NRC Publications Archive Archives des publications du CNRC

Determination of vanadium in biological fluids using HR-ICP-MS

Yang, L.; Sturgeon, R.; Prince, D.; Gabos, S.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. /
La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version
acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien
DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1039/b207563f>

Journal of Analytical Atomic Spectrometry, 17, 10, pp. 1300-1303, 2002

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=c3dbbb89-a5c0-4ac2-9cb3-5d30383da7de>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=c3dbbb89-a5c0-4ac2-9cb3-5d30383da7de>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the
first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la
première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez
pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

National Research
Council Canada

Conseil national de
recherches Canada

Canada

Determination of vanadium in biological fluids using HR-ICP-MS

Lu Yang,^{*a} Ralph E. Sturgeon,^a Dennis Prince^b and Stephan Gabos^c

^a*Institute for National Measurement Standards, National Research Council Canada, Ottawa, Ontario, Canada K1A 0R6. E-mail: Lu.Yang@nrc.ca*

^b*DS Prince Consulting Ltd., Edmonton, Alberta, Canada*

^c*Alberta Health and Wellness, Edmonton, Alberta, Canada*

Received 2nd August 2002, Accepted 28th August 2002

First published as an Advance Article on the web 9th September 2002

A simple, fast and sensitive method is described for the determination of vanadium in biological fluids using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). Samples were diluted 20-fold in 0.3% HNO₃. Spectral interference from ³⁵Cl¹⁶O⁺, present in urine or serum matrices, was completely resolved from the vanadium major isotope of ⁵¹V⁺ at medium resolution of 4000. Quantitation of V in urine and serum was achieved by the method of additions using standards prepared in a pooled urine or serum sample. Mass offset and lock mass (⁴⁰Ar¹⁶O⁺) options were employed for more accurate and precise results. Better than 2.0% RSD was obtained with a 200 pg ml⁻¹ V spike added to a 20-fold diluted urine or serum matrix, reflecting one day stability. Vanadium concentrations in the range of <10 to 1500 pg ml⁻¹ and <10 to 760 pg ml⁻¹ were determined in the urine and serum samples, respectively. Good day-to-day precision of less than 4.0% RSD was typical for both sample matrices. Spike recoveries of 99 ± 2 and 98 ± 3% (one standard deviation, *n* = 3) were obtained in the urine and serum, respectively. An in-house CRM (SLRS-4: river water) was analyzed daily, yielding a V concentration of 0.337 ± 0.010 ng ml⁻¹ (one standard deviation, *n* = 35), in good agreement with the certified value of 0.32 ± 0.03 ng ml⁻¹. A detection limit (3 *s*) of 10 pg ml⁻¹ was estimated for V in reconstituted urine and serum samples.

Introduction

Vanadium is released in large quantities into the environment, mainly from the combustion of fossil fuels and from various industrial processes which use V as a catalyst, as an alloying agent in steel and in welding and metal plating processes.¹ Direct inhalation exposure to V leads to respiratory symptoms ranging from a mild cough to severe bronchitis.² In Canada, a comprehensive exposure study program was initiated from June 1997 to November 1998 by a multi-stakeholder group, including industry, government and non-government organizations. This arose as a result of public concerns in Fort McMurray (36 400 population, Alberta, Canada) over emissions from the oil sands industry, which currently produces 500 000 barrels per day (bpd) and is scheduled to expand production to over 1 500 000 bpd in the next ten years. Vanadium was detected in air samples collected from this area. For the comprehensive exposure study, V levels in population serum and urine were required to be determined in order to ascertain any relationship to exposure and the use of the data as a biomarker.

