Determination of ultra-trace amounts of prosthesis-related metals in whole blood using volumetric absorptive micro-sampling and tandem ICP – mass spectrometry

Eduardo Bolea-Fernandez[a], Kim Phan[a], Lieve Balcaen[a], Martín Resano[b] and Frank Vanhaecke[a,*]

[a]Ghent University, Department of Analytical Chemistry, Campus Sterre, Krijgslaan 281-S12, 9000 Ghent, Belgium

[b]University of Zaragoza, Aragón Institute of Engineering Research (I3A), Department of Analytical Chemistry, Pedro Cerbuna 12, 50009 Zaragoza, Spain

[*]Corresponding author: Frank.Vanhaecke@UGent.be – Tel:+32(0)92644848 – Fax: +32(0)92644960

ABSTRACT

This paper reports on an evaluation of the suitability of a novel sample collection approach, volumetric absorptive micro-sampling (VAMS), in the context of the determination of ultra-trace concentrations of prosthesis-related metals (Al, Ti, V, Co, Cr, Ni, Sr and Zr) in whole blood. In a first phase, a simple dilute-and-shoot approach (100-fold dilution) followed by tandem ICP – mass spectrometry (ICP-MS/MS) analysis was developed for the accurate and sensitive determination of the target elements. The ICP-MS/MS method relies on the use of mass shift reactions proceeding when pressurizing the collision/reaction cell (CRC) with CH_3F/He for dealing with spectral overlap. Limits of detection (LoDs) between 0.3 and 30 ng L^{-1} were attained in a multi-element approach. The accuracy of the method was demonstrated *via* successful analysis of the reference materials Seronorm Whole Blood Levels 1 and 3, and real venous blood samples, spiked with the target elements at different concentration levels (5 – 50 µg L^{-1}). Although the implementation of VAMS devices introduced contamination problems for Al, Cr and Ni, VAMS followed by ICP-MS/MS analysis shows potential for future real-life routine applications when assessing levels of Ti, V, Co, Sr and/or Zr.

Keywords: Prosthesis, implant, VAMS, whole blood, ICP-MS/MS, spectral interference, CH_3F

1. INTRODUCTION

The most common and well-known method of sampling blood is venipuncture. However, if regular monitoring of a patient's blood is required or only limited amounts of blood can be taken, more straightforward ways of blood sampling would be helpful. The use of "dry sampling" approaches, such as the already widely used dried blood spot (DBS) method, and the more recently introduced volumetric absorptive micro-sampling (VAMS), are considered potential alternatives to overcome the problems related with the traditional venipuncture approach.[1, 2] These sampling methods offer inherent advantages, such as (i) minimally invasive collection of capillary blood *via* a finger or heel prick, (ii) improved sample conservation under ambient conditions due to the stabilizing effect of DBS and VAMS, and (iii) easier transport of the samples to the laboratory.[3] In a very short period of time, these benefits have drawn the attention of both the pharmaceutical and medical communities. The low amount of sample required in comparison with the venipuncture approach could be advantageous in animal studies during the early stage of drug discovery, and the simplicity of the procedure may facilitate unsupervised sample collection, thus reducing the number of mandatory visits to the doctor or hospital for those patients that need a regular follow-up. Nowadays, DBS sampling is widely used for many applications (e.g., neonatal screening,[4, 5] drug development,[6] pharmacokinetic studies,[7] medical diagnosis,[8] and toxicological[9] and forensic[10] studies). However, the use of DBS still suffers from a number of pitfalls that need to be addressed. Despite the development of several strategies for coping with the differences in hematocrit content (i.e. the fraction of red blood cells in the blood sample, expressed as the percentage of the total volume), this variability can still be considered as the most important factor hampering a wider application of DBS, as it influences viscosity and spreadability of blood spotted on filter paper.[11-13] In addition, issues related with difficulties for sample deposition and DBS formation, e.g., influence of the substrate, blood volume spotted and blood temperature, have been reported in literature too.[14] Recently, VAMS has been suggested as a promising alternative for the DBS method.[15, 16] The VAMS device consists of a holder on which an absorptive porous substrate is attached. This substrate takes up a fixed volume of blood when exposed to a liquid blood sample (see Figure 1). This sampler is designed to be simple and

ergonomic, and resembles a pipette tip, which enables it to be integrated into an automated sample preparation procedure. VAMS shares the benefits of DBS sampling in terms of easiness, sample stability, and transport efficiency, while in contrast to DBS, VAMS has been proven to overcome the issue of the variable HCT level.[17-19] However, VAMS is still in its infancy, and although some works reported on the successful use of VAMS for bioanalysis (especially in the context of drug analysis and pharmacokinetics),[20-22] to the best of the authors' knowledge no works to date have reported on the use of VAMS for subsequent (ultra-)trace element analysis in biofluids. We have evaluated the use of VAMS in the context of determination of prosthesis-related metals in whole blood.

