The Use of Collision/Reaction Cell ICP-MS for the Simultaneous Determination of 18 Elements in Blood and Serum Samples

Application

Clinical

Authors

R. Wahlen, L. Evans, J. Turner, and R. Hearn
LGC Limited, Queens Road
Teddington, Middlesex, TW11 0LY
UK

Abstract

This study describes the development of a robust high-throughput analytical method for the determination of 18 elements (15 trace elements and 3 electrolytes) in blood and serum samples using an Agilent Technologies 7500ce collision/reaction cell ICP-MS system. The only sample preparation necessary was dilution using an alkaline diluent containing ammonia, EDTA Triton X-100, and butan-1-ol. Instrument calibration was performed using external calibration with internal standardization. The performance of the method exceeded a previously-used magnetic sector HR-ICP-MS method by at least a factor of three in terms of sample throughput and matched the precision and detection limits of that method.

Introduction

The analysis of metals in clinical fluids such as whole blood, serum, and urine has been used for many years to provide information on toxicity, work-place exposure, and nutrient availability, and as a diagnostic tool for a number of ailments. The fact that many trace metals are present at variable and often low concentrations (sub ng/mL range) in different clinical sample types has presented clinical analysts with a variety of challenges. In addition, matrix components, such as organic compounds, proteins, or electrolyte salts that may interfere with the analysis of trace elements, are often present at elevated levels (mg/mL or above). The matrix to be analyzed, the amount of sample that can be taken and the means of sampling may also impose restrictions. Sufficient volumes of urine can normally be obtained from patients with noninvasive techniques, whereas the collection of whole blood or serum samples usually involves use of needle and syringe and generally yields smaller sample volumes (often only µL or mL) for analysis. The analysis technique employed should therefore provide the following capabilities: sufficiently low detection limits (DLs), ability to overcome matrix related interferences, sufficient linearity to measure a wide concentration range in unknown samples, simultaneous multi-elemental determinations, and ability to cope with small sample volumes.

Analysis of clinical matrices by inductively coupled plasma mass-spectrometry (ICP-MS) is becoming more widespread since ICP-MS meets a number of the above requirements, namely very low DLs for many trace metals (sub ng/mL), relative freedom from interferences, simultaneous multi-elemental determination, and suitability for small sample volumes, as well as providing isotopic information and the possibility of employing isotope dilution mass spectrometry (IDMS) as a high-caliber reference calibration technique. When analyzed by ICP-MS, many of the elements of interest suffer from mass spectral interferences derived from the sample matrix. Before the development of sufficiently sensitive collision/reaction cell (CRC) quadrupole ICP-MS instruments, matrix-based spectral interferences were overcome by the use of sector field or high-resolution ICP-MS (HR-ICP-MS) [1] or by non-mass spectroscopic techniques such as atomic fluorescence (AF) [2] or atomic

absorption spectroscopy (AAS) [3]. Another way of overcoming matrix-effects is the use of sample digestion using concentrated acids or ashing techniques [4]. These techniques can be expensive, time-consuming, and/or less suitable for high sample throughput.

In our laboratory, magnetic sector HR-ICP-MS (Element 1, ThermoFinnigan) was used [1, 5] for monitoring post- and pre-operation samples from patients with metal-on-metal hip replacements. After 1:20 dilutions of blood and serum samples with approximately 0.7-mM ammonia, 0.01-mM EDTA, and 0.07% (v/v) Triton X-100 or 1:15 dilutions of urine samples with 1% HNO_3, the elements such as Al, V, Co, Cr, Mo, Ni, and Ti were analyzed. The main drawbacks of this technique were cost, practicality, and duration of instrument set-up, as well as instrument down-time and matrix tolerance during analytical runs containing more than ~30 blood or serum samples.

Objectives

The aim of this work was to develop a robust ICP-MS methodology based on CRC quadrupole ICP-MS (CRC-ICP-MS), capable of measuring a wide range of elements in a single analysis after only a simple dilution of the samples.

