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**Analysis of whole blood through ICP-MS equipped with
a high temperature total sample consumption system**

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Abstract

This work evaluates the performance of a high temperature Torch Integrated Sample Introduction System (hTISIS) in Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the multielemental analysis of whole blood. Two different quadrupole-based spectrometers were tested and the optimization studies were done with a reference whole blood material. Sample dilution factor and hTISIS temperature were taken as variables. The sample was introduced following two diverse procedures: continuous aspiration and air-segmentation. The optimum performance of the system in terms of both analytical figures of merit and accuracy was found for a 200 °C hTISIS temperature and either 1:10 or 1:25 dilution factor, depending on the ICP-MS device used. An improvement in terms of sensitivity and detection limits was obtained with the hTISIS as compared to a conventional Cyclonic spray chamber. Thus, for 1:25 diluted blood samples, hTISIS improved the sensitivity by a 2.0 to 4.5 factor. Meanwhile, the limits of detection for hTISIS at 200 °C were from 1.1 to 8.4 times lower than for Cyclonic spray chamber. This analytical parameter was in the ng L⁻¹ range for the detected elements. Matrix effects in turn became less severe as the hTISIS temperature went up (from room temperature to 80 °C). All these results were obtained without a severe degradation of the plasma fundamental parameters. In fact, under optimum conditions BaO⁺/Ba⁺ and Ba⁺⁺/Ba⁺ ratios were 1.2 % and 2.0 %, respectively. The method developed was applied to the analysis of low volumes (*c.a.*, 2.5 µL) of real blood samples after minimally invasive collection using Volumetric Absorptive Microsampling. The hTISIS is an easy-to-implement sample introduction system that can be employed for routine analysis through ICP-MS. highlighted

Introduction

Biochemical reactions in humans depend on essential elements such as cobalt, copper, magnesium, manganese, selenium, sodium or zinc and are interfered by toxic elements such as arsenic or lead. Deficiencies in essential elements must be treated and toxics must be removed.^{1,2,3} This kind of information can be obtained through the analysis of blood, as blood is the medium for the transport of essential and toxic trace elements.¹

The elemental determination in blood samples has been carried out through techniques such as atomic absorption spectrometry,^{4,5,6,7,8} total reflection X-ray fluorescence (TXRF),^{5,9} or hydride generation atomic fluorescence spectrometry (HG-AFS)¹⁰. However, due to the increasing demand in the number of elements to be determined in blood, alternative analytical methods are needed.

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES),^{5,11,12} and, particularly, Mass Spectrometry (ICP-MS)^{5,13,14,15,16,17} offer multi-elemental measurement capability, low detection limits,¹⁸ wide dynamic range and short analysis time.¹⁶ However, these techniques suffer from spectral as well as non-spectral interferences that may degrade the accuracy of blood analysis.¹⁹

Interferences arising from the atomic and molecular ions generation in the plasma through the combination of argon and matrix constituents hinder ICP-MS quantification.¹⁷ In the particular case of blood analysis the most severe specific spectral interferences are ascribed to the presence of chloride, sodium, sulfur or carbon.^{17,19,20,21,22,23} These interferences can be minimized or even removed by using a reaction and/or collision cell.^{14,17,18,24,25}

Non-spectral interferences can also be found when carrying out the analysis of blood samples. Therefore, high concentrations of inorganic and organic solutes may cause changes in the aerosol generation, transport and/or the properties of the plasma. Furthermore, dissolved salts may lead to a partial blocking of the injector torch or the ICP-MS cones.¹⁰ In order to overcome some of these problems, internal standardization or standard addition are classically employed.¹⁵

Matrix effects can also be minimized by lowering the sample consumption rate, on the one hand, and using a total sample consumption system, on the other. In this way, the matrix plasma load decreases and the interferences associated to the sample introduction system may be removed. The latter assessment is based on the fact that, with such system, the analyte transport efficiency is close to 100% regardless the sample nature.^{26,27} The so called Torch Integrated Sample Introduction System working at high temperatures (hTISIS) consists of a micronebulizer associated to a low inner volume single pass spray chamber.^{26,28,29} The main advantages of the hTISIS over a conventional spray chamber are: as stated before (i), analyte transport efficiencies are virtually 100%; as a result, (ii) very low sample volumes (c.a., 2-5 μL) are required to perform the analysis; thus, (iii), the solvent plasma load is also low; (iv) memory effects are practically negligible; and, (v) virtually no waste is generated.³⁰ Besides, the extent of the matrix effects caused by both organic and inorganic concomitants is less pronounced as compared to a conventional Cyclonic spray chamber.²⁸ Up to now, hTISIS has been applied to accurate analysis of a few environmental, biological and clinical reference materials.^{27,31,32}