The increasing use of metal-on-metal (MoM) prostheses has aroused serious concern about the elevated metal concentrations released in case of degradation and/or malfunction of these devices. Regular monitoring of the blood of implanted patients is required, which necessitates the development of efficient, fast and reliable analytical approaches for ultra-trace determination of various prosthesis-related metals (e.g., Al, Ti, V, Cr, Co, Ni, Sr and Zr) in biofluids.[23-27] Recent clinical works aiming to assess prosthesis-related problems reported concentrations of ~2 μg L^{-1} of Co and Cr in well-functioning devices, while concentrations as high as ~400 and 200 μg L^{-1} of Co and Cr, respectively, were reached in patients with clear malfunctions of their prosthesis. Although there are important knowledge gaps about the level of metal concentrations that should raise medical concern, and there is not a clear consensus about critical values, in the case of Co and Cr, 7 μg L^{-1} was selected as the clinical action level that requires a follow-up of the patient's status.[28, 29] The use of dry approaches for sample collection may help in the periodic control of patients with reduced mobility. Dry sampling approaches also bring about specific challenges, such as the very low sample volume available, and the necessity to develop very sensitive (and preferably multi-element) analytical methodologies.[30-33]

Inductively coupled plasma-mass spectrometry (ICP-MS) is the technique of choice for the determination of (ultra-)trace concentrations of many elements in biological fluids owing to its low detection limits, multi-element capabilities, high sample throughput and low sample consumption. However, ICP-MS is strongly affected by the occurrence of spectral interference, i.e. overlap of the

signals of the target analyte ions with those of other atomic (isobaric nuclides, doubly charged ions) or polyatomic ions with the same nominal mass-to-charge (m/z) ratio.[34] Thus, interference-free measurement of prosthesis-related metals is not self-evident for a blood sample, which contains many matrix elements at high concentrations (e.g., C, Ca, Cl, K, Mg, Na, P and S), while the levels of the analyte elements are often extremely low. The use of high resolution sector field ICP-MS (HR-SF-ICP-MS) is an elegant option to deal with spectral overlap, but the increase in mass resolution is also accompanied by a significant reduction in sensitivity (1 – 2 orders of magnitude).[35, 36] Alternately, quadrupole-based ICP-MS instruments equipped with a collision-reaction cell (ICP-CRC-QMS) can be used to separate analyte and interfering ions with the same m/z ratio *via* gas phase ion-molecule processes.[37-40] ICP – tandem mass spectrometry (ICP-MS/MS) is a recently introduced and very powerful tool that should be capable to deal with these complex situations,[41-43] as it offers enhanced control over the reactions taking place in the cell, enabling the use of very reactive gases to monitor the target analytes as molecular reaction product ions at a mass/charge ratio that is free from interference.[44-46]

This study aimed at the development and evaluation of an analytical approach whereby VAMS is used for whole blood sampling, with subsequent ICP-MS/MS analysis for the determination of prosthesis-related metals at ultra-trace levels.

2. EXPERIMENTAL

2.1. *Instrumentation*

All measurements were carried out using an Agilent 8800 ICP-MS/MS instrument (ICP-QQQ, Agilent Technologies, Japan). The sample introduction system consisted of a MicroMist nebulizer (400 µL min^{-1}) mounted onto a Peltier-cooled (2 °C) Scott-type spray chamber. This instrument is equipped with two quadrupole units (Q1 and Q2) with an octopole collision/reaction cell system (ORS^3) located in-between. In this work, both quadrupoles were used as mass filters (MS/MS mode) and the cell was pressurized with a methyl fluoride/helium (CH_3F/He - 10:90) mixture as reaction gas. The mass flow controller can be adjusted from 0 to 100%, corresponding to gas flow rates of 0 to 1 mL min^{-1} (mass

flow controller calibrated for O_2). The reaction product ions selected for both methods (100-fold dilution and VAMS) are indicated in Table 1.

A Thermo Element XR sector-field ICP-MS instrument (Thermo Scientific, Germany) was used for validation purposes for those target analytes for which reference values were not available (or in the case of suspected contamination). Table 1 shows the instrument settings and data acquisition parameters for ICP-MS/MS and SF-ICP-MS measurements.

2.2. *Samples and reagents*

Only high-purity reagents were used throughout this work. Ultrapure water (resistivity > 18.2 MΩ.cm) was obtained using a Milli-Q element water purification system (Millipore, France). Pro-analysis 12 M HCl (ChemLab, Belgium) was further purified by sub-boiling distillation. 1 g/L single-element standard solutions of Al, Co, Cr, Ni, Sr, Ti, V and Zr (Instrument solutions, The Netherlands) were appropriately diluted and used for method development, validation and calibration purposes. External calibration was relied on for quantification, with Rh (Inorganic Ventures, The Netherlands) as an internal standard. Standards and samples were prepared in 0.3 M HCl. The VAMS devices (lot: 41106A) were obtained from Phenomenex (Torrance, USA) and are available under the brand name Mitra™. Such a sampling device consists of a hydrophilic polymer with an absorption volume of ~10 µL (10.5 µL for whole blood according to the instructions of the manufacturer). This absorption volume has been experimentally validated and the sampling reproducibility of these devices for blood collection has been systematically assessed under different circumstances by Denniff *et al*.[2] Two blood reference materials, Seronorm Trace Elements Whole Blood Level 1 and Level 3 (Sero, Norway; Reference 210105 and 210305, respectively), with certified or indicative values for the elements targeted in this study, were analyzed for method validation purposes. In order to further evaluate the methods developed, spiking experiments were carried out with real venous blood taken from control patients at the University Hospital Miguel Servet (Zaragoza, Spain). The blood was collected in plastic tubes (BD Vacutainer, U.S.A.) containing EDTA as anti-coagulant.