Quantitation of trace levels of vanadium in biological fluids (present at the pg ml⁻¹ level) is a difficult analytical task, due not only to the very low analyte concentrations that need to be addressed, but also because of the relatively complex sample matrix. Inductively coupled plasma mass spectrometry (ICP-MS) remains popular for trace element determinations in a variety of biological samples as a consequence of its high sensitivity and superior detection power. Unfortunately, a major polyatomic interference by ³⁵Cl¹⁶O⁺ (arising from the sample matrix) on the major ⁵¹V⁺ isotope hampers accurate determination of V in such matrices as urine and serum when using quadrupole-based ICP-MS. As a result, separation of the analyte from such interference prior to Q-ICP-MS determination is often needed to ensure accurate results. Thus, electrothermal vaporization³ and cryogenic desolvation⁴ techniques have been used with ICP-MS to reduce the interferences

for V determination in urine or serum. Detection limits in the range of 45 to 100 pg ml⁻¹ have been reported using these approaches. More recently, a dynamic reaction cell (DRC) ICP-MS method⁵ has been reported for V determination in serum and urine with a detection limit of 28 pg ml⁻¹.

High resolution sector field inductively coupled plasma mass spectrometry (HR-ICP-MS) not only possesses high sensitivity, sub-pg ml⁻¹ instrumental detection limits, a large dynamic range and multi-element capability, but also has the capability to resolve a significant number of spectral interferences, including that from ³⁵Cl¹⁶O⁺ on ⁵¹V⁺. Thus, this technique has gained popularity recently for the determination of trace elements and/or their isotope ratios in biological fluids.^{6–14} Detection limits in the range 12 to 100 pg ml⁻¹ for V in biological fluids have been reported using HR-ICP-MS.^{9,10,12,14} In 2001, ThermoFinnigan released software, version 2.1, highlighting new mass offset and lock mass features, which permit correction for mass shift (due to hysteresis within the magnet material) and mass drift (due mostly to thermal effects) previously observed with both their Element 1 and Element 2 HR-ICP-MS instruments. These improvements not only eliminate the need for repeat mass calibrations during daily operation, especially at medium and high resolution, but also provide more stable instrument performance, resulting an improved accuracy and precision of analytical results.

The objective of this study was to take advantage of the high sensitivity, superior detection power and stability features offered by the Element 2 HR-ICP-MS instrument for the rapid, accurate and precise determination of V in urine and serum samples that were simply diluted 20-fold in 0.3% HNO₃. In this manner, complex sample preparation was avoided and possible contamination introduced from reagents or internal standards minimized. A simple, one-point standard additions calibration was used to compensate for matrix effects and to ensure accurate quantitation of V in the urine and serum samples. The method was applied to the determination of V in 400 samples of urine and serum which had been collected for the exposure study.

Experimental

Instrumentation

The HR-ICP-MS used in this work was a ThermoFinnigan Element 2 (Bremen, Germany) equipped with a Scott-type double-pass glass spray chamber and a Conikal nebulizer (Glass Expansion, West Melbourne, Victoria, Australia). A plug-in quartz torch with a sapphire injector and a Pt guard electrode were used. Optimization of the Element 2 was performed as recommended by the manufacturer. Typical operating conditions are summarized in Table 1.

A Gilson Microman M250 piston pipette (Mandel Scientific Company Ltd., Guelph, Ontario, Canada) was used to pipette urine or serum samples. Polypropylene screw cap tubes, 8 and 30 ml volume, used for the storage and preparation of urine and serum samples, were purchased from Sarstedt Inc. (St-Leonard, Quebec, Canada).

Reagents and solutions

Nitric acid was purified in-house by sub-boiling distillation of reagent grade feedstock in a quartz still prior to use. Ultra-clean water was obtained from a Nanopure ion exchange reverse osmosis system (Barnstead/Thermolyne, Boston, MA, USA). A 1000 $\mu\text{g ml}^{-1}$ stock solution of V was prepared by dissolution of the high purity metal (Johnson, Matthey & Co. Limited, London, UK) in HNO_3 . An intermediate working solution of 100 ng ml^{-1} , used for preparing calibration standards, was prepared by a serial dilution of the stock with 1% HNO_3 solution.