The goal of the present work was thus to test the suitability of the hTISIS operated at high temperature as a system for the analysis of sample of very complex nature but of great importance, such as blood, through ICP-MS. Relevant parameters such as the hTISIS chamber temperature and sample dilution were optimized for blood reference samples. The analytical figures of merit were compared against those obtained by using a conventional sample introduction system.

2. Experimental

2.1. Chemicals and samples

Ultra-pure water prepared from a Mili-Q system (Merck, Darmstadt, Germany) was employed in all experiments. All the standards and samples were prepared in 1% nitric acid (HNO₃ 65% p.a EMSURE ISO, Merck).

Single element stock solutions (Certipur, Merck) containing 1,000 mg element L⁻¹ were used to prepare the standards (10 µg L⁻¹ and 1 µg L⁻¹). These standards also contained 10 µg L⁻¹ Ba. Furthermore, Rh at 25 µg L⁻¹ was employed as internal standard.

The reference materials analyzed were Seronorm Trace Elements Whole Blood L-2 and Seronorm Trace Elements Whole Blood L-3 (Sero, Billingstad, Norway; Reference 210205 and 210305, respectively). These lyophilized human whole blood materials were reconstituted with 5 mL Milli-Q water, according to the instructions reported in the certificate. Then, aliquots of the L-2 solution were 1:10, 1:25, 1:50 and

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3 1:100 diluted with a (1%) $\text{HNO}_3\text{:H}_2\text{O}$ solution and L-3 was 1:10 diluted. Real samples
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5 were taken from three different adult individuals.
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9 The sampling procedure was carried out through the so-called Volumetric
10 Absorptive Micro-Sampling (VAMS) method. A puncture with a safety lancet was
11 performed on the finger of several individuals and a small volume (10.5 μL) was
12 retained on the absorptive tip of the VAMS micro-sampler (Mitra^{TM}, Neoteryx LLC,
13 Torrance, USA). Afterwards, the samples were dried under ambient conditions for 4
14 hours. The following step was to extract the absorbed samples with a 100 μL 1% HNO_3
15 solution using an ultrasonic bath (generator power: 50W, frequency: 50Hz) for 1 hour
16 to promote the analytes extraction. Finally, the analysis of the resulting solutions was
17 performed by means of external calibration with a set of plain water standards.
18 Anonymous samples were provided by the Hospital Universitario Miguel Servet,
19 Zaragoza. The principles outlined in the Declaration of Helsinki for all human or animal
20 experimental investigations have been followed. In addition, informed consent has
21 been obtained from the participants involved.
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43 2.2 Instrumentation

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45 A NexION 300X ICP-MS spectrometer (Perkin-Elmer, Waltham, USA) was used. This
46 instrument is equipped with a quadrupole cell that can be used either as a collision
47 cell, in combination with kinetic energy discrimination, or as a dynamic reaction cell.
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49 The system contains a triple cone interface, with an additional hyper skimmer cone,
50 providing a more gradual pressure reduction within the interface, which results in less
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dispersion of the ion beam. A quadrupole ion deflector deviates the ion beam over a 90-degree angle, focusing it into the cell. Table 1 summarizes the ICP-MS operating parameters. A pneumatic concentric nebulizer (Type C, Meinhard Glass Products, Santa Ana, USA) was fitted to the hTISIS by means of a Teflon adapter whereas a Cyclonic spray chamber (Quartz chamber, 50 cm³ inner volume, Perkin-Elmer) was used as the reference chamber for comparative studies.

Additionally, an Agilent 7700x ICP-MS (Agilent, Santa Clara, USA) was also used. The instrument is equipped with a High Matrix Introduction (HMI) accessory that increases the tolerance to salty matrices as compared to conventional ICP-MS instruments. The sample introduction system used with this instrument was the hTISIS and a High Efficiency Nebulizer (HEN, Meinhard Glass Products).