2.3. Sample preparation

Seronorm Trace Elements Whole Blood Levels 1 and 3 reference materials are provided as a lyophilized material and were reconstituted in Milli-Q water following the instructions of the manufacturer. In addition to these reference materials, aliquots of 1 mL of real whole blood were spiked with small volumes of a multi-element solution in order to obtain added concentrations of 5, 10, 25 and 50 μg L^{-1} of the analyte elements. This concentration range was selected taking into account the critical concentration level for prosthesis-related metals in whole blood, and for evaluating the capabilities of VAMS at different levels. Both the reference materials (after reconstitution) and the real venous blood samples (with and without spiking) were subjected to the same sample preparation procedure. In this work, two different methods for sampling and analysis of blood were compared, i.e. (i) the direct measurement of the samples after appropriate dilution, and (ii) the use of VAMS for sample collection, followed by extraction and ICP-MS/MS analysis. In the first case, whole blood was diluted with Milli-Q water (in metal-free 15 mL polypropylene centrifuge tubes, VWR, Belgium) and subsequently acidified with 12 M HCl. Rh (final concentration of 1 μg L^{-1}) was also added as an internal standard. The resulting 100-fold diluted solution (in 0.3 M HCl) was homogenized and centrifuged (Centrifuge 5702, Eppendorf AG, Germany) during 5 minutes at 4400 rpm. The supernatant was separated for subsequent ICP-MS-MS analysis to prevent possible nebulizer clogging. For the VAMS approach, the sampler device was dipped into the sample for a couple of seconds (2-4 s; following the procedure described by the manufacturer). The VAMS device was subsequently dried under ambient conditions (>2 hours). Thereafter, the absorption probe was removed from the sampler device and added into a metal-free polypropylene centrifuge tube. The analytes were extracted in approximately 1 mL of Milli-Q water with the aid of a vortex mixer (VWR, Belgium, 2 minutes). The solution thus obtained was acidified, as described earlier, and Rh was added as an internal standard. The final volume of 1 mL was considered as the minimum volume needed for ICP-MS measurement.

3. RESULTS AND DISCUSSION

3.1. Development of a multi-element ICP-MS/MS method for interference-free determination of (ultra-) trace amounts of prosthesis-related metals in whole blood

The determination of ultra-trace amounts of prosthesis-related metals in complex samples, such as whole blood, can be seriously hampered by the occurrence of spectral interferences from concomitant matrix elements (e.g., C, Ca, Cl, K, N, Na, Mg, P and S) present at high concentrations. Thus, the development of a multi-element method for the interference-free determination of Al, Co, Cr, Ni, Sr, Ti, V and Zr in whole blood is a challenging task. The use of ICP-MS/MS can provide interference-free conditions *via* reaction of the target analyte ions with a properly selected reaction gas, as a result of which they are converted into reaction product ions that can be measured interference-free at different m/z ratios (mass-shift). This approach has already been evaluated (and proven successful) for other applications and/or sample types.[43, 44] In this work, a CH_3F/He mixture was selected as reaction gas in ICP-MS/MS[45, 47-49] and *via* product ion scanning (PIS) (at different flow rates of CH_3F/He within the range of 0 to 1 mL min^{-1}) the optimum reaction product ions, i.e. those giving the higher signal-to-background ratios, were identified. As the aim was to develop a multi-element method, compromise conditions were sought for and they were reached at the maximum gas flow rate (1 mL min^{-1}). Under these conditions, appropriate reaction product ions were selected for further use. In the case of Al, Cr, Ni and Co, these reaction product ions resulted from molecular addition: $^{27}AlCH_3F^+$ (m/z = 61), $^{52,53}Cr(CH_3F)_2^+$ (m/z = 120, 121), $^{58,60}Ni(CH_3F)_2^+$ (m/z = 126, 128) and $^{59}Co(CH_3F)_2^+$ (m/z = 127). In the cases of Sr and Zr, the reaction product ions were formed *via* F atom transfer: $^{86,87,88}SrF^+$ (m/z = 105, 106, 107) and $^{90,91}ZrF^+$ (m/z = 109, 110). For Ti and V, finally, the reaction product ions were formed upon a combination of F atom transfer and multiple CH_3F addition: $^{47,49}TiF_2(CH_3F)_3^+$ (m/z = 187, 189) and $^{51}VF_2(CH_3F)_3^+$ (m/z =191). The reaction product ions selected were monitored in all subsequent method development and ICP-MS/MS analyses (instrumental parameters for ICP-MS/MS measurements are shown in Table 1) and the figures of merit obtained under these conditions are compiled in Table 2. It needs to be stressed that these LoDs and LoQs are purely instrumental LoDs/LoQs, such that the effect of sample pretreatment needs to be taken into account for assessing the final detection capabilities in the case of analysis of real samples (*vide infra*).

3.2. A simple dilute-and-shoot approach for the determination of (ultra-)trace amounts of prosthesis-related metals in whole blood

3.2.1. Results obtained for Seronorm Trace Elements Whole Blood L-1 and L-3

Seronorm Trace Elements Whole Blood L-1 and L-3 reference materials can be considered as representative for real venous blood samples, containing (ultra-)trace metal concentrations of the analyte elements in a similar matrix composition. The results obtained after a simple dilute-and-shoot approach, consisting of a 100-fold dilution of the reconstituted material, followed by ICP-MS/MS analysis, were evaluated. This dilution needs to be taken into account for assessing the LoDs and LoQs attainable (see Table 2). External calibration was used for quantification purposes, with Rh as an internal standard, to correct for matrix effects, instrument instability and signal drift. Several aliquots (n = 5) of both reference materials were analyzed. The results are presented in Table 3, which shows the average result for 5 aliquots of both reference materials, each measurement consisting of 10 consecutive replicate measurements. The validation of the results obtained in this work was performed by comparison with certified or indicative values and results obtained using SF-ICP-MS in the case of absence of reference values or in those cases possibly affected by contamination of the reference material (see Table 1 for the instrumental parameters used in the SF-ICP-MS method).

