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Inductively Coupled Plasma Mass Spectrometry ICPMS-2040/2050 LF

Application News

Determination of 20 Nutritional, Essential and Toxic Elements in Blood Serum by ICP-MS using Alkaline Dilution

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User Benefits

- Using serum calibrators and serum control samples of ©Recipe simplifies working routine while reducing risk of errors
- Offering gas switching times <5 sec and using ProActive Rinsing function, a sample cycle time of ~2.0 min. can be achieved, avoiding the need of additional fast rinse accessories / sample loops
- Of great importance is the comparable low serum volumes required per specimen (~ 75 µL Serum)

■ Introduction

Elemental analysis of blood serum is crucial for understanding the biochemical composition of the human or animal body and its implications for health. Several scientific studies have provided evidence supporting the importance of element analysis in various aspects of healthcare, whether it can be nutritional assessment, toxic element exposure or medical conditions.

In recent times, ICP-MS is state of the art choice for routine analysis of elements in blood serum. Different aspects like robustness and throughput form the principles of this method, while not compromising reliability.

Important aspects to take into consideration are highlighted within this note, especially aiming to reduce sample volume and measurement time, allowing to quantify more than 20 elements in ~2.0 min. while avoiding more complex sample injection techniques.

Fig. 1 ICPMS-2040 LF with AS-20 Autosampler

■ Sample Preparation and Calibration

Blood serum is the liquid supernatant, after a clotted blood sample was centrifugated. Thus, it does not contain any blood cells or clotting factors, but still represents a difficult sample matrix. Beside appr. 91 % water, it is a carbon rich matrix of 7 % proteins and the remaining 2 % is made up of electrolytes, nutrients, waste materials (such as urea) and hormones.

To analyze a blood serum sample by ICP-MS, it requires some step of sample preparation which can be destruction or dilution.

Due to being labor- and time intensive, a complete microwave assisted acid digestion was not selected, even though it represents the most ideal sample preparation: Commonly, nitric acid is applied for digestion resulting in proteins and other carbon sources to be destroyed and finally separated as CO_2.

The other type of sample preparation is simple dilution in acid- or alkaline media. While acid media might cause denatured proteins which increase the risk of e.g. clogging the sample line or nebulizer, alkaline dilution (Table 1) was selected in this note.

Table 1 Components of Alkaline Diluent prepared in Water*1

Reagent	Description	Purpose
Ammonia	25% (v/v), Suprapur®, Merck, Germany	adjusts alkaline pH
Triton®X-100	p.A., Merck, Belgium	Homogenizes sample & supports lyse of cells
EDTA (Na-free)	>99.999% Sigma-Aldrich, USA	stabilizes ions in solution
Isopropanol	≥99.999%, Sigma-Aldrich, USA	compensates carbon enhancement effect*2

*1 All solutions are prepared using ASTM Type 1 ultrapure water (18.2 MΩ resistivity, Arium® Pro VF, Sartorius AG).
*2 Carbon present in sample will otherwise increase response of less ionized elements (e.g. As, Se), resulting in false positives.

For matrix matched calibration, Serum Calibrator Level 1-4 (Recipe® ClinMass® 9950, table 2) was selected. For quality control, Serum Control Level 1-2 (Recipe® ClinMass® 8882) is measured in user-defined intervals. Calibrators, controls as well as samples are simply 20-fold diluted by alkaline diluent and well homogenized (vortex mixing). This 20-fold dilution in combination with using low total sample volumes (e.g 1.5 ml per measurement), allows to reduce the required blood serum volumes down to ~ 75 µL, which is very helpful in case the available volume is limited.

If in contrast 3 ml volume per specimen would be necessary while using less dilution (e.g. 10-fold), then 300 µL Serum or more would be required. In addition, calibrators or controls following this preparation would be consumed 4 times quicker.

Table 2 Recipe® 9950 Calibration Levels, µg/L, Batch dependent; Excerpt

Element	Blank	L1	L2	L3	L4
Ag	0	0.555	3.68	10	29
As	0	2.24	5.15	11	28.5
Ba	0	15.1	23.1	39.3	88.7
Be	0	0.51	2.65	6.97	19.9
Bi	0	0.797	2.27	5.23	14
Cd	0	0.52	1.53	3.58	9.73
Co	0	0.601	2.05	4.96	13.6
Cu	0	750	846	1042	1626
Fe	0	839	943	1156	1805
Hg	0	0.527	1.74	4.18	11.5
I	0	40.7	51.9	74.8	143
Mg	0	15769	17763	21563	32789
Mo	0	0.539	1.84	4.49	12.2
Pt	0	1.83	217	650	1975
Sb	0	0.876	2.26	5.08	13.5
Se	0	60.6	72.7	98.1	174
Sn	0	1.01	2.89	6.71	18.2
Tl	0	0.519	1.53	3.55	9.62
V	0	0.501	1.53	3.56	9.68
Zn	0	1206	1315	1534	2203