The sample was supplied to the nebulizer both in continuous and discrete modes by means of the spectrometer peristaltic pump using 0.19 mm i.d. flared end PVC-based tubing (Glass Expansion, Melbourne, Australia). In the case of the segmented-flow injection mode, air was continuously aspirated and, at a given time, 2.5-10 µL of either samples or standards were injected by adapting the nozzle to the flared end tubing. The solution was thus driven towards the nebulizer thus avoiding sample dispersion, as the carrier stream was simply air.

3. Results and discussion

When working under air segmentation, a transient signal is obtained. Due to the fast data acquisition capability of modern ICP-MS devices, (a 20 ms dwell time was used),

the transient signal contained a plateau. Under these circumstances, the dispersion of the sample plug was negligible. Obviously, this was due to the absence of liquid carrier stream, which reduced the extent of sample re-nebulization from the chamber walls and the sample dispersion in liquid phase.

3.1. Effect of hTISIS temperature on the sensitivity and comparison with the Cyclonic spray chamber

As expected, the higher the chamber temperature, the greater the ICP-MS sensitivity. This trend has been previously observed when working with aqueous and organic standards in ICP techniques and can be assigned to an increase in the analyte transport rate.²⁶ However, a comment should be made regarding ICP-MS because this technique is more sensitive to operational changes than ICP-OES, and somewhat different trends have been observed with different ICP-MS spectrometers. In fact, under a continuous flow aspiration regime and for a spectrometer equipped with a dynamic reaction cell, the sensitivity for plain water solutions peaked at hTISIS temperatures going from 135 to 180 °C.²⁷ Meanwhile, for a multicollector ICP-MS system, the ionic signal was constant from 55 to about 75 °C and then decreased.³³ For organic solutions, under air segmented mode, it was found that the peak height increased with temperature up to 110 °C. Above this value, a drop in sensitivity was observed.³⁴

With the NexION 300X instrument used in the present work, the effect of the hTISIS temperature was tested under continuous aspiration for four different reference whole blood dilution factors (1:100, 1:50, 1:25 and 1:10). It was found that

the most diluted samples provided a steep increase in signal with temperature. However, in the case of the 1:10 diluted blood samples, a maximum in sensitivity was achieved at 80 °C. This was likely due to the degradation of the nebulizer performance and the ion transfer efficiency to the mass spectrometer. In fact, at 200 °C a solid deposit was found at the nebulizer tip and the sampler cone.

The hTISIS is particularly indicated to handle low liquid sample volumes. By working under the segmented-flow injection mode, solid deposits observed at the interface were less severe than in continuous mode. Figure 1 shows the effect of the temperature on sensitivity under segmented-flow injection. Peak areas obtained for hTISIS were divided by those encountered for the conventional Cyclonic spray chamber. The improvement factor, in terms of sensitivity, incorporated by the former device increased with temperature, but depended on both the analyte atomic mass and the sample dilution factor. Thus, for instance, the relative area was higher for light (^{59}Co , ^{63}Cu , ^{66}Zn) than for heavy (^{110}Cd , ^{208}Pb) isotopes. This might suggest that the mass discrimination phenomena for light elements over heavy ones were less marked at high chamber temperatures than at room temperature. Note that, for a conventional sample introduction setup, the presence of salty matrices induces space charge effects that decrease the sampling efficiency of light ions.³⁵ Meanwhile, heavy ions remain preferentially in the center of the ion beam and are more efficiently transferred to the mass spectrometer through the interface. The higher peak area enhancement factor found for light elements when increasing the temperature suggests that they became efficiently focused under these operating conditions.

It was interesting to notice that, as expected, the dilution factor played an extremely important role in terms of hTISIS performance over the reference chamber. Figure 2 plots the normalized area with respect to the Cyclonic spray chamber for five different nuclides and the four evaluated dilution factors. In general terms it appeared that, despite the low injected sample volume (*c.a.*, 5 μL), the normalized area was the lowest for the 1:10 dilutions. This effect was much more marked when the hTISIS was set at 200 °C than when it was employed at room temperature. Obviously, the introduction of highly concentrated samples resulted detrimental from the point of view of hTISIS-ICP-MS signal production because of the above mentioned formation and growth of solid deposits at the interface, thus giving rise to a decrease in the ion transmission efficiency.

