Challenge

A sample preparation procedure that allows for accurate analysis of a wide range of elements in biological samples.

Solution

Alkali sample preparation using a single, external calibration.

Multi-element Analysis of Biological Materials by ICP-MS using Alkali Dilution

Introduction

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is today the instrument of choice when it comes to elemental analysis of biological materials including urine, whole blood, plasma and serum. The fast, multi-element capability of the technique and wide dynamic range are key benefits that allow the measurement of major, minor, essential and toxic elements, covering the concentration range from sub parts-per-trillion (ppt) to percent levels in a single analysis. This places greater pressure on the sample preparation procedure as the chemistry of elements will define the range of elements and level of accuracy that can be achieved. For example:

- Acidification of biological samples to improve elemental stability will cause proteins to coagulate and potentially retain certain elements, producing lower than expected results.
- The degree of ionization (and hence signal strength) of As and Se within the plasma is influenced by the carbon content in the samples and the effect must be minimized for accurate measurement.
- Volatile forms of iodine are formed at lower pH, greatly affecting elemental recovery during preparation and washout properties within the sample introduction system.
- The addition of gold has been reported to improve the washout properties of mercury, reducing rinse times for faster analysis especially after a high recording.

This application study evaluates an alkali sample preparation procedure that allows for the broadest range of elements to be measured accurately and precisely for different biological samples using a single, external calibration. While at the same time, maintaining method simplicity and keeping sample contamination to a minimum. To achieve this, the following steps were taken.

- Samples were simply diluted to reduce sample preparation times and minimize the potential for contamination by eliminating the need for complete microwave acid digestion
- Samples were prepared in an alkali mixture using ammonium hydroxide to break down the cell membrane (Lysis), preventing coagulation and allowing complete release of all elements
- Propanol was added to buffer the enhancement effects of variable carbon content in the matrix on the important elements of As and Se
- In the absence of acidification, EDTA was added to stabilize the elements
- 200ppb Au was added to improve the washout properties of Hg within the sample introduction system.

Instrumentation

PlasmaQuant MS with ASPQ 3300 autosampler and ESI injection valve were used for the analysis of various clinical standard reference materials including whole blood, blood plasma, serum and urine. The instrument operating conditions are summarized in Table 1, including the integrated Collision Reaction Cell (iCRC) modes using helium and hydrogen gases to remove problematic spectroscopic interferences on important elements like, As, Se, Cr and Fe (Table 2). The integrated Nitrox accessory was also utilized, allowing a small flow of nitrogen gas to be added online to the ICP, enhancing the signal of and reducing carbon-related matrix-effects on As and Se.

Table 1: PlasmaQuant MS operating conditions

Parameter	Specification
Plasma Gas Fow	9 L/min
Auxiliary Gas Flow	1.25 L/min
Nebulizer Gas Flow	1.08 L/min
Nitrogen Gas Flow (via Nitrox)	0.03 L/min (for As and Se)
Plasma RF Power	1.3 kW
iCRC Gas Flows	H_2 - 120 mL/min for ^{27}Al, ^{56}Fe, ^{75}As, ^{78}Se, ^{114}Cd, ^{121}Sb, ^{127}I, ^{202}Hg, ^{205}Tl H_2 - 180mL/min for ^{52}Cr He - 120 mL/min for all other elements
Sampling Depth	5 mm
Ion Optics	Auto-optimized
Detector Attenuation	Medium (Li, Mg, Fe) None (for all other elements)
Dwell Time	20 ms
Scans per Replicate	20 (peak hopping at 1pt/pk)
No. of Replicates	3
Pump Rate	10rpm - Sample line - black/black PVC pump tubing Internal standard –orange/green PVC pump tubing
Nebulizer Type	MicroMist (quartz concentric)
Spraychamber Type	Glass Scott
Spraychamber Temperature	3°C

Table 2: Major interferences on important analyte isotopes used in clinical analyses and the recommended collision-reaction gas

Analyte Isotope	Potential Interference	Recommended Collision/Reaction gas
^{52}Cr	$^{40}Ar^{12}C$, $^{36}Ar^{16}O$, $^{35}Cl^{16}O^{1}H$	H_2
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$	H_2
^{75}As	$^{40}Ar^{35}Cl$, $^{40}Ca^{35}Cl$	H_2
^{78}Se	$^{40}Ar^{38}Ar$, $^{40}Ca^{38}Ar$	H_2