■ Configuration & Measurement Condition

System configuration of ICPMS-2040 LF was selected according to Table 3. To highlight is the increased sampling depth of 6 mm instead of highly sensitive 5 mm. In addition, argon dilution was applied. Both measure let less sample (blood serum) enter the cone interface to gain optimum system robustness while keeping sufficient sensitivity.

Table 3 ICPMS-2040/2050 LF Configuration and Basic Method Settings

Autosampler	:	AS-20 Round Table / Dual Rinse with 5x spacer for low dead volume
Spray Chamber	:	Cyclonic Twister
Chamber Temperature	:	5 °C
Nebulizer	:	Coaxial 07UES, ~ 0.4 ml/min
Peristaltic Pump	:	4 Channels, 12-roller @ 12 rpm
Internal Standard Tubing	:	PVC, flared-end, orange-blue
Sample Tubing	:	PVC, flared-end, black-black
Drain	:	Automatic overflow type
Torch	:	Shimadzu Minitorch
Cone Material	:	Ni
Sampling Depth	:	6.0 mm
RF-Power	:	1.2 kW
Plasma Gas	:	Ar 9.00 L/min
Auxiliary Gas	:	Ar 1.10 L/min
Carrier Gas	:	Ar 0.47 L/min
Dilution Gas	:	Ar 0.38 L/min
Cell Gas	:	He 6.0 mL/min
Cell Voltage	:	-21.0 V
Energy Filter	:	7.0 V

More than 20 elements have been selected for quantification (Table 4) whereat some of the selected isotopes are partwise affected by polyatomic interferences. To eliminate this negative effects, the ICPMS-2040 LF highly efficient He-collision mode was applied.

The alkaline diluent was measured as blank sample. In most cases the trace region is of interest, thus all calibration curves (Fig. 2) are weighted inversely (1/I) to improve the precision in the low-end trace region.

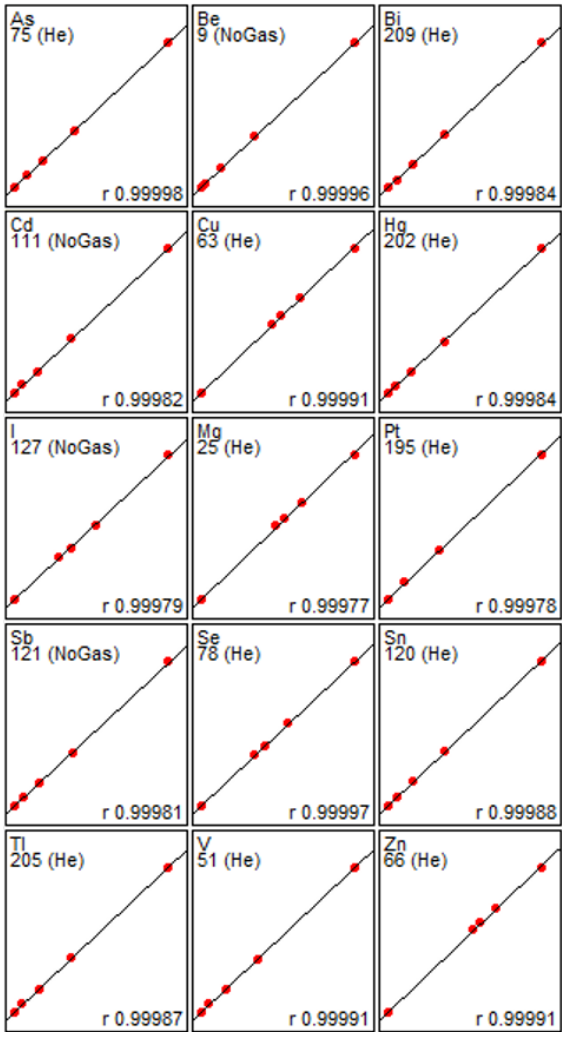

Fig. 2 Calibration Curves (examples)