Limits of detection in blood reference samples (Table 2) were calculated according to the $3s_b$ criterion (where s_b was the standard deviation of a series of five consecutive blank measurements). In the present situation, sensitivity was taken as the peak area divided by the analytical concentration for a reference material. It was observed that the hTISIS provided generally lower LODs than the Cyclonic spray chamber when the diluted reference blood sample was analyzed. Table 2 also shows that the 7700x ICP-MS instrument provided similar LODs to those calculated for the Nexlon 300X system. Note, that the injected sample volume was two times lower for the former spectrometer. Consequently, the absolute LODs would be lower when working with the 7700x system. These results could be assigned to the fact that, as mentioned in the experimental section, a HEN was employed in this case, whereas the studies with the NexION 300X spectrometer were done with a C-type pneumatic

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concentric nebulizer. As the HEN is a more efficient nebulizer, it was able to provide similar LODs as the C-type with a lower injected sample volume. In the case of ^{66}Zn and the 7700x system it was observed that high LODs were obtained. It was verified that the background signal for this isotope was actually high. This isotope suffers from polyatomic spectral interferences caused by the presence of sulfur and calcium as concomitants (*i.e.*, $^{34}\text{S}^{16}\text{O}_2$, $^{32}\text{S}^{34}\text{S}$, $^{33}\text{S}_2$, $^{48}\text{Ca}^{18}\text{O}$).³⁶ The same reason can be argued to explain why a decrease in the dilution factor, two last columns in Table 2, improved the limits of detection neither for ^{66}Zn nor for ^{63}Cu (likely interfered by $^{40}\text{Ar}^{23}\text{Na}^+$). Apparently, the collision cell operating conditions had to be further optimized to accurately determine this element with the mentioned spectrometer. As this was not the goal of the present work, all the analyses were performed under the conditions summarized in Table 1.

Interestingly, it was verified that an increase in the sample concentration yielded increased LODs (see the two last columns in Table 2). In conclusion, when the Nexlon 300X ICP-MS was used, a 1:25 dilution was advisable to obtain the lowest LODs whereas the 1:10 was the dilution factor recommended when working with the 7700x system.

3.2. Comparison between continuous and air segmented-flow modes

When considering the benefits of the hTISIS with respect to the Cyclonic spray chamber, it was verified that the former improved the analytical figures of merit in both aspiration modes. As Figure 3 reveals, the signal enhancement factor was higher

when the sample was introduced according to the segmented mode. These results revealed that the high temperature single pass spray chamber was a more appropriate device for the analysis of low sample volumes than a Cyclonic design, especially with segmented-flow injection mode. This was especially interesting for the analysis of blood samples in situations for which the available volume was low and/or when minimally-invasive home-based collection protocols are implemented.³⁷

3.3. Effect of the sample introduction system on the signal stability

Table 3 summarizes the RSD values for all elements analyzed in continuous as well as in segmented-flow injection mode. In the first case, the relative standard deviation was calculated from five consecutive independent signal measurements whereas for segmented injection the RSDs were obtained from the peak areas of five replicates. By comparing both datasets, it may be observed that, as expected, the RSD values were lower when the sample was continuously aspirated than when working under discrete sample aspiration mode. This trend could be due to small changes in the injected sample volume into the air carrier stream. From Table 3 it is also obvious that, particularly in discrete mode, the RSDs for the hTISIS were of the same order as those for the Cyclonic spray chamber. An ANOVA analysis was applied and the results revealed that statistically significant differences existed under continuous mode, for the two sample introduction systems ($P = 0.0033$, $\alpha=0.05$) although both data sets were acceptable. Regarding the discrete mode, the use of the hTISIS did not degrade the signal stability with respect to a conventional sample introduction system.

3.4. Matrix effects

ICP-MS matrix effects may be caused by the sample introduction system as well as by the plasma and the interface. In the former case, the mass of analyte leaving the spray chamber varies as a function of the matrix considered. In the latter situation, the ion transmission is affected by the matrix. Space-charge effects in turn can be considered as processes occurring in and just after the interface. According to these effects, heavy elements are less affected by the presence of easily ionized elements than light isotopes.^{38,39,40,41,42} The NexION 300X ICP-MS Instrument used in this work displays a three-aperture interface to cope with the space charge effects. With this interface the pressure is gradually reduced in steps smaller than for other instruments equipped with only two apertures (sampler and skimmer). This fact limits the ion beam divergence and prevents the deposition of the sample on the spectrometer inner surfaces by reducing the extent of space charge effects.^{43,44}

