–40	0.5	0.167
^{127}I	0.9992	1–300	1	0.333
^{137}Ba	0.9998	1–300	1	0.333
^{195}Pt	0.9999	0.1–2,000	0.1	0.033
^{197}Au	0.9999	0.1–1,000	0.1	0.033
^{202}Hg	0.9983	0.5–40	0.5	0.167
^{205}Tl	0.9969	0.1–30	0.1	0.033
^{209}Bi	0.9966	0.1–30	0.1	0.033

The data showed exceptional accuracy with %CV and %Bias within range (CV < 15% and Bias ± 20%) between target and measured concentrations for the INSTAND e.V. Proficiency Test Samples #206 and #207 for plasma and serum, respectively (Table 4). The %CV for intra-assay precision was < 8.5% for all the trace elements (Table 5). The maximum %CV for inter-assay precision for all the trace elements was 9.8% (Table 6).

Table 4. Results of INSTAND e.V. proficiency test samples #206 and #207 for plasma and serum

Analyte	Measurement mode	Sample type	Sample #	Target value [µg/L]	Measured value [µg/L]	CV [%]	Bias [%]
^{9}Be	N/A	Serum	Sample 207	0.535	0.488	4.6	-8.8
^{9}Be	N/A	Serum	Sample 207	5.90	5.96	2.9	1
^{27}Al	NH_3	Plasma	Sample 206	63.7	69.3	10.2	8.8
^{27}Al	NH_3	Plasma	Sample 206	115	133	7.3	15.4
^{27}Al	O_2	Plasma	Sample 206	63.7	60.8	7.4	-4.6
^{27}Al	O_2	Plasma	Sample 206	115	112	5.8	-2.7
^{51}V	KED	Serum	Sample 207	1.11	1.07	2.2	-4
^{51}V	KED	Serum	Sample 207	9.82	9.35	1.1	-4.8
^{51}V	O_2	Serum	Sample 207	1.11	1.08	4.4	-2.7
^{51}V	O_2	Serum	Sample 207	9.82	9.19	1.3	-6.4
^{52}Cr	O_2	Plasma	Sample 206	6.46	5.56	5.0	-13.9
^{52}Cr	O_2	Plasma	Sample 206	10	8.81	4.7	-11.9
^{55}Mn	KED	Plasma	Sample 206	3.01	2.85	2.3	-5.4
^{55}Mn	KED	Plasma	Sample 206	5.87	5.57	1.6	-5.1

Table 4 (continued). Results of INSTAND e.V. proficiency test samples #206 and #207 for plasma and serum