Firstly, Seronorm Trace Elements Whole Blood L-3 was selected on the basis of the concentrations of the target analytes expected in whole blood of patients with increased concentrations owing to prosthesis-related problems. As can be seen, within the corresponding uncertainty, the results obtained using ICP-MS/MS are in agreement with the certified or indicative values reported by Seronorm, except for a minor difference in the case of both Ti isotopes (t-test, $t_{experimental}$ = 10.998 and 7.092 > $t_{critical}$ = 2.776, for ^{47}Ti and ^{49}Ti, respectively) and for a clear deviation in the case of ^{58}Ni (t-test, $t_{experimental}$ = 14.328 > $t_{critical}$ = 2.776), as indicated after statistical evaluation using a one-sample t-test at a level of confidence of 95%. For Zr, certified and/or indicative values were not available. In addition, no significant variation was noticed between the results obtained *via* ICP-MS/MS and SF-ICP-MS, respectively (except for ^{58}Ni), which further demonstrates the accuracy of the method developed for all target nuclides, including Ti and Zr. For ^{58}Ni, additional experiments with a solution containing Fe demonstrated that the results obtained using ICP-MS/MS were clearly affected by isobaric overlap of the signals of $^{58}Fe^{+}$ and $^{58}Ni^{+}$, as Fe reacts in the same way as Ni, leading to the reaction product ion

$^{58}Fe(CH_3F)_2^+$ (m/z = 126). Therefore, the high Fe content in the samples prohibits an interference-free determination of Ni *via* ^{58}Ni. However, accurate Ni concentrations could be obtained using another isotope of Ni (e.g., ^{60}Ni).

In order to further evaluate the capabilities of the method developed under the most challenging conditions, Seronorm Trace Elements Whole Blood L-1, containing very low concentrations of the target elements, was analyzed as described above. The experimental results obtained using ICP-MS/MS are within the range of certified or indicative values in the case of Al, Ti, Co, ^{52}Cr and ^{60}Ni. The LoQ was not sufficiently low to allow for quantification of Cr *via* ^{53}Cr and isobaric overlap of the signal of ^{58}Ni with that of ^{58}Fe hindered the determination of Ni *via* this isotope. A small discrepancy was found in the case of V (t-test, $t_{experimental} = 13.149 > t_{critical} = 2.776$), but the difference between experimental and indicative value can be considered negligible for the purpose of this work. Further validation of the ICP-MS/MS results by comparison with the SF-ICP-MS result was not possible for V due to the higher LoQ obtained *via* SF-ICP-MS, but for Sr and Zr, possible bias in the indicative results was revealed. This hypothesis is also supported by cross-validation using different isotopes of the same element. Other inaccurate certified values have been reported in the literature for Seronorm samples (e.g., Se in Seronorm Trace Elements Serum L-2 [50]). These differences were hypothetically attributed to unknown factors causing variations between the batch measured and those originally prepared for evaluation and certification. Although at first sight the difference between the reference value and the ICP-MS/MS result seems quite large for Al, the ICP-MS/MS result is within the acceptable range indicated by the manufacturer, due to the high uncertainty accompanying the reference value. The positive bias may also be the result of possible Al contamination coming from the vial, as indicated by the manufacturer.

3.2.2. Results obtained for real venous blood samples

In order to further evaluate the method developed for the analysis of whole blood after a simple dilute-and-shoot approach, spiking experiments were carried out to simulate the condition of a prosthesis failure and the resulting increase in concentration of the target elements. Therefore, small amounts of a multi-element standard solution were added to 4 aliquots of a real blood sample to obtain spike

concentrations of 5, 10, 25 and 50 µg L^{-1} of all the elements studied. The sample without spike ("blank blood" – containing concentrations below the LoQ for the elements targeted in this study) and the spiked samples were measured after 100-fold dilution following the procedure described in the previous section for the Seronorm reference materials; the results thus obtained are shown in Figure 2. The recoveries were calculated after subtracting the intensity for the "blank blood" sample from the intensity of the corresponding spiked sample. The results were within the range of 91% – 109%; 97% – 109%; 96% – 103% and 98% – 106% for spike concentrations of 5, 10, 25 and 50 µg L^{-1}, respectively. Generally speaking, the results obtained are in excellent agreement with the amounts added (within the typical acceptance criterion of a bias <15% for clinical QC analysis).[51, 52] Furthermore, no significant differences were found as a function of the spike concentration, which demonstrates the accuracy of the method developed at levels as low as 5 µg L^{-1}. The fact that, in the case of Ti, Cr, Sr and Zr, accurate results can be obtained, relying on different isotopes of the same element indicates that also isotope dilution could be used for calibration purposes, if necessary. As described before, the signals obtained for ^{58}Ni are affected by isobaric overlap with ^{58}Fe; nevertheless, the results for both Ni isotopes - in terms of spike recovery - are in good agreement. This finding supports the aforementioned hypothesis about spectral overlap of the signals of ^{58}Ni with ^{58}Fe. Also, special attention needs to be paid to the uncertainty of those measurements, and clearly, it can be seen that a higher standard deviation was found in the case of the low abundant Cr isotope (^{53}Cr), while better precisions were obtained for Co and V. These variations are related with the different sensitivities and detection capabilities of the method developed for the different target nuclides (see Table 2). Overall, the satisfactory results obtained using the simple dilute-and-shoot approach followed by ICP-MS/MS analysis suggest that it could be used in the case of a small sample volume, as obtained using VAMS.