In order to evaluate the magnitude of matrix effects, the signal found for the 1:25 diluted sample was divided by that measured for the 1:10 dilution one. These were the two most concentrated samples, thus the interferences were expected to be more severe than for the remaining cases. The effect of the hTISIS temperature is considered in Figure 4 for both continuous (Figure 4.a) and air-segmented (Figure 4.b) modes. In absence of interferences the ratios obtained should be equal to the ratio between dilution factors, irrespectively of the element and matrix considered. Taking into account the dilutions considered and by assuming a 10% deviation as acceptable, the target value was set at 0.40 ± 0.04 . In order to mitigate solid deposit formation at

the ICP-MS interface, the injected volume was 5 μL . When the sample was continuously delivered to the nebulizer at room temperature (Figure 4.a), the ratios were generally very close to 0.4. Furthermore, the increase in the chamber temperature up to 60-80 $^{\circ}\text{C}$ also led to acceptable results. Regarding the results found in air-segmented injection mode (Figure 4.b) matrix effects were observed (*i.e.*, average ratios higher than 0.4) when the hTISIS was operated at room temperature. However, these effects became less severe as the hTISIS temperature went up. This observation was in agreement with the fact that the aerosol transport interferences were mitigated when working at 80 $^{\circ}\text{C}$.

3.5. Oxides and doubly charged ions

When the hTISIS temperature was increased, the amount of solvent delivered to the plasma went up. As a result, the oxide production in the plasma also increased (Figure 5). When considering room temperature conditions, it was noticed that the increase factor in terms of oxide ratios was similar to that observed for the analytical signals. Thus, for instance, in segmented-flow injection mode the oxide ratios increased by a factor of 1.4 as the TISIS chamber was used at room temperature with respect to the Cyclonic chamber. Meanwhile, ^{63}Cu and ^{110}Cd signals increased by 1.3 and 1.1 respectively. Therefore, under these conditions the increase in oxide ratio was due to an enhancement in the amount of solution reaching the plasma. However, at 200 $^{\circ}\text{C}$ the BaO^+/Ba^+ ratio was nine times as high as that encountered at room temperature whereas there was a 4.2 and 3.0-fold ionic intensity improvement for ^{63}Cu and ^{110}Cd , respectively. These discrepancies at high temperature could be partially attributed to

the slight decrease in the doubly charged ion ratios as the TISIS temperature went up. Surprisingly, the double charged ions fraction only decreased by about a 30%, which could be assigned to the low sample consumption rates chosen.

3.6. Analysis of blood reference material (RM)

According to the data previously obtained, the best conditions in terms of sensitivity corresponded to the TISIS operated at 200 °C. In addition, a 1:25 dilution factor represented a good compromise in terms of sensitivity and matrix effects. Higher sample contents caused the formation of solid deposits on both the inner chamber walls and the spectrometer interface. Meanwhile, higher sample dilution factors resulted detrimental from the point of view of sensitivity. A blood reference material was analyzed using such dilution factor.

Table 4 summarizes the concentrations found for the Cyclonic spray chamber and the hTISIS in both continuous and discrete modes. In order to quantify these elements, a series of plain water standards were employed. By taking into account the confidence intervals, it could be observed that in the case of the Cyclonic spray chamber, only copper and lead provided acceptable results in continuous mode, whereas in segmented-flow injection mode, acceptable results were obtained for cobalt, copper and zinc. In the case of the hTISIS operated in continuous sample aspiration, it was noticed that concentrations for cadmium, cobalt and zinc were different with respect to the reference values. The appearance of matrix effects and/or the too high LODs could be the main reasons to justify eventual deviations from the

reference values. Finally, when the hTISIS was operated in segmented-flow injection mode at 200 °C, concentrations similar to the reference ones were obtained for the five elements studied.

3.7. Analysis of real samples

Nowadays a current trend in analysis of biological fluids, such as blood, by means of clinical laboratories are the so-called home-based collection protocols, giving rise to the so called dried blood spots.^{37, 45} The involved procedures, which are minimally invasive, are especially interesting for screening of newborns, for controlling people with limited mobility or living in areas with restricted access to Hospitals/clinical labs. Once the blood samples are taken (a few droplets deposited on a clinical filter paper or a similar device) and dried, they can be sent by ordinary mail to the laboratory, as they are stable and easy to transport and store.