A simple dilution of the samples was selected as the preferred sample preparation method, as acid digestion techniques can increase the sample turnaround time, cost, and the potential for contamination.

In order to achieve the required sample throughput of up to 100 samples per batch, the quantitation method had to be based on external calibration. Minimal instrument drift was therefore paramount in order to reduce the need for frequent recalibration or drift correction.

The target elements included the trace metals Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, V, and Zn as well as the electrolytes K, Mg, and Na.

The sensitivity achieved needed to match previously obtained DLs using HR-ICP-MS in our laboratory of 0.2 ng/mL (for example, Co, Mo) and 1.0 ng/mL (Ni) in the undiluted samples.

Sample Preparation

All samples, standards, and quality control (QC) materials were diluted 20-fold using a solution containing approx. 0.7-mM ammonia, 0.01-mM EDTA, and 0.07% (v/v) Triton X-100. Butan-1-ol was added to the diluent as a carbon source at 1.5% (v/v) in order to improve matrix matching between standards and samples and thereby increase the accuracy for analytes such as As and Se, whose ionization behaviors in the plasma are affected by the carbon content [6]. In order to keep the chemistry of the sample introduction system stable throughout the run, the diluent was also used for pre- and post-analysis rinse functions. Commonly used rinse acids such as HNO_3, even at dilute levels of 1%, can lead to coagulation or precipitation of clinical matrix components and result in tubing or nebulizer blockages.

The selection of internal standard (IS) elements and the IS concentration is very important. The choice of elements is often restricted in clinical analysis due to the presence of many of the elements that are usually used in environmental applications at ng/mL levels in clinical samples. Blood and serum samples were analyzed in semi-quantitative mode to determine the most suitable IS elements, that is, those which were not present or present at the lowest levels in relative terms. For elements that were present in the samples, such as Sc, the concentration of the IS was added at such a level that the contribution from Sc in the sample to the total ^{45}Sc signal would be negligible. The chosen IS elements (Sc, Ge, Rh, In, and Tl) were added to the diluent at a concentration of 20 ng/mL. Addition of the IS in this way negated possible mixing problems if online addition of the IS via a T-piece was used.

Instrumentation

An Agilent 7500ce Octopole Reaction System (ORS) ICP-MS was used in three different gas modes: hydrogen, helium, and standard or no-gas mode. The ICP-MS conditions and the isotopes, integration times and gas modes for the multi-elemental determination are given in Tables 1 and 2. Quantitation on all isotopes was performed using the three central points of the spectral peaks.

A 100-µL/min PFA microflow nebulizer was used and sample uptake and washout times were reduced using the larger diameter peristaltic pumps of the Integrated Sample Introduction System (ISIS). The pump speed was set at 0.1 rps during the analysis and washout in order to minimize overloading of the sample introduction system and the plasma with matrix components. The torch was equipped with a 2.5-mm diameter injector and the Shield Torch system was used. Nickel (Ni) cones were used at all times.

The total acquisition time per sample was 208 s. This included the sequential loading of the H_2, He, and Std tune files and a 40 s equilibration and stabilization time between the different gas modes. Each sample/standard solution was analyzed sequentially in all gas modes before the autosampler probe moved to the next sample. After each sample, the autosampler probe was rinsed for 5 s using 5% HNO_3 and the sample introduction system was then rinsed using the diluent for 30 s.

Table 1. ICP-MS Parameters Used in the Different Gas Modes

	H_2	He	Std
Rf Power (W)	1500	1500	1500
Carrier gas (L/min)	0.87	0.87	0.87
Make up gas (L/min)	0.17	0.17	0.17
Spray chamber temp (°C)	2	2	2
Gas flow (mL/min)	4	4	Not used