3.3. Volumetric absorptive micro-sampling (VAMS) followed by ICP-MS/MS analysis for the determination of (ultra-)trace amounts of prosthesis-related metals in whole blood

Once the ICP-MS/MS method was successfully developed and validated using whole blood reference materials and real samples (spiking experiments), the capabilities and limitations of VAMS were

evaluated in the context of ultra-trace determination of prosthesis-related metals in whole blood. Therefore, special attention was paid to (i) the optimization of a procedure to extract the blood from the VAMS devices, and (ii) the reduction of the total sample volume needed for analysis (as only ~ 10 µL of whole blood is retained in the VAMS sampling devices). For the extraction of blood from the VAMS, a simple extraction method, allowing one to obtain accurate results without compromising the sample throughput, was aimed at. The samples were extracted from the VAMS devices with ~1 mL of Milli-Q water, followed by addition of Rh as an internal standard and subsequent acidification with HCl (25 µL of 12 M HCl). After centrifugation, the supernatant was subjected to ICP-MS/MS analyses. Thus, the blood sample was diluted ~100-fold, resulting in a final volume of 1 mL (0.3 M HCl and 1 µg L^{-1} of Rh). For measuring the VAMS samples, the method was adapted to lower measurement times due to the low volume available, and thus, only 1 isotope per element (that leading to accurate results with the lowest LoQ possible) was monitored, while 5 measurement replicates were selected (see Table 1).

3.3.1. VAMS contamination

An important issue when measuring ultra-trace concentrations is to control the level of contamination introduced during sample collection and/or preparation. In this method, metal-free material and high-purity reagents were used, and sample preparation steps were reduced to the minimum. However, contamination originating from the sampler device cannot be excluded, and therefore, a careful evaluation of the level of the target elements extracted from blank VAMS devices was required. Five blank VAMS devices were subjected to the complete sample preparation procedure, and the resulting solutions were analyzed *via* ICP-MS/MS. The corresponding results are shown in Table 4, which provides the average and the standard deviation (n = 5) for Al, Ti, V, Cr, Co, Ni, Sr and Zr in blank VAMS devices, also taking into account the 100-fold dilution. The results for Ti, V, Co and Sr show very low contamination levels, below the LoQ in the case of Ti, V and Co, thus the measurement of these elements using VAMS should not be affected to a large extent by contamination issues. In the case of Zr, the concentration was considered relatively high for a “blank” device. As however the contamination seems to remain constant within the different VAMS devices, blank subtraction still

provided accurate results. However, the situation is different in the case of Cr, Ni and Al. The high levels of Al and Ni could be tentatively explained by the introduction of those elements during the manufacturing, as the absorptive probe is stated to be made of a hydrophilic polyolefin, which may involve the use of Al and Ni catalysts for the polymerization process. Consequently, Al, Cr and Ni could not be measured accurately using the VAMS approach and these 3 target analytes were not taken into account in further experiments with the VAMS devices. Further investigation is required in order to improve the manufacturing process of the VAMS devices and/or to develop cleaning steps under controlled conditions prior to the sampling, thus avoiding contamination.

3.3.2. Results obtained for Seronorm Trace Elements Whole Blood L-1 and L-3 using VAMS followed by ICP-MS/MS analysis

The capabilities and limitations of the VAMS approach in the case of prosthesis-related metals in whole blood were also evaluated by measuring the reference materials Seronorm Trace Elements Whole Blood Level 1 and Level 3. Table 5 shows the average concentrations obtained for 3 replicate analyses (reference material sampled with 3 different VAMS devices) for both reference materials (every analysis consisting of 5 consecutive measurements). The average and standard deviation of 3 different analyses may provide an idea on the variability due to contamination issues. Blank VAMS were also analyzed every measurement session in order to assess contamination. The results obtained using VAMS followed by ICP-MS/MS analysis were compared to the recommended values, i.e. the certified/indicative values provided by the manufacturer or the results of the measurements using SF-ICP-MS in the case of absence of reference values, possible contamination, or suspect reference values (as indicated in section 3.2.1). As can be seen, the experimental results are within the recommended range in all cases. However, although no significant differences were found for Co in the Level 1 material (t-test, $t_{experimental} = 2.706 < t_{critical} = 4.303$), high uncertainty was observed, which needs to be attributed to the low amount of Co in the reference material. Under such conditions, the small contribution of contamination becomes more important due to the closeness of the concentration level to the LoQ, which probably indicates the limitation of the method developed. However, concentrations below 1 µg L^{-1} can be considered as normal levels in whole blood and do not indicate any metal

release. In addition, the VAMS-based method only shows slight differences in precision compared to the simple dilute-and-shoot approach, except in the case of Zr, for which the deviation seems to be higher as a consequence of the contamination described in the previous section and indicated in Table 4.

3.3.3. Results obtained for real venous blood samples using VAMS followed by ICP-MS/MS analysis

Aiming at a further evaluation of the method developed using VAMS followed by ICP-MS/MS analysis, the same spiked real venous blood samples as analyzed in section 3.2.2 (5, 10, 25 and 50 µg L^{-1} of the target elements selected for this method) were also measured following the VAMS approach. Three replicate analyses were performed for every spiked sample. The results are shown in Figure 3, which represents the recoveries resulting after subtracting the contribution from the "blank" real venous blood. The precision (indicated as error bars in Figure 3) corresponds with the standard deviation of 3 replicate analyses for every sample (3 VAMS devices). As can be seen, within the experimental uncertainty, no significant differences were found between the results obtained using the VAMS approach followed by ICP-MS/MS analysis and the expected results (bias <15% as clinical QC analysis criterion). The accuracy and precision improved with the increase in concentration, which is clearly related with possible slight contamination of the VAMS devices and/or introduced during the sample preparation steps, although the closeness to the LoQ cannot be excluded as possible source of uncertainty. For samples containing 25 and 50 µg L^{-1} spikes, the results are accurate and precise for all target nuclides, without significant differences compared to the simple dilute-and-shoot approach. In addition, for Co and V, the results are not even affected at lower concentration levels, which can be related with the lower contamination in the VAMS devices and the better LoQ for both target elements. In the case of Ti and Zr, higher uncertainty was obtained for lower concentrations, i.e. 5 and 10 µg L^{-1}, which is in good agreement with the results obtained for the analysis of the reference materials following the same procedure, and definitively seems to point to the relatively high contamination, already described in section 3.3.1 for Zr. However, based on the accuracy and precision of the results obtained for all elements at the different concentration levels of the spike, it

may be assumed that the VAMS approach could be used for sampling of whole blood with the aim of diagnosing failing prostheses based on increased metal levels in the patient's blood.