Among the different devices used for such protocols, Volumetric Absorptive Microsampling (VAMS) is a new one that is particularly promising, as it permits to collect a constant and known amount of blood in a very simple way.^{46,47} Furthermore, the collected sample volume is independent of the hematocrit level.⁴⁸ It has been very recently demonstrated that, when aiming at multi-elemental analysis, this methodology allows the recovery of the sample in a simple way by applying a single extraction step with 1% HNO₃ without any further manipulation.⁴⁹ The use of a conventional spray chamber and nebulizer, however, requires a 100-fold dilution to be able to obtain sufficient sample for analysis. Using the hTISIS, in principle, a much

lower value could be used, with the subsequent improvement in the LODs, even for labs for which an ICP-MS/MS device (used in ref. 46) is not available.

As mentioned before, the hTISIS was also coupled to a spectrometer containing an aerosol phase dilution accessory (*i.e.*, High Matrix Introduction, HMI, system). Unlike for the Nexlon, with the 7700x spectrometer it was possible to perform the quantification of the 1:10 whole blood diluted samples by lowering the injected sample volume down to 2.5 μL . This was also possible because in this case, a micronebulizer (HEN) having a narrow capillary (80–100 μm id) and, hence, a low dead volume was employed. The studies with this device were extended to 11 elements. Again, aqueous standards were employed for calibration. It could be positively concluded that, for all the evaluated elements the obtained concentrations were in agreement with the reference ones (Table 5).

According to data in Table 5, it was observed that the method developed could be applied to the analysis of blood samples by using a 1:10 dilution factor. Furthermore, quantitative data corresponding to 11 elements were acquired with a single 2.5 μL injection volume. This made the hTISIS especially suitable for the analysis of blood microsamples and fully compatible with home-based collection protocols.

The developed method was finally applied to the analysis of three real blood samples (Table 6). The elemental quantification was performed in two different ways: (i) using aqueous standards; and, (ii) single standard calibration where the reference sample acted as a matrix matched standard. The results revealed an acceptable

concordance between the concentrations provided by both methods for ^{51}V , ^{75}As , ^{78}Se , ^{121}Sb and ^{205}Tl . Only for copper results differed significantly depending on the calibration method employed. This could be due to the differences between the matrices of a real blood sample and the reference one. In a different experiment, real samples were spiked and the recoveries were obtained by applying external calibration with plain water standards. According to the results, this parameter ranged from 96% (^{55}Mn) to 109% (^{121}Sb) range thus giving an additional proof of the accuracy of the employed procedure.

Finally, by comparing the elemental concentrations determined in the blood real samples with those of the reference one (i.e., containing pathological levels of elements), it was concluded that all three individuals contained metal levels below the pathological ones.

As a final step, potential contaminations from the VAMS tip were evaluated. In this case, the hydrophilic polyolefin tips were washed with 100 ml of the nitric acid solution and the elemental concentrations in the resulting extracts were determined through ICP-MS. The corresponding results are shown in Table 7. As previously stated in ref. 46 elements such as vanadium, chromium and aluminum were present in the solid sorbent. According to the data obtained in the present work, although significant concentrations of seven elements were detected in the obtained solutions, it was possible to quantify all of them in the samples with an acceptable accuracy (see Table 5).

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4. Conclusions

The present work has demonstrated the applicability of the hTISIS to the analysis of low volumes of whole blood under two different working modes: continuous and air-segmented. The results reveal that, in discrete (2.5 – 10 µL) sample introduction mode, the hTISIS improves the performance over a conventional sample introduction system in terms of sensitivity and detection limits. Besides, the use of the hTISIS do not degrade the signal stability and matrix effects are mitigated thus making it possible to perform calibration with a set of plain water standards. Moreover, the methodology requires minimal sample preparation involving only the sample evaporation, extraction and dilution. Although a single sample preparation requires more than 1 hour, many assays could be easily done in parallel, hence increasing the actual sample throughput. Regarding the quantification of whole blood reference material, the best results are achieved when working under the segmented-flow injection mode. By using such a total sample consumption system, it is possible to perform microsample analysis following a home-based collection protocol.

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Table 1 ICP-MS operating conditions.