Table 2. Analysis Parameters for the Analytes of Interest

Analyte	Isotope monitored (*m/z*)	Integration time per mass (s)	Internal standard used (*m/z*)	Gas mode used
Na	23	0.3	45	He
Mg	24	0.3	45	He
Al	27	3.0	45	He
K	39	0.3	45	He
V	51	1.5	45	He
Cr	53	3.0	45	He
Mn	55	0.9	45	Std
Fe	56	0.3	45	H_2
Co	59	1.5	45	He
Ni	60	1.5	45	He
Cu	65	0.9	72	He
Zn	66	0.3	72	He
As	75	1.5	72	He
Se	78	1.5	72	H_2
Mo	95	1.5	103	Std
Cd	111	1.5	115	Std
Sb	121	0.9	115	Std
Pb	Sum of 206, 207 and 208	0.9	205	Std

Method Performance and Robustness

The stability of the proposed methodology was tested by running blood and serum samples in a sequence over a 10-hour period (a total of 90 samples, including calibration standards and QC checks) and monitoring the behavior of IS elements, calibration slopes, and check standards.

Instrument Stability - Signal Variation for IS Elements

Typical signal variation for the IS elements of choice (Sc, Ge, Rh, In, Tl) was 4.8%–9.3% in hydrogen mode, 5.5%–8.2% in helium mode, and 6.7%–10.0% in standard mode. This was assessed during a 90-sample sequence of blood and serum samples. Figure 1 shows the variation for the IS elements throughout the 10-hour run. Sc is present in some clinical sample types at ng/mL levels, which can be seen here after sample 8.

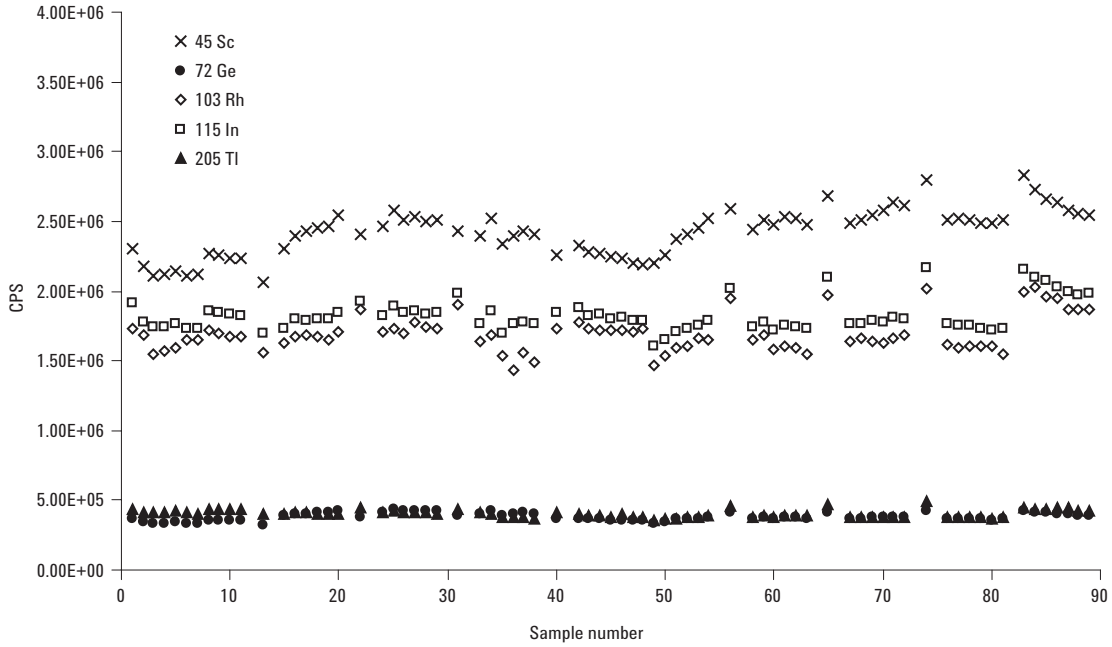


Figure 1. Variation of the IS signals in standard mode throughout the 10-hour run.