Samples and Reagents

Table 3: List of reagents and samples used

Reagents	Samples and reference materials
Milli-Q deionized water (>18.2 MΩ.cm, Millipore MilliQ)	RECIPE ClinChek Control - Urine Level 1 & 2
NH_4OH (Carl Roth)	RECIPE ClinChek Control - Whole Blood Level 1 & 3
Propanol (VWR)	RECIPE ClinChek Control - Plasma Level 2
Triton X-100 (VWR)	SERO Seronorm Trace Elements - Serum L1 & L2
1000 mg/L Gold (Au) Standard (Inorganic Ventures)	80 biological samples including a variety of urine, plasma, serum, whole blood and red blood cells
EDTA (VWR)	

Sample Preparation

After reconstituting the aforementioned reference materials according to the provided instructions, sample solutions and certified reference materials were then prepared for analysis by diluting 20-fold in an alkali mixture of 2% (v/v) NH_4OH, 1 g/L EDTA, 1% (v/v) Propanol , 0.05% (v/v) Triton X-100 and 200ppb Au. Calibration, blank, internal standard and rinse solutions were also prepared in the same alkali mixture.

Results and Discussion

Tables 4-10 show the average concentrations determined for elements in Clinchek Control certified reference materials of urine, whole blood and plasma, and Seronorm trace elements in serum.

A review of the data suggests that it is possible to accurately determine most, if not all certified elements, including iodine, in various biological samples following simple dilution in the alkali mixture. The majority of results were found to fall within ±10% of the certified value. While there were a small number of outliers on some elements, the behavior was not consistent across all solutions.

Elements with direct polyatomic interferences formed by argon and sample matrix elements (see Table 2), including As, Se, Cr and Fe, did not appear to be biased, demonstrating effective interference removal. While higher than expected recoveries were observed for Cr in both serum control standards, this behavior was not consistent with other control standards. In particular, for whole blood where Cr levels are similarly low and the matrix high in carbon and chloride.

Table 4: Results for Clinchek Urine Level 1

Analyte Isotope	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (Lot 923) (µg/L)
^{27}Al	**94.6**	95.5	71.6 – 119
^{75}As	**43.9**	43.5	34.8 – 52.2
^{114}Cd	**2.2**	2.1	1.71 – 2.57
^{59}Co	**2.1**	2.1	1.70 – 2.56
^{52}Cr	**10.8**	10.5	7.88 – 13.1
^{65}Cu	**62.8**	60.8	48.6 – 73.0
^{56}Fe	**36.3**	40.6	32.5 – 48.7
^{202}Hg	**3.3**	3.5	2.79 – 4.19
^{55}Mn	**2.7**	3.1	2.46 – 3.68
^{95}Mo	**27.1**	27.2	21.8 – 32.6
^{60}Ni	**6.6**	5.5	4.14 – 6.90
$^{206..208}$Pb	**23.1**	24.6	18.5 – 30.6
^{105}Pd	**1.6**	1.5	1.14 – 1.90
^{121}Sb	**13.0**	11.8	9.44 – 14.2
^{78}Se	**28.6**	28.3	22.6 – 34.0
^{118}Sn	**5.4**	4.9	3.94 – 5.90
^{205}Tl	**7.8**	7.3	5.82 – 8.74
^{51}V	**21.2**	19.9	15.9 – 23.9
^{66}Zn	**244**	236	177 – 295