	Perkin-Elmer NexION 300X	Agilent 7700x
Chamber temperature	RT-200 °C / RT	200 °C
Nebulizer gas flow rate	1.1 L min ⁻¹	0.5 L min ⁻¹ /0.56 L min ⁻¹ HMI
Sample uptake rate	33 µL min ⁻¹	
RF power	1600 W	1600 W
Plasma gas flow rate	18 L min ⁻¹	15 L min ⁻¹
Auxiliary gas flow rate	1.2 L min ⁻¹	0.90 L min ⁻¹
Cell gas flow rate (He)	0.8 mL min ⁻¹	4.3 mL min ⁻¹

Table 2 Limits of detection (ng L^{-1}) obtained for the different elements in the reference material (Seronorm Trace Elements Whole Blood L-2) under segmented injection mode obtained for the Cyclonic chamber and the hTISIS with the two ICP-MS spectrometers.

	Cyclonic	hTISIS 200 °C	hTISIS 200 °C	hTISIS 200 °C
	segmented *	segmented *	segmented #	segmented @
^{59}Co	59	7	6	29
^{63}Cu	699	617	748	945
^{66}Zn	4357	3300	19000	21000
^{111}Cd	29	6	4	15
^{208}Pb	113	17	102	136

* 1:25 diluted sample (NexION 300X ICP-MS Instrument). Injected Volume: 10 μL

1:25 diluted sample and the (7700x ICP-MS Instrument). Injected Volume: 5 μL .

@ 1:10 diluted sample (7700x ICP-MS Instrument). Injected Volume: 5 μL .

Table 3 Signal stability for the two sample introduction systems tested (NexION 300X ICP-MS Instrument).*

RSD (%)	Cy		TISIS	
	Segmented-flow		Segmented-flow	
	Continuous	injection	Continuous	injection
⁵⁹ Co	4.2	16.2	6.4	5.0
⁶³ Cu	0.5	5.2	4.2	5.2
⁶⁶ Zn	0.6	10.1	2.7	8.0
¹¹¹ Cd	1.5	9.0	3.5	8.9
²⁰⁸ Pb	1.2	7.5	4.0	4.9

* 1:25 diluted blood sample; hTISIS temperature: 200 °C; sample injected volume in air-segmented injection mode: 10 µL.

Table 4 Elemental contents ($\mu\text{g L}^{-1}$) found for whole blood in continuous aspiration and air-segmented-flow injection modes.*

Element	Reference values	Cyclonic Continuous	hTISIS continuous 200 °C	Cyclonic segmented	hTISIS segmented 200 °C
Co	5.8 ± 1.2	8.0 ± 0.7	1.9 ± 0.7	5.3 ± 0.9	5.5 ± 1.2
Cu	1330 ± 270	1286 ± 8	1695 ± 115	1375 ± 74	1160 ± 82
Zn	6500 ± 300	8775 ± 63	9085 ± 392	6137 ± 831	6509 ± 863
Cd	5.8 ± 0.2	1.3 ± 0.4	8.8 ± 0.5	12 ± 3	7 ± 2
Pb	310 ± 62	366 ± 3	324 ± 23	443 ± 19	376 ± 30

* NexION 300X; 1:25 sample dilution factor; confidence intervals were calculated as $\pm$

$(t s)/(N)^{1/2}$ being $N = 5$.

Table 5 Elemental contents (in $\mu\text{g L}^{-1}$) and recoveries found for the 1:10 whole blood diluted reference sample. Injected Volume: 2.5 μL .

Isotope	Seronorm L-3. Reference values.	Seronorm L- 3. This work.	Recovery (%)
^9Be	12.5 ± 2.5	13.6 ± 2.2	109
^{51}V	5.7 ± 1.1	5.9 ± 0.6	104
^{53}Cr	23.2 ± 4.7	31.0 ± 4.0	134
^{55}Mn	47.3 ± 9.5	40.0 ± 7.0	86
^{63}Cu	2470 ± 250	2890 ± 170	117
^{65}Cu	2470 ± 250	2930 ± 220	119
^{75}As	30.4 ± 7.3	28.9 ± 2.2	95
^{78}Se	272 ± 55	265 ± 28	97
^{111}Cd	12.1 ± 1.3	12.2 ± 2.5	100
^{121}Sb	22.4 ± 4.5	24.3 ± 1.0	109
^{205}Tl	34.1 ± 6.9	36.1 ± 1.8	106