Table 4 Elements and Isotopes

Element	Isotope	Integration time [s]	Cell Gas	Internal Standard
Ag	107	0.5	He	^{103}Rh
As	75	0.5	He	^{74}Ge
Ba	137	0.1	-	^{103}Rh
Be	9	1.0	-	^{45}Sc
Bi	209	0.2	He	^{187}Re
Cd	111	0.5	-	^{103}Rh
Co	59	0.5	He	^{74}Ge
Cu	63	0.1	He	^{74}Ge
Fe	56	0.1	He	^{74}Ge
Hg	202	1.0	He	^{187}Re
I	127	0.1	-	^{103}Rh
Mg	25	0.1	He	^{74}Ge
Mo	95	0.2	He	^{103}Rh
Pt	195	1.0	He	^{187}Re
Sb	121	0.2	-	^{187}Re
Se	78	5.0	He	^{74}Ge
Sn	120	2.0	He	^{103}Rh
Tl	205	0.2	He	^{187}Re
V	51	2.0	He	^{45}Sc
Zn	66	0.1	He	^{74}Ge

■ Results and Discussion

Method Stability

The method was stable over the full 60 samples runtime indicated by internal standard recovery, standardized to the lowest calibration curve reference level (L1 = 100 %, Fig. 3). At the same time, serum control L1 and L2 included to middle, and end of batch have been traced back to given values within specified reference ranges.

The reference values may be entered to the LabSolutions™ ICPMS method directly, allowing for quick assessment of the quality of the results obtained from each run, as outliers will be highlighted automatically.

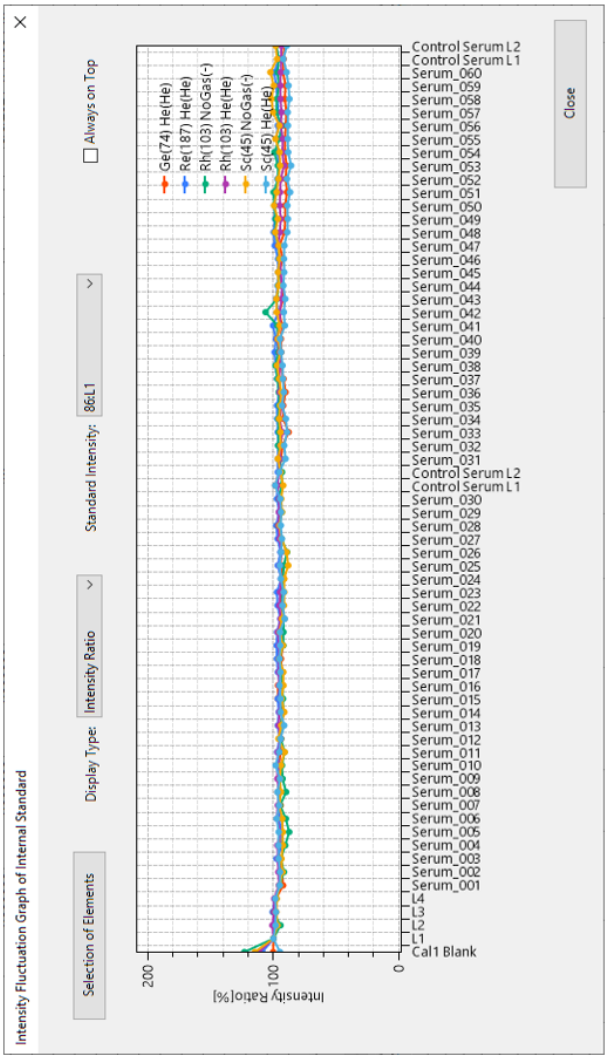

Fig. 3 Internal Standard Stability Plot

Method Sensitivity

In general, the method is developed to be stable while allowing to analyze the usual reference ranges (Table 5). The sensitivity is determined by standard deviation of the calibration curve blank sample. Because samples and reference samples are treated in the same way, the dilution factor can be disregarded, allowing to use the given limit of quantification (LOQ) as approximation for the method quantification limit (MQL).

Table 5 ICPMS-2040 LF Method Sensitivity [µg/L]

Element	LOD (3s) ≈ MDL	LOQ (10s) ≈ MQL	Common Reference Range[1)]	ICPMS-2040 LF suitable?
Ag	0.31	1.04	< 2	●
As	0.06	0.21	< 12	●
Ba	0.16	0.55	< 2.9	●
Be	0.01	0.05	< 0.5	●
Bi	0.03	0.11	< 2.5	●
Cd	0.04	0.15	< 0.3 - 0.65	●
Co	0.02	0.07	< 0.5	●
Cu	0.37	1.22	200 - 1900	●
Fe	0.71	2.36	220 - 1680	●
Hg	0.04	0.14	< 2.0	●
I	0.20	0.65	40 - 80	●
Mg	19.0	63.3	17000 - 24000	●
Mo	0.21	0.70	< 1	●
Pt	0.03	0.10	< 0.2	●
Sb	0.02	0.05	< 10	●
Se	0.07	0.24	16 - 118	●
Sn	0.12	0.39	< 2	●
Tl	0.03	0.11	< 10	●
V	0.03	0.11	< 0.7	●
Zn	1.41	4.70	500 - 1200	●

To further increase sensitivity for individual elements, a simple approach can be to increase its integration time.