Table 5: Results for Clinchek Urine Level 2

Analyte Isotope	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (Lot 923) (µg/L)
^{27}Al	**140**	157	126 – 188
^{75}As	**82.2**	82.1	65.7 – 98.5
^{114}Cd	**13.7**	15.0	12.0 – 18.0
^{59}Co	**34.2**	34.6	27.7 – 41.5
^{52}Cr	**34.7**	35.4	28.3 – 42.5
^{65}Cu	**114.6**	118	94.4 – 142
^{56}Fe	**205**	213	170 – 256
^{202}Hg	**39.9**	39.5	31.6 – 47.4
^{55}Mn	**14.0**	21.2	17.0 – 25.4
^{95}Mo	**102**	109	87.2 – 131
^{60}Ni	**43.7**	44.1	33.1 – 55.1
$^{206..208}$Pb	**62.2**	64.5	48.4 – 80.6
^{105}Pd	**8.5**	9.9	7.94 – 11.9
^{121}Sb	**49.6**	45.9	36.7 – 55.1
^{78}Se	**69.7**	76.5	61.2 – 91.8
^{118}Sn	**10.3**	9.4	7.54 – 11.3
^{205}Tl	**19.4**	18.8	15.0 – 22.6
^{51}V	**50.0**	48.4	38.7 – 58.1
^{66}Zn	**528**	556	445 – 667

Table 6: Results for Clinchek Whole Blood Level 1

Analyte Isotope	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (Lot 227) (µg/L)
^{75}As	**6.9**	5.52	4.42 – 6.62
^{114}Cd	**1.4**	1.32	1.06 – 1.58
^{59}Co	**1.6**	1.90	1.52 – 2.28
^{52}Cr	**1.8**	1.69	1.35 – 2.03
^{65}Cu	**735**	689	551 – 827
^{202}Hg	**2.4**	1.49	0.969 – 2.01
^{25}Mg	**28000**	26700	24000 – 29400
^{55}Mn	**8.9**	7.87	6.30 – 9.44
^{60}Ni	**2.3**	1.9	1.52 – 2.28
$^{206..208}$Pb	**62.2**	58.4	46.7 – 70.1
^{78}Se	**89.1**	74.3	59.4 – 89.2
^{205}Tl	**0.9**	0.89	0.714 – 1.07
^{66}Zn	**4770**	4630	3940 – 5320

Table 7: Results for Clinchek Whole Blood Level 3

Analyte Isotope	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (Lot 923) (µg/L)
^{75}As	**22.8**	19.6	15.7 – 23.5
^{114}Cd	**6.80**	6.54	5.23 – 7.85
^{59}Co	**13.6**	13.6	10.9 – 16.3
^{52}Cr	**13.1**	11.9	9.52 – 14.3
^{65}Cu	**1753**	1610	1370 – 1850
^{202}Hg	**8.7**	7.98	6.38 – 9.58
^{25}Mg	**45300**	42500	38300 – 46800
^{55}Mn	**23.9**	19.9	15.9 – 23.9
^{60}Ni	**14.0**	13.8	11.0 – 16.6
$^{206..208}$Pb	**468**	427	363 – 491
^{78}Se	**189**	162	130 – 194
^{205}Tl	**9.70**	9.12	7.30 – 10.9
^{66}Zn	**8339**	8020	6620 – 9220

Table 8: Results for Clinchek Plasma Level 2

Analyte Isotope	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (Lot 423) (µg/L)
^{27}Al	**61.8**	55.6	41.7 – 69.5
^{75}As	**54.3**	46.5	34.9 – 58.1
^{9}Be	**20.5**	17.7	14.2 – 21.2
^{209}Bi	**4.9**	4.4	3.5 – 5.2
^{114}Cd	**11.6**	10.7	8.6 – 12.8
^{59}Co	**10.1**	9.7	7.7 – 11.6
^{52}Cr	**12.2**	10.6	8.5 – 12.7
^{65}Cu	**1269**	1220	976 – 1460
^{56}Fe	**1232**	1110	888 – 1330
^{202}Hg	**10.3**	9.4	7.6 – 11.3
^{127}I	**116.9**	98.4	78.7 – 118.0
^{7}Li	**9164**	8460	7610 – 9310
^{25}Mg	**29800**	29400	26500 – 32300
^{55}Mn	**16.9**	15.5	12.4 – 18.6
^{95}Mo	**6.8**	6.3	5.1 – 7.6
^{60}Ni	**16.3**	13.9	11.1 – 16.7
^{105}Pd	**8.2**	7.7	6.1 – 9.2
^{195}Pt	**5.0**	4.3	3.5 – 5.2
^{121}Sb	**7.1**	5.9	4.7 – 7.1
^{78}Se	**131**	120	96 – 144
^{118}Sn	**8.4**	7.6	6.1 – 9.1
^{49}Ti	**54.1**	46.8	37.4 – 56.2
^{205}Tl	**11.7**	10.8	8.6 – 13.0
^{51}V	**11.0**	9.9	7.9 – 11.8
^{66}Zn	**1676**	1540	1230 – 1850