²⁰⁶ Pb	447 ± 46	452 ± 17	101
²⁰⁸ Pb	447 ± 46	410 ± 17	92

Table 6 Elemental contents (in µg L⁻¹) found for three real samples.*

Isotope	Real Sample 1		Real Sample 2		Real Sample 3	
Calibration standards	reference material	Aqueous	reference material	Aqueous	reference material	Aqueous
⁹ Be	< 2	< 2	< 2	< 2	< 2	< 2
⁵¹ V	1.5 ± 0.4	2.1 ± 0.5	1.5 ± 0.5	2.4 ± 0.8	1.6 ± 0.6	2.5 ± 0.8
⁵³ Cr	< 5	< 5	< 5	< 5	< 5	< 5
⁵⁵ Mn	< 31	< 31	< 31	< 31	< 31	< 31
⁶³ Cu	1522 ± 93	2029 ± 116	1242 ± 54	1863 ± 150	713 ± 80	865 ± 134
⁶⁵ Cu	1552 ± 95	2028 ± 112	1294 ± 94	1905 ± 198	714 ± 83	973 ± 163
⁷⁵ As	2.3 ± 0.3	3.1 ± 0.3	8.4 ± 0.5	12.2 ± 1.2	3.1 ± 0.7	4.3 ± 0.8
⁷⁸ Se	145 ± 20	195 ± 22	124 ± 18	188 ± 29	119 ± 22	155 ± 23

¹¹¹ Cd	< 2	< 2	< 2	< 2	< 2	< 2
¹²¹ Sb	1.3 ± 0.3	1.7 ± 0.4	2.2 ± 0.3	3.1 ± 0.4	1.7 ± 0.5	2.1 ± 0.5
²⁰⁵ Tl	18.0 ±	23.5 ±	23.5 ±	33.1 ±	27.3 ±	35.2 ±
	3.8	4.3	3.5	5.8	4.2	4.1
²⁰⁶ Pb	< 36	< 36	< 36	< 36	< 36	< 36
²⁰⁸ Pb	< 26	< 26	< 26	< 26	< 26	< 26

* Injected Volume: 2.5 µL.

Table 7 Elemental contents ($\mu\text{g L}^{-1}$) found in the VAMS tips.*

Isotope	Concentration
^{51}V	5.9 ± 0.7
^{53}Cr	24.4 ± 1.6
^{55}Mn	89 ± 18
^{63}Cu	714 ± 349
^{65}Cu	759 ± 359
^{111}Cd	3.4
^{205}Tl	7.4
^{206}Pb	100 ± 12
^{208}Pb	98 ± 9

* Concentration after taking into account the 10-fold dilution. Confidence intervals were calculated as $\pm (t s)/(N)^{1/2}$ being $N = 5$

Fig. 1 Effect of the hTISIS temperature on the sensitivity (using peak area values) normalized with respect to the Cyclonic spray chamber. A dilution factor of 1:50 and air-segmented injection mode (Injected Volume: 5 μ L) were used. Experiments were carried out with a NexION 300X ICP-MS... Error bars were calculated according to error propagation (n=5).

Fig. 2 Effect of the dilution factor on the sensitivity (using peak area values) normalized with respect to the Cyclonic spray chamber at (a) room temperature and (b) 200 $^{\circ}$ C hTISIS temperature. The air-segmented (5 μ L) injection mode was used Experiments were carried out with a NexION 300X ICP-MS

Fig. 3 Sensitivities obtained for the hTISIS referred to those encountered for the Cyclonic spray chamber in continuous and air segmented-flow regimes. Blood samples were 1:25 diluted with deionized water. The hTISIS temperature was set at 200 $^{\circ}$ C. The black bars represent the continuous mode, while the white bars thesegmented (10 μ L) flow injection mode. A NexION 300X ICP-MS was used for this experiment. Error bars were calculated according to error propagation (n=5).

Fig. 4 Intensity ratios obtained for two different sample dilutions (1:25 and 1:10) under different operating conditions in (a) continuous injection and (b) 5 μ L air-segmented injection. A NexION 300X ICP-MS was used for this experiment. Error bars were calculated according to error propagation (n=5).

Fig. 5 Variation of ^{63}Cu , ^{110}Cd (values for Cd have been multiplied by a factor of 1000) signal intensities, and barium oxide and doubly charged ions ratios, with the TISIS temperature in air-segmented mode (Injected Volume: 10 μL). A NexION 300X ICP-MS was used for this experiment. Error bars were calculated according to the standard deviation obtained from the absolute standard deviation (n=5).

Figure 1

Figure 2

(a)

(b)

Figure 3

Figure 4

(a)

(b)

Figure 5