

# Comparison of Zeeman Background Corrected Atomic Absorption Spectrometric and Inductively Coupled Plasma Mass Spectrometric Detection of Trace Elements in Electrothermally Vaporized Serum

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**Abstract:** There is continued interest in the measurement of degradation products of metallic implants in biological tissues and fluids. A study was conducted to compare analytical results obtained by the Zeeman Background Corrected Atomic Absorption Spectrometric and Inductively Coupled Plasma Mass Spectrometric techniques on a uniform set of triple-element (Ti, Al, and V) spiked human serum specimens over concentration ranges up to 20 ppb (20 ng/ml). The results indicate that the two methods are comparable. The positive and negative aspects of each method of instrumental analysis are discussed. © 1999 John Wiley & Sons, Inc. *J Biomed Mater Res (Appl Biomater)* 48: 90–93, 1999

**Keywords:** titanium; aluminum; vanadium; Zeeman background corrected atomic absorption spectrometry; electrothermal vaporization inductively coupled plasma mass spectrometry

## INTRODUCTION

The study of trace element concentrations in biological tissues and fluids in relation to the chronic use of metallic implants in human clinical practice has been made possible by the advances in analytical capability. The first major step was the improvement of atomic absorption spectrometry by the replacement of a gas flame by a black-body furnace for direct vaporization and the introduction of a succession of background correction techniques, most recently that of Zeeman correction. This has produced the modern Zeeman background corrected atomic absorption spectrometry (ZAAS), which has been available since the 1980s. More recently, the preferred technique for trace element analysis has gradually shifted toward inductively coupled plasma mass spectrometry (ICP-MS), which is capable of simultaneous determination of elements at parts per trillion levels. An electrothermal vaporization (ETV) attachment is sometimes used as a sample introduction system to enhance the instrument's capability of analyzing microliter size samples

and/or to facilitate the removal of sample matrix and hence minimize interferences.

In our studies of the release, distribution, and excretion of organometallic species from titanium-base alloy implants in animals and patients, we have used and recently reported<sup>1,2</sup> both techniques for the detection of titanium (Ti), aluminum (Al), and vanadium (V) in serum. It is generally believed that ICP-MS has the advantages of increased sensitivity, speed, superior detection limits, and reduced matrix interferences in comparison to ZAAS. The use of the ETV instead of conventional pneumatic nebulization has enabled its use for the determination of trace elements in serum because the electrothermal cycle allows the removal of many deleterious effects from the bulk matrix and thus reduces spectral interferences; it eliminates the need for elaborate chemical separations, preconcentrations, or digestions that increase the likelihood of contamination and usually result in higher detection limits; it allows the analysis of small sample volumes generally encountered in clinical samples; and it has lower detection limits. Our study<sup>2</sup> showed that when the ETV is used, simple dilutions of 1:2 with matrix modifiers that are used to prevent the loss of analytes during the electrothermal cycle and are also used to enhance the transport efficiency of the analytes into the ICP-MS was sufficient for the successful determination of Ti, Al, and V in serum. We report a brief

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study done to compare the results of the two techniques on spiked serum specimens.

## METHODS AND MATERIALS

Using cleanliness and preparation precautions previously reported,<sup>3</sup> a pool of human serum was prepared. Pooled serum was dispensed to a final volume of 5 mL into 21 vials. In three vials nothing was added; in three vials 60  $\mu\text{L}$  of 0.1%  $\text{HNO}_3$  was added to approximate the acid conditions in the spiked samples. In the next five sets of three vials each, 20, 40, 60, 80, and 100  $\mu\text{L}$  of standard was added. This gave serum spiked samples of no addition; addition of acid only; 4, 8, 12, 16, and 20 ng/mL Ti; and 2, 4, 6, 8, and 10 ng/mL of Al and V. All specimens were thoroughly mixed and then split into two duplicate 21-specimen sets. Each laboratory had three subsets of spiked samples that each contained two "unspiked serum samples" that served as serum blanks (pooled serum baseline values) and five spiked serum samples. The standard solution used to spike the serum samples had concentrations of 1000 ng/mL Ti and 500 ng/mL Al and V in 0.1%  $\text{HNO}_3$  prepared from 1000 mg/mL certified atomic spectroscopy standards (Fisher). Serum samples were frozen after preparation. Samples were thawed prior to analysis and diluted according to laboratory protocols.<sup>2,3</sup>