Table 9: Results for Seronorm Serum Level 1

Analyte Isotope	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (Lot 0903106) (µg/L)
^{27}Al	**55.1**	33.6	31.7 – 35.5
^{59}Co	**1.3**	1.2	1.0 – 1.4
^{52}Cr	**2.4**	1.5	1.3 – 1.7
^{65}Cu	**1696**	1691	1607 – 1775
^{56}Fe	**1332**	1390	1310 – 1470
^{202}Hg	**0.70**	0.73	0.63 – 0.83
^{7}Li	**5599**	5741	5420 – 6062
^{25}Mg	**19300**	20100	18800 – 21400
^{55}Mn	**15.4**	15.0	14.1 – 15.9
^{60}Ni	**7.1**	5.8	5.1 – 6.5
^{78}Se	**118**	107	100 – 114
^{66}Zn	**2067**	1738	1667 – 1809

Table 10: Results for Seronorm Serum Level 2

Analyte Isotope	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (Lot 0903106) (µg/L)
^{27}Al	**110**	104	98 – 110
^{59}Co	**2.9**	3.2	3.0 – 3.4
^{52}Cr	**6.5**	4.8	4.4 – 5.2
^{65}Cu	**2696**	2887	2788 – 2986
^{56}Fe	**1923**	2030	1900 – 2160
^{202}Hg	**1.74**	1.87	1.74 – 2.00
^{7}Li	**10772**	10950	9981 - 11919
^{25}Mg	**37100**	40800	36100 – 45500
^{55}Mn	**19.8**	19.9	18.8 – 21.0
^{60}Ni	**11.1**	9.8	9.2 – 10.4
^{78}Se	**168**	163	153 – 173
^{66}Zn	**2523**	2520	2314 – 2726

Measurement of Iodine

In a separate study, iodine was measured in serum and urine Clinchek Control samples using the same sample preparation procedure and instrument setup.

Iodine is an essential micronutrient for normal thyroid function with approximately 90% of excess iodine excreted in the urine. Accepted minimum adequate level of urinary iodine is 100 µg/L and levels above this are considered normal. Urinary iodine concentrations below 25 µg/L are classified as severe deficiency. The volatile HI and I_2 forms are avoided in the alkali matrix, thereby minimizing Iodine loss during preparation as well as greatly reducing memory effects within the sample introduction.

The results of this separate analysis, along with iodine measured in blood plasma from the original multi-element study are shown in Table 11.

Table 11: Result for Iodine in Urine, Plasma and Serum Clinchek Control Standards

ClinCheck Control	Measured Value (µg/L)	Certified Value (µg/L)	Certified Range (µg/L)
Urine Level 2 (lot 432)	**466**	497	373 – 622
Plasma Control L2 (lot 423)	**117**	98.4	78.7 – 118
Serum Level 2 (lot 347)	**157**	133	106 – 160

Stability Performance

Once the method was prepared and validated, real human biological samples were analyzed. Figure 1 shows the measured internal standard ratios for each sample and demonstrates the importance of internal standard correction when switching between biological matrices. Note that only data collected in He iCRC mode is presented, although H_2 iCRC mode showed similar behavior within the same analysis. The internal standard ratio ranges between 75 - 125% of the initial reading over the duration of the analysis, while the stability within the same sample matrix was excellent, demonstrating the stability of the entire system.