4. CONCLUSION

In this work, the capabilities and limitations of a novel volumetric absorptive micro-sampling (VAMS) approach combined with tandem ICP – mass spectrometry for the determination of (ultra-)trace amounts of prosthesis-related metals in whole blood were evaluated. Firstly, a method enabling the simultaneous and interference-free measurement of ^{27}Al, $^{47,49}Ti$, ^{51}V, $^{52,53}Cr$, ^{59}Co, ^{60}Ni, $^{86,87,88}Sr$ and $^{90,91}Zr$ was developed, relying on chemical resolution (mass-shift approach) using CH_3F/He in the CRC of an ICP-MS/MS instrument, with attainable (instrumental) LoDs ranging from 0.3 to 30 ng L^{-1}. Subsequently, a simple dilute-and-shoot approach (100-fold dilution of the samples – ICP-MS/MS analysis with external calibration and Rh as internal standard), the method was successfully applied to the analysis of two reference materials (Seronorm Trace Elements Whole Blood Level 1 and Level 3) and to that of spiked real venous blood samples with different spike concentrations (in the range of 5 to 50 µg L^{-1}). In the last stage, it was evaluated whether the method developed can be combined with VAMS as an alternative sample collection approach. Except for the elements inherently affected by the contamination of such devices, i.e. Al, Cr and Ni, VAMS was demonstrated to be suitable in the context of the determination of (ultra-)trace concentrations of the selected elements in whole blood. The possibility to successfully determine ultra-trace amounts of metals in such small sample volumes is owing to the high detection power and multi-element capabilities and the creation of interference-free conditions using chemical resolution in an MS/MS approach of ICP-MS. Future efforts have to aim at the development of metal-free devices when aiming at determining such low concentration levels for the analytes currently affected by contamination. To the best of the authors' knowledge, this is the first work in which VAMS is studied as a sampling approach for whole blood with the aim of (ultra-)trace element determination, and the potential suitability of this approach for future real life and/or routine applications has been demonstrated.

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Figure captions:

Fig. 1 Volumetric absorptive micro-sampling (VAMS) device (Mitra™), before and after sampling blood.

Fig. 2 Recoveries obtained for the spiking experiments with real venous blood using a simple dilute-and-shoot approach. The error bars indicate the standard deviation of 10 consecutive measurement replicates. The solid and dashed red lines indicate 100% recovery and ±15% bias as clinical QC analysis criterion, respectively.

Fig. 3 Recoveries obtained for the spiking experiments with real venous blood using the VAMS approach. The error bars indicate the standard deviation of 3 replicate analyses. The solid and dashed red lines indicate 100% recovery and ±15% bias as clinical QC analysis criterion, respectively.