The river water CRM SLRS-4 (NRCC, Ottawa, Canada) was used to check the accuracy of the method.

Samples and sampling

Samples of blood and urine were obtained from study participants who reported to hospital laboratories for collection of the first full emission of morning urine. Care was taken during sampling to avoid possible contamination. The detailed sampling procedure and contamination control measures will be reported later by Alberta Health and Wellness. In brief, an individual's urine sample was first collected in a Labcraft plastic container (Anachemia Science, Montreal, Quebec, Canada), the sample was thoroughly shaken and a 30 ml aliquot poured into a screw-capped conical polypropylene centrifuge tube. The sample was frozen immediately and then shipped frozen to the NRC for analysis.

For sampling serum, venipuncture was first performed using a stainless phlebotomy needle with a plastic hub. The required volume of blood was slowly drawn into a 7 ml Monoject trace element blood collection tube with royal blue stopper, this being a no additive, silicone coated tube (Sherwood Medical Canada). The blood sample was left for 30–60 min (maximum) to allow clotting. The serum was transferred to an 8 ml polypropylene screw-capped tube after centrifugation. The serum

samples were frozen immediately and shipped frozen to the NRC laboratory for analysis.

Sample preparation and measurement procedure

To reduce the risk of contamination, all plastic labware was cleaned by leaching with 1 + 1 diluted reagent grade HNO_3 for a week, rinsed with ultra-clean water and filled with 2% high purity HNO_3 for a further week of leaching. Pipette tips were leached in 5% high purity HNO_3 and rinsed several times with ultra-clean water before use. All plastic tubes were pre-screened for contamination before use for the preparation of samples.

Urine and serum samples were prepared in a class 100 fume hood. Aliquots of 0.250 ml each of urine or serum were delivered using a Gilson Microman M250 piston pipette into pre-cleaned 8 ml polypropylene tubes and diluted with 0.3% HNO_3 to a total of 5.0 ml prior to analysis.

Each set of calibration standards, Std1 and Std2, was prepared as follows: aliquots of 2.5 ml each of pooled urine or serum sample were used with addition of 0 and 0.100 ml of 100 ng ml^{-1} V standard solution and diluted to 50 ml with 1% HNO_3 , resulting in concentrations of 0 and 200 pg ml^{-1} , respectively. Before starting a measurement sequence, Std1 was aspirated for 5–10 min to permit the system to stabilize. The Mass offset values for all isotopes used were then obtained using Std2. After transferring the mass offset values in the method and activating the lock mass at $^{40}\text{Ar}^{16}\text{O}^+$, the measurement sequence was started and data collected in the order Std1 and Std2 followed by three sample reagent blanks and seven urine samples or serum samples. Both Std1 and Std2 were repeatedly measured after every 10 samples. A measurement sequence usually contains fifty samples. A standard additions calibration method was used for the final quantitation of V in all samples.

Results and discussion

Optimization

Optimization of the Element2 was performed daily, as recommended by the manufacturer, using a 1 ppb multi-element standard solution. A Pt guard electrode was used to achieve high sensitivity. The nebulizer gas flow rate was tuned to obtain the most stable signal, at acceptable sensitivity, and low level (5–6%) oxide formation (UO/U) rather than for absolute intensities of Li, In and U. Mass offset values for isotopes of interest for the determination of V were then determined immediately after mass calibration at medium (*i.e.*, 4000) resolution. It was determined that aspirating a 20-fold diluted urine or serum sample for 5–10 min before starting the measurement sequence was necessary to stabilize the system and achieve better accuracy and precision of results. For this purpose, 20-fold diluted urine and serum samples, spiked at a concentration of 200 pg ml^{-1} V, were used to access the instrument stability under chosen experimental conditions. One hour short term stabilities of 1.4 and 1.5% RSD ($n = 20$) were obtained with these standard solutions of urine and serum, respectively. Six hour stabilities were estimated from six repeat measurements of Std2 (200 pg ml^{-1} V spike) in a measurement sequence, yielding better than 2% RSD ($n = 6$) for both urine and serum.

