www.analytic-news.com

Publication Date: 12.04.2017

Preparing Biological Samples for Elemental Analysis by ICP-MS

Andrew Ryan

Analytik Jena AG

Introduction

The transition from Atomic Absorption Spectroscopy (AAS) to Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) has been gradual for the clinical industry over the past two decades. Both flame AAS and graphite furnace AAS techniques have served the industry well in hospitals, clinical institutes, universities, health organizations and general industry for research, diagnostic and monitoring purposes. The strength of Atomic Absorption lies in its ability to accurately and precisely measure the major, minor, essential and toxic elements in various biological samples, from % levels down to sub parts-per-billion (<ppb).

Although, the ability of ICP-MS to measure more elements in less time has seen the technique become the preferred choice in the clinical industry for elemental analysis. With a large dynamic range and ultra-low detection limits, ICP-MS is able to meet the growing demands for more information in less time and with less complexity.

In replacing flame and furnace AAS systems, the Inductively Coupled Plasma provides a significantly hotter heat source in decomposing the sample into its individual atoms (and ions), offering reduced matrix effects in comparison and true multi-element analysis. And while graphite furnace AAS offers the advantage of low volume handling at microliter volumes, the lower detection limits of ICP-MS allows for greater dilution volumes. Although, the demand for better handling of low-volume samples has seen the introduction of more efficient sample loading accessories in ICP-MS.

Before samples can be analyzed, whether they be whole blood, blood serum, blood plasma, urine or some other biological material, they first must be prepared for analysis. The simplest preparation is to analyze directly, although the high matrix of biological samples usually exceeds the total dissolved solids (TDS) limit that an ICP-MS can routinely analyze long term. While the variable matrix of biological samples can influence the accuracy of results if not accounted for. Even the pH of the final sample to be analyzed is important in determining which elements can be precisely measured. Therefore, the question often asked "What is the best way to prepare biological samples for analysis by ICP-MS".

Biological samples naturally have a high organic and salt content, including cells, proteins, clots, bio-fluids and chloride-based salts, predominately of sodium. The elemental composition within each of these components will vary, as will the elemental form. Some elements will be inorganic and easily extracted, while others are organically bound or may be confined to cell structures and require more aggressive extraction or even acid-digestion to completely decompose and remove the organic matter.

This article discusses the comparison of three sample preparation techniques for the analysis of biological solutions, with emphasis on whole blood. While other matrices, including urine, plasma and serum were also evaluated, whole blood is typically the most complex of these matrices when it comes to sample preparation and analysis by ICP-MS. The study involved preparing and analyzing various plasma, serum, urine and whole blood control standards and determining the concentration of various elements in each matrix from a single, external calibration. This work was conducted over a 12-month period by different analysts from Analytik Jena on PlasmaQuant MS systems located in Europe. The sample preparation methods evaluated include:

1. Complete acid digestion using closed-vessel microwave digestion
2. Acidic dilution in 0.5%HNO_3, 1% Propanol, 0.1% Triton X-100 and 200ppb Au
3. Alkali dilution in 2% NH_4OH, 1g/L EDTA, 1% Propanol, 0.05% Triton X-100 and 200ppb Au

Sample preparation method 1 provides complete decomposition of all organic matter and offers arguably, the best general purpose sample preparation procedure for many types

PlasmaQuant® MS

of biological samples. The closed vessel capability of microwave digestion reduces the loss of volatile elements, although can perhaps increase the possibility of contamination through increased sample handling. The main limitation of microwave digestion is time. The time it takes to complete a digestion cycle and the time it takes to prepare a large batch of samples. While open digestion systems can hold larger sample numbers, the potential loss of volatile elements including Hg, As and Se is of concern.

Sample preparation method 2 at first appears to be reasonably complex given the number of reagents involved, actually requires simple dilution of the sample once the diluent is created in volume. It is important though that high-purity reagents are sourced to keep contamination to a minimum. While the addition of nitric acid is anticipated to coagulate proteins, the addition of Triton X-100 surfactant assists in breaking-down cell membranes and maintaining sample homogeneity. Nitric acid is added to stabilize elements, although is kept to a minimum as the coagulated material can potentially block the nebulizer of the ICP-MS sample introduction system.

It is widely reported that the presence of carbon can influence the ionization efficiency of poorly ionized elements, including important clinical elements in Arsenic and Selenium. As carbon from biological material will vary

significantly in samples, so too will the apparent signal for these two elements as their degree-of-ionization within the plasma is affected. Therefore, a miscible organic solvent such as propanol is added to buffer this effect by saturating all measured solutions with carbon. Additionally, gold is often added at approximately 200ppb as it is widely reported to reduce the memory effects of Hg and improve rinsing times.