Fig. 1

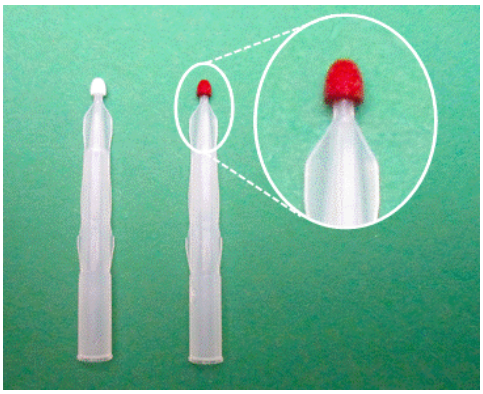

Fig. 2

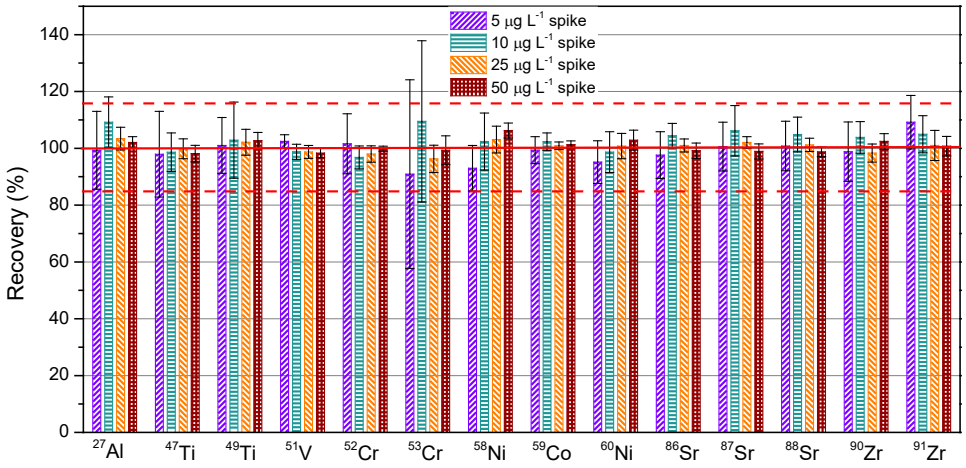

Fig. 3

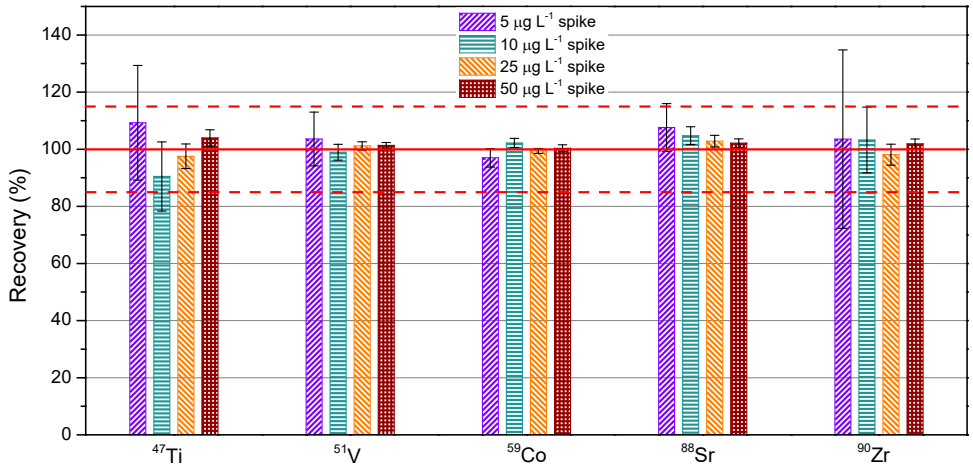

Table 1. Instrument settings and data acquisition parameters for ICP-MS/MS and SF-ICP-MS measurements

Agilent 8800			**Element XR**	
	100-fold dilution method	**VAMS method**		**100-fold dilution method**
Reaction gas	CH_3F/He		Scan type	Escan
Scan type	MS/MS		Resolution	Medium m/Δm ≈ 4000
Plasma mode	Low matrix		RF power (W)	1200
RF power (W)	1550		Carrier gas flow rate (L min^{-1})	0.975
Carrier gas flow rate (L min^{-1})	1.11		Mass window (%)	125
Reaction gas flow rate (mL min^{-1}, MFC calibrated for O_2)	1.0		Search window (%)	50
Extract 1 (V)	-6.0		Integration window (%)	60
Q1 bias (V)	-1.0		Sample time (s)	0.01
Octopole bias (V)	-5.0		Samples / peak	20
Energy discrimination (V)	-8.0		Nuclides monitored	^{27}Al, ^{47}Ti,^{49}Ti,
Extract 2 (V)	-195.0			^{51}V, ^{52}Cr, ^{53}Cr,
Q2 QP bias (V)	-13.0			^{59}Co, ^{58}Ni, ^{60}Ni,
Q1 →Q2 masses	27 → 61	27 → 61		^{86}Sr, ^{87}Sr, ^{88}Sr,
	47 → 187	47 → 187		^{90}Zr, ^{91}Zr,^{103}Rh
	49 → 189	51 → 191	Total analysis time / sample (s)	142
	51 → 191	52 → 120		
	52 → 120	59 → 127		
	53 → 121	60 → 128		
	59 → 127	88 → 107		
	58 → 126	90 → 109		
	60 → 128	103 → 103		
	86 → 105			
	87 → 106			
	88 → 107			
	90 → 109			
	91 → 110			
	103 → 103			
Wait time offset (ms)	10			
Sweeps / replicate	100			
Integration time / mass (s)	1			
Replicates	10	5		
Total analysis time / sample (s)	150	45		

Table 2. Calibration data and instrumental limits of detection (LoD) and of quantification (LoQ) for the ICP-MS/MS method developed

Nuclide	Reaction product ion	Q1 (amu)	Q2 (amu)	Sensitivity[a] (L µg^{-1})	Intercept[a] (counts s^{-1})	R^2	LoD[b] (µg L^{-1})	LoQ[b] (µg L^{-1})	MDL[c] (µg L^{-1})	MQL[c] (µg L^{-1})
^{27}Al	$^{27}AlCH_3F^+$	27	61	5570 ± 50	3000 ± 86	0.997	0.02	0.06	2	6
^{47}Ti	$^{47}TiF_2(CH_3F)_3^+$	47	187	853 ± 14	80 ± 20	0.99994	0.02	0.06	2	6
^{49}Ti	$^{49}TiF_2(CH_3F)_3^+$	49	189	725 ± 15	50 ± 23	0.99990	0.03	0.09	3	9
^{51}V	$^{51}VF_2(CH_3F)_3^+$	51	191	20700 ± 120	40 ± 160	0.99998	0.0005	0.002	0.05	0.2
^{52}Cr	$^{52}Cr(CH_3F)_2^+$	52	120	6440 ± 28	80 ± 51	0.99997	0.003	0.008	0.3	0.8
^{53}Cr	$^{53}Cr(CH_3F)_2^+$	53	121	773 ± 18	60 ± 12	0.9998	0.02	0.08	2	8
^{58}Ni	$^{58}Ni(CH_3F)_2^+$	58	126	9230 ± 57	300 ± 88	0.99996	0.006	0.02	0.6	2
^{59}Co	$^{59}Co(CH_3F)_2^+$	59	127	34400 ± 120	200 ± 170	0.99998	0.0003	0.001	0.03	0.1
^{60}Ni	$^{60}Ni(CH_3F)_2^+$	60	128	3760 ± 37	100 ± 44	0.99998	0.003	0.009	0.3	0.9
^{86}Sr	$^{86}SrF^+$	86	105	15500 ± 96	96 ± 92	0.999996	0.0008	0.003	0.08	0.3
^{87}Sr	$^{87}SrF^+$	87	106	11300 ± 53	20 ± 83	0.99998	0.003	0.009	0.3	0.9
^{88}Sr	$^{88}SrF^+$	88	107	134000 ± 600	200 ± 670	0.999997	0.0005	0.002	0.05	0.2
^{90}Zr	$^{90}ZrF^+$	90	109	5990 ± 42	-20 ± 56	0.99997	0.006	0.02	0.6	2
^{91}Zr	$^{91}ZrF^+$	91	110	1304 ± 22	10 ± 25	0.99996	0.01	0.04	1	4

[a]Uncertainties expressed as standard deviation (n = 10).