Analyte	Measurement mode	Sample type	Sample #	Target value [µg/L]	Measured value [µg/L]	CV [%]	Bias [%]
^{56}Fe	O_2	Plasma	Sample 206	754	696	3.7	-7.7
^{56}Fe	O_2	Plasma	Sample 206	2614	2285	3.5	-12.6
^{56}Fe	KED	Plasma	Sample 206	754	738	1.2	-2.2
^{56}Fe	KED	Plasma	Sample 206	2614	2465	1.4	-5.7
^{59}Co	KED	Plasma	Sample 206	2.87	2.72	1.5	-5.4
^{59}Co	KED	Plasma	Sample 206	9.02	8.57	1.9	-4.9
^{59}Co	O_2	Plasma	Sample 206	2.87	2.43	4.9	-15.3
^{59}Co	O_2	Plasma	Sample 206	9.02	7.63	4.7	-15.5
^{60}Ni	KED	Plasma	Sample 206	11.8	11.0	2.3	-7.2
^{60}Ni	KED	Plasma	Sample 206	5.63	5.45	3.9	-3.3
^{63}Cu	KED	Plasma	Sample 206	1735	1604	1.1	-7.6
^{63}Cu	KED	Plasma	Sample 206	1277	1185	1.3	-7.2
^{66}Zn	KED	Plasma	Sample 206	3040	2909	1.7	-4.3
^{66}Zn	KED	Plasma	Sample 206	1690	1571	1.5	-7.1
^{66}Zn	O_2	Plasma	Sample 206	3040	2900	2.2	-4.6
^{66}Zn	O_2	Plasma	Sample 206	1690	1569	2.3	-7.2
^{75}As	KED	Plasma	Sample 206	2.58	2.42	2.0	-6.2
^{75}As	KED	Plasma	Sample 206	15.3	14.8	1.2	-3.4
^{75}As	O_2	Plasma	Sample 206	2.58	2.65	3.7	2.6
^{75}As	O_2	Plasma	Sample 206	15.3	15.8	2.7	3.3
^{78}Se	KED	Plasma	Sample 206	140	139	1.8	-0.7
^{78}Se	KED	Plasma	Sample 206	101	102	1.4	0.5
^{80}Se	O_2	Plasma	Sample 206	140	137	1.3	-1.9
^{80}Se	O_2	Plasma	Sample 206	101	100	1.6	-0.9
^{95}Mo	KED	Serum	Sample 207	1.75	1.73	4.3	-1.1
^{95}Mo	KED	Serum	Sample 207	1.13	1.08	3.2	-4.5
^{111}Cd	KED	Plasma	Sample 206	0.477	0.485	4.9	1.7
^{111}Cd	KED	Plasma	Sample 206	0.945	0.968	2.2	2.4
^{120}Sn	KED	Serum	Sample 207	1.48	1.42	1.4	-3.8
^{120}Sn	KED	Serum	Sample 207	13.4	13.5	2.2	0.8
^{121}Sb	KED	Serum	Sample 207	5.51	5.32	1.8	-3.4
^{121}Sb	KED	Serum	Sample 207	9.03	8.86	2.6	-1.9
^{127}I	KED	Plasma	Sample 206	181	176	2.1	-2.7
^{127}I	KED	Plasma	Sample 206	65.9	64.8	2.9	-1.6
^{195}Pt	KED	Plasma	Sample 206	45.5	43.4	2.7	-4.6
^{195}Pt	KED	Plasma	Sample 206	540	525	2.2	-2.7
^{197}Au	KED	Plasma	Sample 206	148	142	2.3	-3.9
^{197}Au	KED	Plasma	Sample 206	873	863	1.2	-1.2
^{209}Bi	KED	Serum	Sample 207	4.51	4.53	1.3	0.5
^{209}Bi	KED	Serum	Sample 207	2.37	2.30	1.3	-2.9

Table 5. Intra-assay precision (%CV) results of recipe controls at three levels {serum controls (level I + II) and plasma controls (level III)}

	^{7}Li (N-A)	^{9}Be (N-A)	^{25}Mg (N-A)	^{25}Mg (KED)	^{27}Al (NH_3)	^{27}Al (O_2)	^{48}Ti (NH_3)	^{51}V (KED)	^{51}V (O_2)
Level I	1.6	1.7	1.5	1.4	5.9	3.3	3.2	2.3	2.6
Level II	1.7	1.6	1.7	1.8	4.1	3.6	3	2.4	1.6
Level III	1.6	2	1.5	1.5	5	5.7	2.5	2	1.9
	^{52}Cr (O_2)	^{55}Mn (KED)	^{56}Fe (O_2)	^{56}Fe (KED)	^{59}Co (KED)	^{59}Co (O_2)	^{60}Ni (KED)	^{63}Cu (KED)	^{66}Zn (KED)
Level I	6.2	3.2	1.6	1.6	1.9	2.2	3	1.8	1.3
Level II	1.6	2.8	1.9	1.6	1.9	1.9	1.3	2.2	1.6
Level III	4.9	2.5	1.4	1.3	1.7	2.7	2.3	1.3	1.3
	^{66}Zn (O_2)	^{75}As (KED)	^{75}As (O_2)	^{78}Se (KED)	^{80}Se (O_2)	^{95}Mo (KED)	^{105}Pd (KED)	^{107}Ag (KED)	^{111}Cd (KED)
Level I	2	1.8	2.1	1.6	1.8	2.9	1.7	1.3	1.9
Level II	1.5	1.7	2.2	1.7	1.9	3.8	1.9	1.9	2
Level III	1.6	1.8	1.6	2.1	1.4	2.6	1.7	1.5	1.7
	^{120}Sn (KED)	^{121}Sb (KED)	^{127}I (KED)	^{137}Ba (KED)	^{195}Pt (KED)	^{197}Au (KED)	^{202}Hg (KED)	^{205}Tl (KED)	^{209}Bi (KED)
Level I	6.4	1.9	2.1	1.8	1.4	1.8	2.2	1.9	2.2
Level II	3.1	1.8	2	1.7	1.9	1.9	1.8	1.6	1.5
Level III	8.5	1.7	2.1	1.9	1.6	1.5	1.5	1.5	1.5