For whole blood samples in this acidic matrix, it is common to see red bloods cells and clots settle at the bottom of sample tubes. Placing the tubes in an ultrasonic bath can help to break these up to some extent. For analysis, it is best to avoid the uptake of these particulates as they are likely to cause blockage of the nebulizer.

Sample preparation method 3 utilizes ammonium hydroxide, producing an alkaline matrix and preventing the biological material from coagulating. EDTA is added as a complexing agent to stabilize the elements of interest. As explained above, propanol, Triton x-100 and 200ppb Au are added for the same reasons. The alkaline matrix also allows for the measurement of Iodine by stabilizing the element and reducing memory effects.

Analysis of Whole Blood

The three sample preparation procedures and subsequent analysis by ICP-MS were performed on a variety of biological control standards, including urine, plasma, serum and whole blood.

A further challenge faced in the analysis of biological samples resulting from matrix components is in the formation of spectroscopic interferences on important analyte isotopes commonly used in ICP-MS. Table 1 lists the major polyatomic interferences observed on As, Se and Cr resulting from the presence of sample matrix and argon ions in the inductively coupled plasma. Fortunately, most modern ICP-MS instruments incorporate collision-reaction gas systems that effectively remove these interferences, improving detection limits well into the parts-per-trillion (ppt) range.

Table 2 lists the results for ClinChek Whole Blood 3 Control Standard (from RECIPE Chemicals) that was independently prepared using the three sample preparation methods, and by three independent analysts on different Analytik-Jena PlasmaQuant MS instruments. Dilution methods 2 and 3 involved a 20-fold dilution while the final dilution factor of method 1 following acid digestion was 100-fold.

A review of the data demonstrates that all three methods achieved excellent recoveries, well within the certified range for most elements. Exceptions include Cu and Zn following acidic dilution with measured values at the lower end of the range, while alkali dilution was in the mid-to-upper range and closer the mean value.

Tab 1: Major interferences on important analyte isotopes in clinical analyses and the recommended collision-reaction gas

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

Tab. 2.: Averaged results for ClinChek Whole Blood 3 using sample preparation method 1, 2 and 3

Analyte Isotope (iCRC gas)	Complete Digestion* (µg/L)	Acidic Dilution (µg/L)	Alkali Dilution (µg/L)	Certified Mean Value (Lot 227) (µg/L)	Certified Range (Lot 227) (µg/L)
^{75}As	21.2	21.9	22.8	19.6	15.7 - 23.5
^{114}Cd	6.70	6.24	6.80	6.54	5.23 - 7.85
^{59}Co	13.6	11.7	13.6	13.6	10.9 - 16.3
^{52}Cr	14.3	15.9	13.1	11.9	9.52 - 14.3
^{63}Cu		1340	1753	1610	1370 - 1850
^{202}Hg	12.0*	12.8	8.70	7.98	6.38 - 9.58
^{25}Mg		45908	45300	42500	38300 - 46800
^{55}Mn		21.1	23.9	19.9	15.9 - 23.9
^{60}Ni	11.9	12.3	14.0	13.8	11.0 - 16.6
$^{206..208}Pb$	438	438	468	427	363 - 491
^{121}Sb		12.5	13.4	NA	NA
^{78}Se	166.0	172.5	188.7	162	130 - 194
^{118}Sn		9.1	10.0	NA	NA
^{205}Tl		9.59	9.70	9.12	7.30 - 10.9
^{66}Zn		7029	8339	8020	6620 - 9220

*WB3 Lot 445 used. Has similar ranges to WB3 Lot 227 with the exception of Hg (mean 12.1, range 9.64 - 14.5µg/L)

Adding nitrogen gas to the plasma

Propanol is added to buffer the effects of carbon on As and Se due to the variable organic content in biological samples. The presence of carbon is known to enhance the signal due to an increase in As and Se ion formation. As tables 3-5 demonstrate, when propanol is not added to all solutions, including samples and calibrants, the variable carbon content within the samples is clearly evident. Measured As and Se concentration values are well above the reported mean and range, particularly in whole blood, which has the highest carbon content of all the analyzed Clinchek Control Standards.

The addition of a small flow of nitrogen gas has also been shown to influence ionization of As and Se within the ICP. As part of this study, the effect of adding nitrogen gas to the plasma was studied and found to be beneficial for both As and Se. A small flow of approximately 60mL/min nitrogen gas added to the argon auxiliary gas line not only enhances the signal, but was also found to buffer the effects of variable carbon content. This is evident in the similar results obtained when adding propanol only and nitrogen only.