Contamination control

Preliminary measurements suggested that V concentrations were as low as 10 pg ml^{-1} in some urine and serum samples. Reagent, plastic bottles and tubes were therefore screened for possible contamination prior to use. Polyethylene bottles (15 ml volume) were checked for V content after HNO_3 acid cleaning and leaching. The bottles were filled with different

Table 1 Element2 HR-ICP-MS operating conditions

Rf power	1200 W
Plasma Ar gas flow rate	15.0 l min^{-1}
Auxiliary Ar gas flow rate	1.000 l min^{-1}
Nebulizer Ar gas flow rate	0.990 l min^{-1}
Sampler cone (nickel)	1.1 mm
Skimmer cone (nickel)	0.8 mm
Resolution	4000
Data acquisition	E-scan, 5 runs $\times$ 30 passes, 100% mass window, 60% search window, 60% integration window, 0.0050 s sample time, $^{40}\text{Ar}^{16}\text{O}^+$ as lock mass
Dead time	17 ns

Table 2 Blanks from selected polyethylene bottles

Leach time	Bottle A in 0.1% HNO ₃	Measured concentration/pg ml ⁻¹			
		Bottle B in 0.5% HNO ₃	Bottle C in 1% HNO ₃	Bottle D in 2% HNO ₃	
10 min	0.3	0.3	0.3	0.3	
2 h	0.3	0.3	0.8	1.3	
4 h	0.3	0.3	1.5	5.5	
24 h	0.4	0.7	2.0	8.8	

concentrations of high purity HNO₃ solution and leached for various time intervals, after which V intensities were recorded at medium resolution. Concentrations of V in leaching solutions were calculated based on external calibration. Increased concentrations of V were detected as the HNO₃ concentration and leaching time increased in some bottles, as shown in Table 2, clearly indicating that the contamination of V arose from this type of polyethylene bottle. Subsequently, 100 polypropylene screw cap tubes of 8 ml volume were tested for V. The intensities of V measured in 0.1 to 2% HNO₃ leaching solutions were all less than 100 cps (<0.1 pg ml⁻¹), indicating no significant contribution to V contamination from these tubes, which were therefore used in the final study.

Matrix effects, interferences and detection limits

In a preliminary study, signal enhancements on ⁵¹V⁺ arising from 10-fold diluted serum and urine samples were 20 and 11%, respectively, compared with that from a standard solution. Sensitivity as a function of sample dilution in the range of 10- to 100-fold was thus investigated in both matrices using solutions spiked at a V concentration of 200 pg ml⁻¹. Intensities were normalized to the intensity obtained from a 200 pg ml⁻¹ standard solution, as shown in Fig. 1 for urine samples. A sensitivity enhancement is clearly evident as the dilution factor is decreased below 50-fold. A similar trend (not shown) was obtained for serum samples, although a slightly higher enhancement at 10- to 40-fold dilution was evident. No blockage of the cones was observed when 20-fold diluted urine or serum was continuously run for 6 h. Consequently, in the interest of maintaining long-term stability without sacrificing sensitivity, a 20-fold dilution was selected for all further measurements.

As noted earlier, a major interference by ³⁵Cl¹⁶O⁺, arising in urine or serum matrices, hampers accurate quantitation of V when using low resolution ICP-QMS. The resolution needed to separate ³⁵Cl¹⁶O⁺ from ⁵¹V⁺ is 2573. As shown in Fig. 2, ⁵¹V⁺ is well separated from ³⁵Cl¹⁶O⁺ at medium resolution (4000) in a 20-fold diluted serum sample spiked to contain

Fig. 1 Sensitivity versus dilution factor for urine. **Fig. 2** Mass spectral scan obtained with a serum sample diluted 20-fold and spiked with V at a concentration of 200 pg ml⁻¹.

200 pg ml⁻¹ V. Final quantitation of V in all urine and serum samples was conducted at medium resolution.