Table 6. Inter-assay precision (%CV) results of recipe controls at three levels {serum controls (level I + II) and plasma controls (level III)}

	^{7}Li (N-A)	^{9}Be (N-A)	^{25}Mg (N-A)	^{25}Mg (KED)	^{27}Al (NH_3)	^{27}Al (O_2)	^{48}Ti (NH_3)	^{51}V (KED)	^{51}V (O_2)
Level I	2.6	2.9	2.3	2.1	6.8	7.9	5.5	3.8	2.6
Level II	2.4	2.3	2	2.5	5.2	3.7	5.3	2.4	2.2
Level III	2.6	3.2	2.6	2.6	9.8	9.3	7.3	6.6	2.9
	^{52}Cr (O_2)	^{55}Mn (KED)	^{56}Fe (O_2)	^{56}Fe (KED)	^{59}Co (KED)	^{59}Co (O_2)	^{60}Ni (KED)	^{63}Cu (KED)	^{66}Zn (KED)
Level I	8	3.2	2.3	1.8	2.1	3.2	4.2	2.1	1.6
Level II	3.1	2.8	2.3	2.2	2.2	2.8	3.1	2.2	1.9
Level III	4.4	4.4	2.2	2.2	2.1	3.8	2.6	2.5	1.9
	^{66}Zn (O_2)	^{75}As (KED)	^{75}As (O_2)	^{78}Se (KED)	^{80}Se (O_2)	^{95}Mo (KED)	^{105}Pd (KED)	^{107}Ag (KED)	^{111}Cd (KED)
Level I	2.2	1.9	3	3.3	2.3	3.5	1.8	2.3	2.3
Level II	2.2	2	2.7	2.7	2.2	4.8	1.9	2.1	2.6
Level III	2.3	2	2.7	2.9	2.2	4.7	2.2	2.5	2.6
	^{120}Sn (KED)	^{121}Sb (KED)	^{127}I (KED)	^{137}Ba (KED)	^{195}Pt (KED)	^{197}Au (KED)	^{202}Hg (KED)	^{205}Tl (KED)	^{209}Bi (KED)
Level I	4.9	2.6	3	2.7	2.4	2.7	2	2.1	3.2
Level II	3	3	3.3	2.9	2	1.9	2	2	2.3
Level III	7.4	3.3	3.2	2.7	3.8	3.2	2.2	2.2	3.7

Conclusion

A robust, reproducible method for the detection of 28 trace elements employing inductively coupled plasma mass spectrometry is reported here. This analytical method uses calibrators and controls from the ClinMass Complete Kit for Trace Elements in Plasma/Serum from RECIPE Chemicals + Instruments GmbH (in preparation), which enable quick, efficient sample preparation. Direct sample injection and detection parameters were analytically validated on an iCAP TQ ICP-MS coupled with an ESI SC-4 DX autosampler. This method offers the capability of monitoring a suite of trace elements in a single measurement using different collision gases with minimal sample preparation and human intervention. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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