Calibration Repeatability and Linearity

Overlaying calibration curves from the beginning, middle, and end of the 10-hour run assessed the robustness of the calibration technique. The correlation coefficients for the mean slope of three calibrations for V, Se, and Pb (Figure 2) during a 10-hour sequence ranged from 0.9997 to 1.0000 and indicate the robustness of the method with these matrices. The calibration coefficients for all elements measured were generally better than r^2 >0.9900.

Check Standards

Check standards at 1 ng/mL level were analyzed throughout the run after every nine samples for the trace metals and were within 10% of the expected value for the elements tested.

Effects of Sample Matrix on the Sample Introduction System

Using dilution factors of 20-fold or less for analysis of these matrices by HR-ICP-MS lead to frequent problems with the sample introduction system, especially blocking of the torch injector. When using quadrupole ICP-MS as described above, dilution factors of 15- and 10-fold could be used without detrimental effects on the sample introduction system (Figure 3) or instrument performance. Reagent blanks were monitored after the analytical run, and no significant deterioration in the DLs or increase in the background levels was observed.

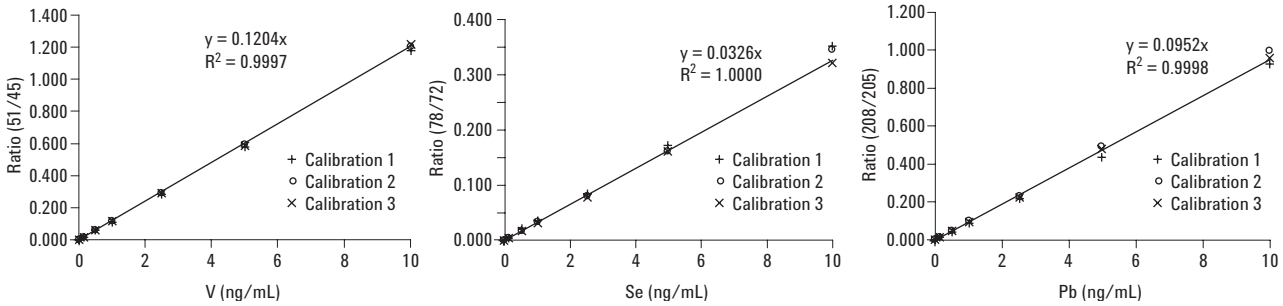


Figure 2. Linearity of overlaid calibration curves for V, Se, and Pb, showing stability of the external calibration approach throughout a 90-sample sequence.

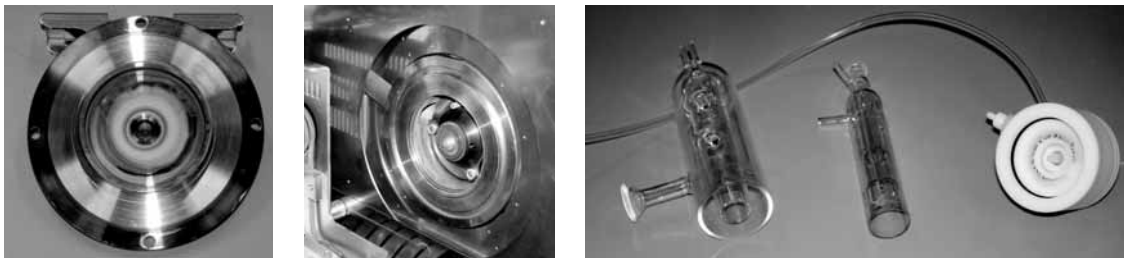


Figure 3. Photos of the interface and sample introduction system after a 90-sample run. Both the sampler and skimmer cones show minor matrix deposits. The 2.5-mm injector torch used was relatively deposit-free. The blood deposits on the spray chamber and the nebulizer block were removed using a sodium hypochlorite solution.