While a slightly higher bias with nitrogen addition was observed when using hydrogen reaction-gas (tables 3, 4), it is effectiveness as an ionization buffer is clearly evident, with the potential to completely eliminate the need for propanol addition or at least significantly reduce the amount required.

As expected, hydrogen reaction-gas is best suited to removing the predominately Ar-based interference on ^{78}Se. Whereas for ^{75}As, which is interfered with by a combination of argon and matrix based interferences, either hydrogen or helium is suitable. Although helium was found to be marginally better overall for the four different matrices analyzed.

Conclusion

This study has compared three sample preparation techniques for the analysis of biological samples by ICP-MS. The general conclusion is that all three methods are effective for the determination of important clinical elements in whole blood, plasma, serum and urine. Possible exceptions include Cu and Zn where alkali dilution gave better recovery for whole blood compared to acidic dilution. These elements may be partially trapped within the coagulated biological material still present in the acidic medium. Unfortunately, this could not be confirmed as these two elements were not measured following complete microwave digestion. Other reports[1] have suggested good agreement for Cu and Zn using complete digestion and alkali dilution.

The second part of this study evaluated the effectiveness of nitrogen gas addition to the plasma in order to both enhance the signal for As and Se, and act as a buffer against variation in the carbon content of the samples. It is clearly evident that the addition of nitrogen is beneficial and almost nullifies the need for adding an additional carbon source, such as propanol, to all solutions prior to analysis.

Reference

1 *Alkali dilution of blood samples for high throughput ICP-MS analysis – comparison with acid digestion. Ying Lu, Maria Kippler, Florencia Harari, Margaretha Grandér, Brita Palm, Helena Nordqvist, Marie Vahter. Clinical Biochemistry 48 (2015) 140-147.*

Tab. 3: Results for Se without propanol or N_2 addition, with propanol only, and with N_2 addition only. H_2 reaction gas was used for all conditions

ClinCheck Control Standard (H_2 reaction gas)	Se78 (µg/L)	Se78 with Propanol (µg/L)	Se78 with N_2 (µg/L)	Mean (µg/L)	Range (µg/L)
Urine Level 1	36.4	24.3	25.3	29.3	23.4 - 35.2
Urine Level 2	81.6	64.3	69.9	79.0	63.2 - 94.8
Plasma Control L1	118.8	85.0	91.5	80.0	64.0 - 96.0
Plasma Control L2	172.9	123.2	133.9	118	94.4 - 142
Serum Level 1	82.5	61.3	67.1	63.3	50.6 - 76.0
Serum Level 2	130.5	97.6	107.1	103	82.4 - 124
Whole Blood L1	141.9	79.1	84.6	74.3	59.5 - 89.2
Whole Blood L3	312.7	172.5	189.7	162	130 - 194

Tab. 4: Results for As without propanol or N_2 addition, with propanol only, and with N_2 addition only. H_2 reaction gas was used for all conditions

ClinCheck Control Standard (H_2 reaction gas)	As75 (µg/L)	As75 with Propanol (µg/L)	As75 with N_2 (µg/L)	Mean (µg/L)	Range (µg/L)
Urine Level 1	46.9	42.0	45.0	44.6	35.7 - 53.5
Urine Level 2	93.9	80.3	89.9	84.5	67.6 - 101
Plasma Control L1	72.1	55.9	58.6	47.8	38.2 - 57.4
Plasma Control L2	146.9	113.7	119.1	92.1	73.7 - 111
Serum Level 1	16.6	13.1	13.7	11.3	9.04 - 13.6
Serum Level 2	28.8	23.4	25.2	19.7	15.8 - 23.6
Whole Blood L1	11.7	8.0	8.4	5.52	4.42 - 6.62
Whole Blood L3	44.3	29.9	31.5	19.6	15.7 - 23.5

Tab. 5: Results for As without propanol or N_2 addition, with propanol only, and with N_2 addition only. He collision gas was used for all conditions

ClinCheck Control Standard (He collision gas)	As75 (µg/L)	As75 with Propanol (µg/L)	As75 with N2 (µg/L)	Mean (µg/L)	Range (µg/L)
Urine Level 1	NA	40.1	40.2	44.6	35.7 - 53.5
Urine Level 2	NA	74.6	80.0	84.5	67.6 - 101
Plasma Control L1	NA	50.7	49.5	47.8	38.2 - 57.4
Plasma Control L2	NA	97.9	99.6	92.1	73.7 - 111
Serum Level 1	NA	12.3	11.7	11.3	9.04 - 13.6
Serum Level 2	NA	22.0	22.6	19.7	15.8 - 23.6
Whole Blood L1	NA	8.1	5.9	5.52	4.42 - 6.62
Whole Blood L3	NA	27.3	23.2	19.6	15.7 - 23.5