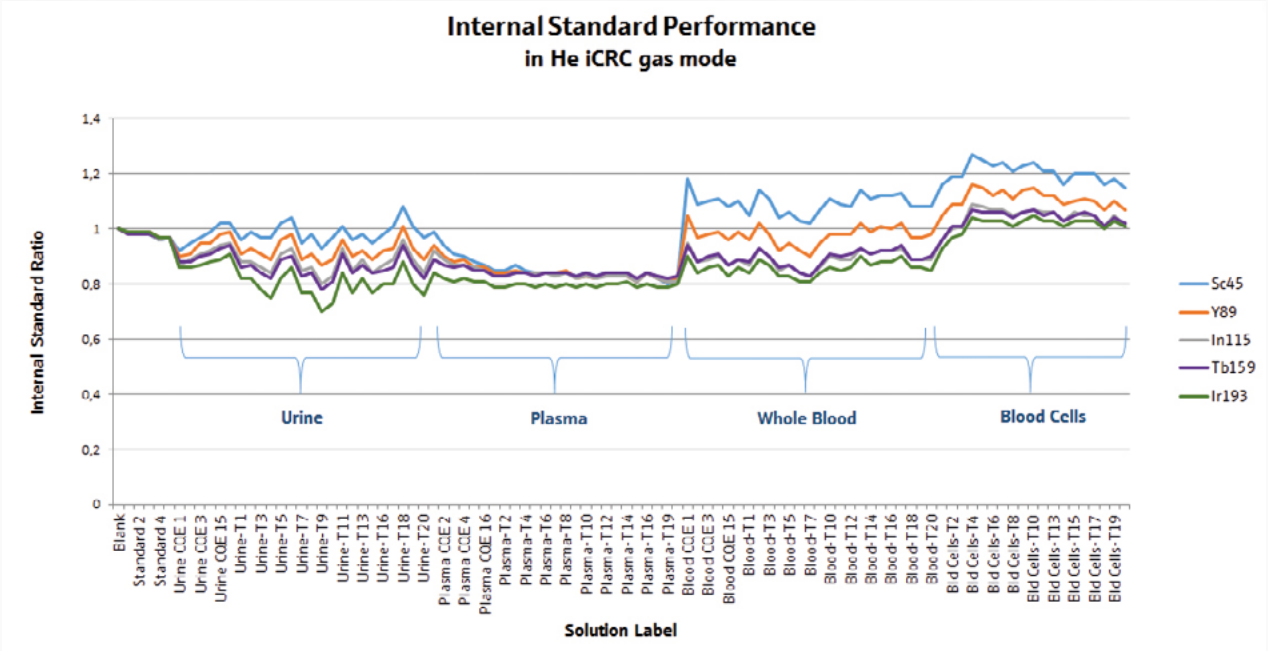

Figure 1: Internal Standard ratio measurement for each sample matrix in He iCRC mode

Conclusion

This study has demonstrated that alkali dilution of biological materials of a clinical nature is an effective sample preparation procedure for multi-element analysis by ICP-MS. The method is suitable for a broad range of elements, including Iodine, which is extremely difficult to measure accurately in an acidic matrix. The alkali matrix prevents the coagulation of biological material that can trap certain elements (Cu, Zn) resulting in lower recovery.
The integrated Collision Reaction Cell of the PlasmaQuant MS plays a key role in eliminating common argon and matrix-related interferences resulting from the combination of plasma gas, sample electrolytes and carbon-rich biological material. The iCRC utilizes both helium collision gas and hydrogen reaction gas for lowest detection limits on key elements including As, Se, Fe and Cr.

The PlasmaQuant MS also includes the option to add a small flow of nitrogen to the plasma gases allowing increased sensitivity on the poorly ionized elements of As and Se. The addition of nitrogen has also shown to reduce carbon-based matrix effects on these elements, potentially eliminating the need to add propanol as a carbon buffer.
With a broad range of elements measured in this study, covering a large concentration range from low µg/L (ppb) to high mg/L (ppm), it's worth mentioning the unique capabilities of the all-digital detection system. While the detector can automatically switch from no attenuation to medium and high attenuation modes, offering a full 10-orders of linear dynamic range, detector attenuation can also be manually set for specific elements during a multi-element scan. Using this unique detector capability, major elements including Li, Mg and Fe were manually set to medium attenuation to avoid detector saturation whilst still covering more than 6 orders of linear dynamic range without the need to ever cross-calibrate attenuation modes.

Last but not least, the EcoPlasma required only 11.33 L/min of argon gas for the analysis, providing significant cost savings. Utilizing a standard Fassel torch, the innovative design of the plasma system does not compromise plasma robustness and stability to achieve low argon gas usage, as is observed with inferior mini-torch designs.

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