[b]Instrumental LoDs and LoQs calculated as 3 and 10 times the standard deviation of 10 consecutive measurements of a blank solution (0.3 M HCl), divided by the slope of the calibration curve respectively.

[c]Method detection and quantification limits (MDL and MQL) correspond with the LoDs and LoQs after taking into account the 100-fold dilution of the samples.

Table 3. Results obtained for Seronorm Trace Elements Whole Blood L-1 and L-3 using a simple dilute-and-shoot approach followed by ICP-MS/MS analysis in comparison with reference values and experimental SF-ICP-MS results

	Seronorm Trace Elements Whole Blood Level 1			Seronorm Trace Elements Whole Blood Level 3		
Nuclide	**Reference value (µg L^{-1})**	**ICP-MS/MS[a] (µg L^{-1})**	**SF-ICP-MS[a] (µg L^{-1})**	**Reference value (µg L^{-1})**	**ICP-MS/MS[a] (µg L^{-1})**	**SF-ICP-MS[a] (µg L^{-1})**
^{27}Al	9.20 ± 6.40	15.47 ± 1.12	< LoQ	105.00 ± 21.00	103.13 ± 6.52	103.18 ± 3.44
^{47}Ti	14.00 ± 2.00	16.45 ± 0.78	14.93 ± 1.56	12.80 ± 0.40	11.11 ± 0.34	11.36 ± 1.30
^{49}Ti	14.00 ± 2.00	16.14 ± 1.22	14.48 ± 1.66	12.80 ± 0.40	10.82 ± 0.63	10.89 ± 1.38
^{51}V	1.30 ± 0.20	0.87 ± 0.07	< LoQ	5.70 ± 1.10	5.75 ± 0.48	5.22 ± 0.72
^{52}Cr	0.86 ± 0.38	0.91 ± 0.19	< LoQ	23.20 ± 4.70	22.20 ± 0.96	21.55 ± 0.70
^{53}Cr	0.86 ± 0.38	< LoQ	< LoQ	23.20 ± 4.70	22.89 ± 1.49	21.82 ± 2.18
^{58}Ni	1.18 ± 0.48	12.92 ± 0.25	---[b]	12.60 ± 2.50	25.03 ± 1.94	---[b]
^{59}Co	0.16 ± 0.06	0.25 ± 0.10	< LoQ	11.40 ± 1.20	11.18 ±0.74	11.31 ± 0.87
^{60}Ni	1.18 ± 0.48	1.48 ± 0.49	< LoQ	12.60 ± 1.20	12.52 ±1.57	< LoQ
^{86}Sr	15.30 ± 1.10	36.78 ± 0.49	37.71 ± 1.00	15.00 ± 0.20	14.86 ± 1.05	14.54 ± 0.30
^{87}Sr	15.30 ± 1.10	36.60 ± 0.60	---[b]	15.00 ± 0.20	14.94 ± 1.08	---[b]
^{88}Sr	15.30 ± 1.10	36.78 ± 0.58	36.63 ± 1.61	15.00 ± 0.20	14.85 ± 1.08	15.38 ± 0.17
^{90}Zr	0.27 ± 0.03	5.44 ± 0.78	5.43 ± 0.96	---[c]	4.61 ± 0.86	4.55 ± 0.32
^{91}Zr	0.27 ± 0.03	5.59 ± 0.64	5.55 ± 1.02	---[c]	4.88 ± 0.79	4.73 ± 0.02

[a]Uncertainties expressed as standard deviation (n = 5).
[b]Not measured.
[c]Not available.

Table 4. Results obtained for blank VAMS devices

Isotope	ICP-MS/MS[a] (µg L^{-1})	ICP-MS/MS x 100[b] (µg L^{-1})
^{27}Al	33 ± 12	3300 ± 1200
^{47}Ti	< LoQ	< LoQ
^{51}V	< LoQ	< LoQ
^{52}Cr	0.30 ± 0.11	30 ± 11
^{59}Co	< LoQ	< LoQ
^{60}Ni	4.9 ± 3.9	490 ± 390
^{88}Sr	0.02 ± 0.01	1.7 ± 0.8
^{90}Zr	0.11 ± 0.01	11 ± 1

[a]Uncertainties expressed as standard deviation (n = 5).
[b]Concentration after taking into account the 100-fold dilution. Uncertainties expressed as standard deviation (n = 5).

Table 5. Results obtained for Seronorm Trace Elements Whole Blood L-1 and L-3 using the VAMS approach followed by ICP-MS/MS analysis compared to recommended values

	VAMS Seronorm L-1		VAMS Seronorm L-3	
Isotope	**Recommended value[a] (µg L^{-1})**	**ICP-MS/MS[b] (µg L^{-1})**	**Recommended value[a] (µg L^{-1})**	**ICP-MS/MS[b] (µg L^{-1})**
^{47}Ti	14.00 ± 2.00	16.46 ± 2.46	12.80 ± 0.40	12.88 ± 1.36
^{51}V	1.30 ± 0.20	0.92 ± 0.24	5.70 ± 1.10	5.61 ± 0.39
^{59}Co	0.16 ± 0.06	0.89 ± 0.62	11.40 ± 1.20	10.82 ±0.48
^{88}Sr	36.63 ± 1.61*	38.82 ± 1.40	15.00 ± 0.20	14.59 ± 0.56
^{90}Zr	5.43 ± 0.96*	5.20 ± 2.64	4.55 ± 0.32*	4.90 ± 1.01

[a]Reference values provided by the manufactures or experimental results determined using SF-ICP-MS (*).
[b]Uncertainties expressed as standard deviation (n = 3).