Method Speed

With ICPMS-2040/2050 Series system generation, features like ProActive Rinsing allow to speed up the method while not requiring additional hardware. The principle behind is to already start rinsing before the actual measurement is finished. This is possible because sample is still left in the capillary and is further injected before rinse solution reaches the nebulizer (Fig. 4). In combination with round table autosampler AS-20, the overall injection capillary length is extremely short, allowing quick sample injection and reducing risks of memory- and carry-over effects. In combination with gas switching times <5 sec, the overall sample cycle time can be reduced to ~2 min.

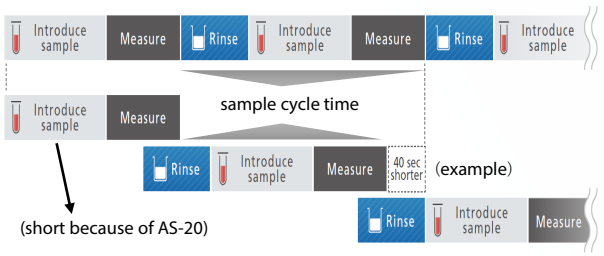

Fig. 4 ProActive Rinsing

Eliminate possible Interferences

A usual troublesome element is Selenium, as the following example indicates: "From a clinical biochemistry point of view, perhaps the most significant example is the interference of gadolinium with selenium. […] Gadolinium isn't normally present in biological samples, however it is present in some intravenous contrast agents used for magnetic resonance imaging (MRI) […]. The presence of gadolinium in a clinical sample can cause an artefactually elevated selenium result"[2)], as double charged ^{156}Gd interferes ^{78}Se.[3)]

This example shows that unforeseen interferences can occur any time and that it can be helpful to use superior techniques to eliminate them. Otherwise interfering substances needs to be steadily monitored to prove its absence.

A good strategy to get rid of e.g. further selenium interferences generated by double charged ions like $^{156}Gd^{++}$ is using hydrogen (H_2) as reaction gas with ICPMS-2050 LF. Some experimental data (Table 6) shows, that using hydrogen, even elevated levels of Gd do not anymore interfere Selenium.

Table 6 Eliminated effect of Gd to Se in H_2-mode (ICPMS-2050 LF)

	Solution A	Solution B	Solution C	Solution D
Gd-level	0 ppm	10 ppm	20 ppm	50 ppm
Se-level	20 ppb	20 ppb	20 ppb	20 ppb
Se-Result H_2-mode	● 19.9 ppb	● 19.7 ppb	● 19.7 ppb	● 19.8 ppb

■ Conclusion

For routine use, the ICPMS-2040 LF is highly suitable for analyzing trace elements in human and veterinary blood serum. The method allows for the measurement of samples from extremely low serum volumes.

To measure Se more sensitive and without double charged ion interferences of Gd, ICPMS-2050 LF is the instrument of choice.

More than 20 elements can be measured at a rate of 30 samples per hour. The low consumption of argon and helium, combined with reduced power consumption for operating the Minitorch plasma and short sample cycle time, makes it exceptionally cost-effective without compromising sensitivity.

Besides the mentioned elements, further ones like nickel (Ni), chromium (Cr) and manganese (Mn) have been successfully evaluated in shorter batches and can be simply added to make the method once more universal.

<References>

1) Trace Elements in Plasma/Serum, Instruction Manual, Recipe Chemicals + Instruments GmbH
2) Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects, Clin Biochem Rev 40 (3) 2019, p. 126.
3) Interference Correction for Doubly Charged Ions, Technical Note SPPD-9059A, Shimadzu Corporation

<Related Applications>

1. Determination of 19 Essential and Toxic Trace Elements in Blood Serum by ICPMS-2030 Series Using Alkaline Dilution, Application News 05-SCA-116-007-EN

05-SCA-116-010-EN First Edition: Nov. 2024

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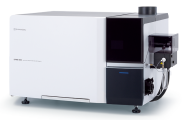

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