A urine and a serum sample containing low V were chosen for estimation of the method detection limit. A standard additions calibration technique was used to compensate for the 6 and 11% enhancements in signal intensity present in 20-fold diluted urine and serum, respectively, for the quantitation of V in these two samples. Detection limits (3 s) were calculated from the standard deviations of 10 measured V concentrations in both matrices, yielding 10 pg ml⁻¹ in both the undiluted urine and serum samples. This detection limit is well suited to the determination of V in these samples since the lowest mean values reported¹ in human urine and serum are 23 and 212 pg ml⁻¹, respectively. The detection limit achieved here is comparable to, or better than, that reported in other studies^{9,10,12,14} using HR-ICP-MS.

Sample analysis

The linearity of the addition calibration curves covering the entire concentration range of samples was examined under optimum experimental conditions using spikes of 0, 100, 200 and 500 pg ml⁻¹ in 20-fold diluted pooled urine and serum samples. Correlation coefficients of 0.9998 and 0.9997 were obtained in the urine and serum, respectively. The final analysis of all urine and serum was performed using a single point addition calibration at a spiked V concentration of 200 pg ml⁻¹, using the measurement sequence described earlier. No significant difference in V concentrations measured in a few selected urine and serum samples was observed when using either a four point calibration or a two point calibration. The system was stabilized after aspirating Std1 for 5–10 min before starting the measurement sequence. No further drift in sensitivity during a day run was observed. Usually, better than 2% RSD, based on six repeat measurements of Std2 (200 pg ml⁻¹ V spike), can be obtained during a day sequence. Therefore, a calibration curve generated from these six repeat measurements was used for the quantitation of V in 50 unknown samples in a sequence. Results from selected urine and serum samples, which were measured on different days (samples were thawed and re-frozen), are reported in Table 3. The highest V concentrations encountered were 1500 ± 40 and 760 ± 20 pg ml⁻¹ in these urine and serum samples, respectively. Good day-to-day precision, better than 4% RSD, was obtained in both sample matrices. Interpretation of these V concentrations is beyond the scope of this study: a full statistical report will be issued by Alberta Health and Wellness in the future.

As no human body fluid CRMs certified for V are available, an in-house river water CRM, SLRS-4, was analyzed daily to evaluate the accuracy of the methodology using a one point addition calibration to a 20-fold diluted sample. A V

Table 3 V concentrations (pg ml⁻¹) in selected urine and serum samples

Sample name	Measured V concentration	RSD (%)
Urine A (<i>n</i> = 3)	< 10	N/A
Urine B (<i>n</i> = 5)	23 ± 1	3.7
Urine C (<i>n</i> = 3)	67 ± 2	3.0
Urine D (<i>n</i> = 11)	134 ± 5	3.7
Urine E (<i>n</i> = 3)	1500 ± 40	2.7
Serum 1 (<i>n</i> = 3)	< 10	N/A
Serum 2 (<i>n</i> = 3)	62 ± 2	3.2
Serum 3 (<i>n</i> = 3)	254 ± 8	3.1
Serum 4 (<i>n</i> = 3)	760 ± 20	2.6

concentration of 0.337 ± 0.010 (one standard deviation, *n* = 35) measured in SLRS-4 over a period of 35 d is in good agreement with the certified value of 0.32 ± 0.03 ng ml⁻¹. Spike recovery experiments (20 pg ml⁻¹ in 20-fold diluted pooled urine and serum samples) were also run. Quantitative spike recoveries of 99 ± 2 and $98 \pm 3\%$ (one standard deviation, *n* = 3) were obtained for urine and serum, respectively. These results suggest that the developed method is capable of providing accurate determination of V in both matrices.

All human urine and serum samples were stored at -2 °C without acidification and samples were restored to this condition after analysis. Good day-to-day precision of less than 4% was obtained for both matrices within a one month analysis period, as shown in Table 3. In addition, no measurable losses of V occurred over a 1 month storage period (e.g., urine D was measured on 11 different days within that month, Table 3, which entailed multiple thawing and re-freezing cycles).