Analysis of Certified Reference Materials (CRMs)

Multiple sub-samples (n=4) of the certified reference material NIST SRM 1598 Bovine serum and the reference material Seronorm MR9067 (whole human blood, level 2) were diluted 20-fold as described above and analyzed using the conditions described in Tables 1 and 2. These materials were chosen because they represented different clinical matrices and contained a wide range of analytes of interest ranging in concentration levels from sub ng/mL to mg/mL. Levels for the same analytes often varied by more than an order of magnitude between the two materials. Certificate data for both materials as well as method DLs (calculated back to the undiluted sample and based on 3 s of the blank concentration) for the method proposed here are shown in Table 3.

The analytical data for both materials were converted to percent recovery data relative to the certified or indicative values and are shown in Figure 4a) and b). The combination of the reference materials chosen for this study provided certified values with uncertainty estimates for all of the elements determined except for Na, where only an indicative value was available. The recovery for Na compared to the indicative value was 99.0%, and the data for the remaining elements measured fell within the uncertainty range for either one or both of the reference materials. Where the certificate values were not achieved (for example, V, Cr, and Cd), the certified concentrations in SRM 1598 were below the DL for the method. Na, As, Ni, and Pb are quoted as indicative values only in SRM 1598 (Table 3.).

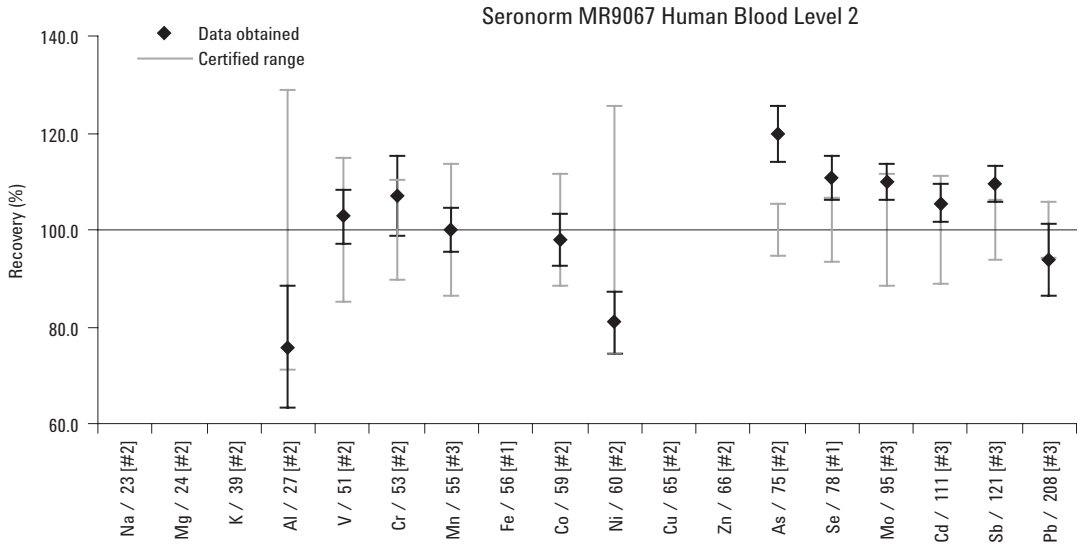
Table 3. Certified Concentrations for the Analytes of Interest in the SRM NIST 1598 and the Reference Material Seronorm MR9067. Method DLs Calculated Back to the Undiluted Sample are Given for Comparative Purposes.

Trace elements	NIST SRM 1598 Bovine serum (ng/g)	Seronorm MR9067 human blood level 2 (ng/mL)	DL (ng/mL)
Al	3.7±0.9	39–71	0.8
As	0.2*	10.6–11.8	0.1
Cd	0.089±0.016	4.8–6.0	0.1
Co	1.24±0.016	4.6–5.8	0.1
Cr	0.14±0.08	5.1–6.3	1.0
Cu	720±40	NA	0.4
Fe	2550±100	NA	19
Mn	3.78±0.32	10.1–13.3	0.1
Mo	11.5±1.1	5.3–6.7	0.1
Ni	0.7*	5.1–8.6	0.2
Pb	0.6*	373–417	0.1
Sb	NA	25–28	0.5
Se	42.4±3.5	114–130	0.2
V	0.06*	3.1–4.2	0.1
Zn	890±60	NA	3.0
Major elements	**(µg/g)**		**(ng/mL)**
K	196±5	NA	100
Mg	20.0±0.4	NA	1.5
Na	3000*	NA	5.0