It was of interest to investigate the long-term stability of V in these samples under such storage conditions. Six urine and six serum samples were selected and re-analyzed following 6 months of storage. Results are summarized in Table 4, and show good agreement with concentrations measured 6 months earlier for all serum samples. Significantly lower concentrations were obtained for all urine samples measured after this time period, evidence that the long-term storage conditions used for this material were inappropriate.

Conclusion

A simple, fast, sensitive and accurate method has been developed for the determination of V in urine and serum samples. A 20-fold dilution in 0.3% HNO₃ in combination with the low detection limit of 10 pg ml⁻¹ makes this method well suited for

Table 4 Long-term storage results

Sample name	V concentration/pg ml ⁻¹ (<i>n</i> = 5)	
	Initial results	Six months later
Urine F	626 ± 15	449 ± 10
Urine G	239 ± 5	205 ± 4
Urine H	66 ± 2	22 ± 1
Urine I	136 ± 4	124 ± 3
Urine J	55 ± 1	40 ± 1
Urine K	41 ± 1	29 ± 1
Serum 10	90 ± 3	93 ± 3
Serum 11	68 ± 3	61 ± 2
Serum 12	339 ± 10	342 ± 10
Serum 13	465 ± 14	473 ± 12
Serum 14	263 ± 7	271 ± 8
Serum 15	251 ± 10	237 ± 8

the quantitation of V in human urine and serum samples. At these levels, special attention should be paid to contamination control from reagents and containers. Optimum long term storage conditions for such samples require further investigation.

References

- 1 E. Sabbioni, J. Kuèera, R. Pietra and O. Vestergery, *Sci. Total Environ.*, 1996, **188**, 49–58.
- 2 R. E. M. Lees, *Br. J. Ind. Med.*, 1980, **37**, 253–256.
- 3 L. Yu, S. R. Koirtiyohann, M. Rueppel, A. K. Skipor and J. J. Jacobs, *J. Anal. At. Spectrom.*, 1997, **12**, 69–74.
- 4 M. G. Minnich, R. S. Houk, M. A. Woodin and D. C. Christiani, *J. Anal. At. Spectrom.*, 1997, **12**, 1345–1350.
- 5 D. E. Nixon, K. R. Neubauer, S. J. Eckdahl, J. A. Butz and M. F. Burritt, *Spectrochim. Acta B*, 2002, **57**, 951–966.
- 6 M. Krachler, A. Alimonti, F. Petrucci, F. Forastiere and S. Caroli, *J. Anal. At. Spectrom.*, 1998, **13**, 701–705.
- 7 S. Stürup, M. Hansen and C. Mølgaard, *J. Anal. At. Spectrom.*, 1997, **12**, 919–923.
- 8 A. T. Townsend, K. A. Miller, S. McLean and S. Aldous, *J. Anal. At. Spectrom.*, 1998, **13**, 1213–1219.
- 9 I. Rodushkin, F. Ödman, R. Olofsson and M. D. Axelsson, *J. Anal. At. Spectrom.*, 2000, **15**, 937–944.
- 10 A. T. Townsend, *J. Anal. At. Spectrom.*, 2000, **15**, 307–314.
- 11 S. Stürup, *J. Anal. At. Spectrom.*, 2000, **15**, 315–322.
- 12 J. Begerow, M. Turfeld and L. Dunemann, *J. Anal. At. Spectrom.*, 2000, **15**, 347–352.
- 13 R. S. Pappas, B. G. Ting, J. M. Jarrett, D. C. Paschal, S. P. Caudill and D. T. Miller, *J. Anal. At. Spectrom.*, 2002, **17**, 131–134.
- 14 I. Rodushkin, F. Ödman and S. Branth, *Fresenius' J. Anal. Chem.*, 1999, **364**, 338–346.