*Is indicative value only

NA Not applicable

a)



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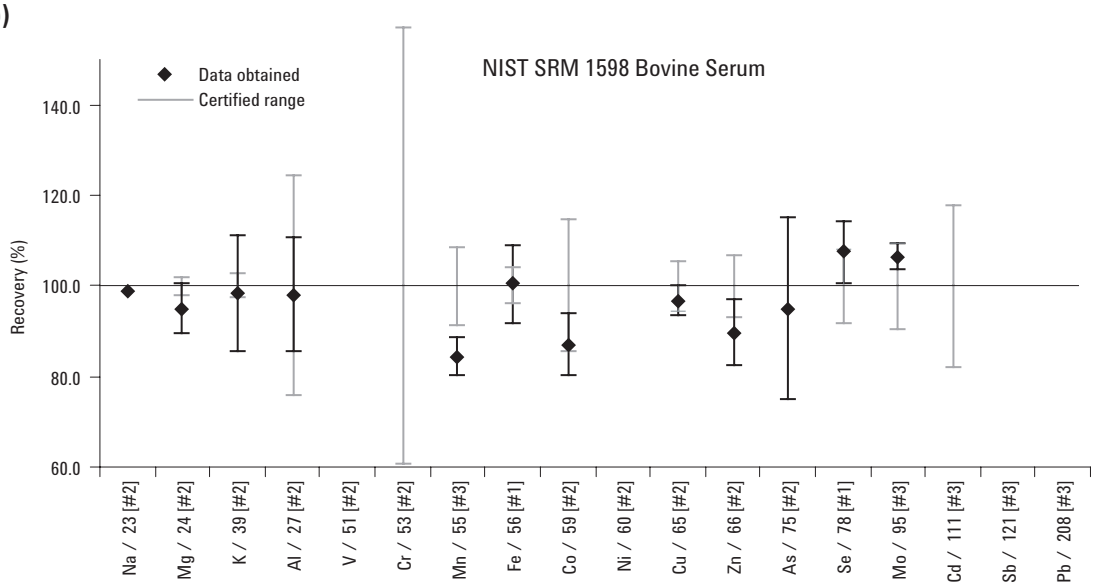

Figure 4. Data obtained expressed relative to the certified data for a) Human blood Seronorm MR9067 and b) Bovine serum SRM NIST 1598. Errors bars represent expanded uncertainties for data obtained and certified ranges for the reference materials. The numbers after the isotopes indicate the tune step used (#1 = H_2, #2 = He, #3 = Std).

Importance of Matrix-Matching and Choice of IS Elements

The data for As and Se in MR 9067 are slightly high compared to the certified mean value, and this could be due to a higher carbon content in this matrix. When increasing the level of butan-1-ol in the diluent from 0% to 3% v/v, recoveries for these analytes decreased and approached 100% (Figure 5). When no butan-1-ol was added to the diluent, recoveries for As and Se were significantly higher than the mean certified values (by 94% and 72% respectively) in comparison to recoveries obtained with butan-1-ol addition at 1.5% (v/v). A complete matrix match was achieved for both samples by using the standard addition technique for As and Se in both reference materials. Recoveries for Se were 95.8% and 99.9% in NIST SRM 1598 and Seronorm MR9067 respectively, and 102.6% for As in Seronorm MR9067. Figure 5 also indicates that the effect of the carbon addition on both elements is slightly different.

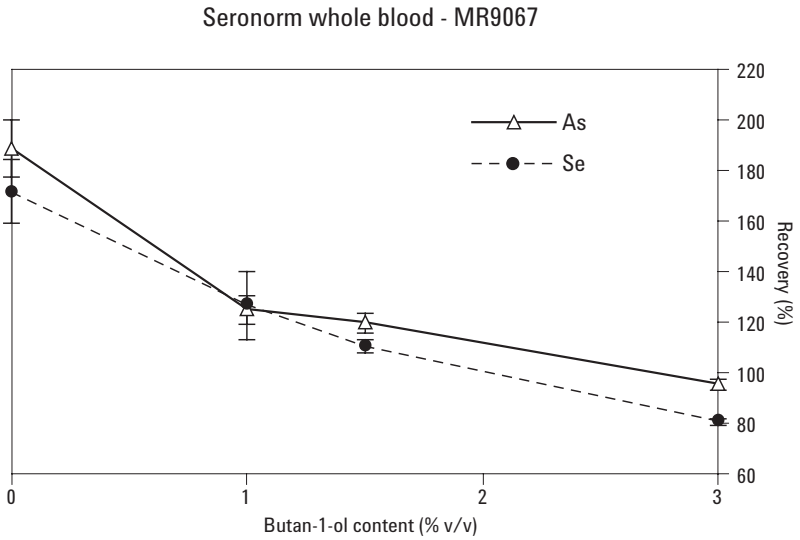

Figure 5. Recovery data for As and Se in Seronorm whole blood (Level 2) with varying levels of butan-1-ol addition to the diluent.

According to the data obtained here, an addition of 3% would be best for As (mean recovery of 95.4 ±2.3%), whereas the ideal volume of butan-1-ol addition for this sample and dilution level for Se is closer to 2%.

For such elements where the ionization is affected by matrix components in the plasma, it is therefore imperative to obtain a good level of matrix matching for the greatest accuracy. If this is not possible, for example if the carbon levels in different samples vary significantly, it may be better to use a different sample preparation procedure such as closed-vessel microwave digestion in order to destroy the organic carbon matrix. However, this can significantly increase the sample turn-around time for large sample batches.

Spike Recovery Data

Spike recovery experiments were performed on both materials for the trace metal analytes at 2–4 different levels with concentrations ranging from 2–5 times of the original analyte concentrations. The only exception to this spiking regime was Fe in MR9067, for which no certificate or indicative value was available before the analysis and where the spike concentration added (20 ng/mL) was not sufficiently high above the determined sample concentration (400 ±5 µg/mL) to give meaningful recovery data. The mean data for all spike levels for the trace metal analytes are shown in Table 4.

Spike recoveries for all elements fell within 100 ±20%, and all except Fe, Se and Mo were within 100 ±10%. The high Se recoveries are thought to be due to the fact that the matrix matching for carbon content consisted of only 1.5% butan-1-ol. High recoveries for Mo were also observed when the samples were analyzed by HR-ICP-MS, and this effect is currently under closer investigation.

Table 4. Mean Spike Recovery Data Obtained for Both Reference Materials

	NIST SRM 1598 bovine serum	Seronorm MR9067 human blood level 2
100% ±5%	Al, V, Cr, Mn, Cu, Zn,Cd, Sb, Pb	Al, V, Cr, Mn, Co,Ni, Cu, Cd
100% ±10%	Co, Ni, As	Zn, As, Sb, Pb
100% ±15%	Fe, Se	Se
100% ±20%	Mo	Mo

Conclusions

A robust CRC-ICP-MS method was developed that is capable of high sample throughput (up to 100 samples per batch) for a large suite of elements in difficult clinical matrices after simple dilution. The method robustness was demonstrated by minimal signal drift during analytical sequences of 10-hour duration, negating the need for frequent recalibration. The method DLs achieved matched those of a previously used HR-ICP-MS method. Further improvements in method DLs can be achieved by reducing the dilution levels of the clinical matrices, which is possible due to the robustness of the sample introduction system. Good agreement within the uncertainty of certificate values was achieved for all of the target analytes in both reference materials where certified data were available across concentration levels ranging from ng/mL–mg/mL level. Spike recoveries for all elements fell within 100 ±20%, and all except Fe, Se, and Mo were within 100 ±10%.

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raimund.wahlen@lgc.co.uk

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