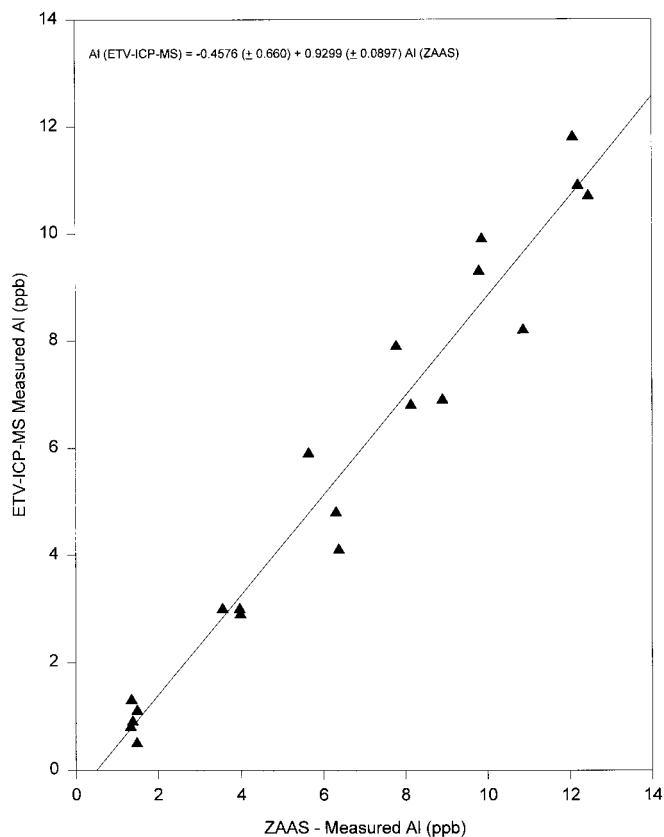
The ZAAS analytical process for Ti, Al, and V in serum has been previously reported.<sup>1,3</sup> The method detection limits in human serum diluted 1:3 with 0.06N  $\text{HNO}_3$ , using standards in reference serum (Life Source<sup>TM</sup>), are 2.11 ppb Ti, 0.17 ppb Al, and 0.81 ppb V.

The ICP-MS analytical process for Ti, Al, and V was described previously.<sup>2</sup> The method detection limits in human serum diluted 1:2 in 0.1 vol%  $\text{HNO}_3$  with suitable matrix modifiers, using high purity standards, are 0.7 ppb Ti, 0.4 ppb Al, and 0.1 ppb V.

## RESULTS AND DISCUSSION

The advantages of ICP-MS as a chemical analytical tool for trace element analysis have been extensively discussed. Of particular value is the ability to perform simultaneous analysis of several elemental species and in many cases through the identity of molecular species, identify the valence of the metallic moiety.<sup>4</sup> Recent reviews<sup>5,6</sup> have discussed its use for trace elemental analysis in human serum and various biological reference materials, such as bovine liver. Despite its relative complexity compared to ZAAS, the use of ICP-MS seemed to be indicated to us because we were aware that many potentially physiologically important trace elements that were previously thought to be present normally in 1 to 10 ppb level concentrations actually have values below 1 ppb in uncontaminated human serum from healthy controls.

The results of the two methods were compared using



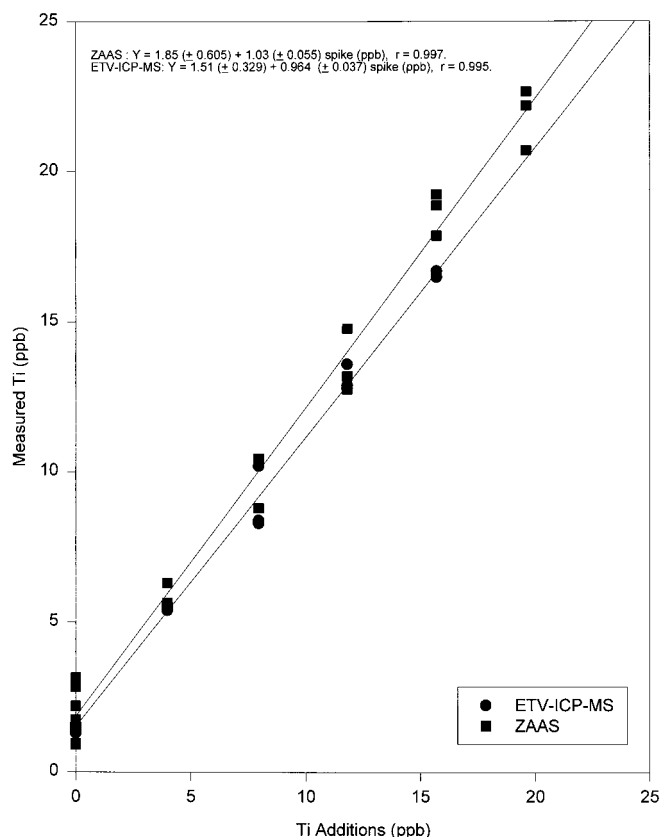
**Figure 1.** Regression equation for aluminum comparing the measured values obtained by Zeeman background corrected atomic absorption spectrometry (ZAAS) vs. electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS). The 95% confidence interval of the slope and y intercept are given in parentheses. Note that the 95% confidence interval of the y intercept includes the desired value of 0 and the 95% confidence interval of the slope includes the desired value of 1.0.

the regression lines design according to Miller and Miller.<sup>7</sup> This design is frequently used to validate a "new method" by applying it to a series of samples already studied by another "reputable standard" method. For Al the ZAAS and ETV-ICP-MS methods can both be considered reputable standard methods because the calibration curves were studied and the certified values in SRM 1598 bovine serum, from the National Institute of Standards and Technology (NIST), were obtained. Neither the ZAAS nor the ETV-ICP-MS method is a reputable standard method for Ti and V because neither method was tested with a reference material (no reference material is available for Ti or V in a matrix similar to serum). In these two cases the classic regression line validation method was modified. Instead of the analytical values from a reputable standard method, the analyte concentrations prepared in the pooled serum were used as reference values. Comparisons were made with ZAAS and the ETV-ICP-MS values versus the prepared concentrations in the pooled serum.

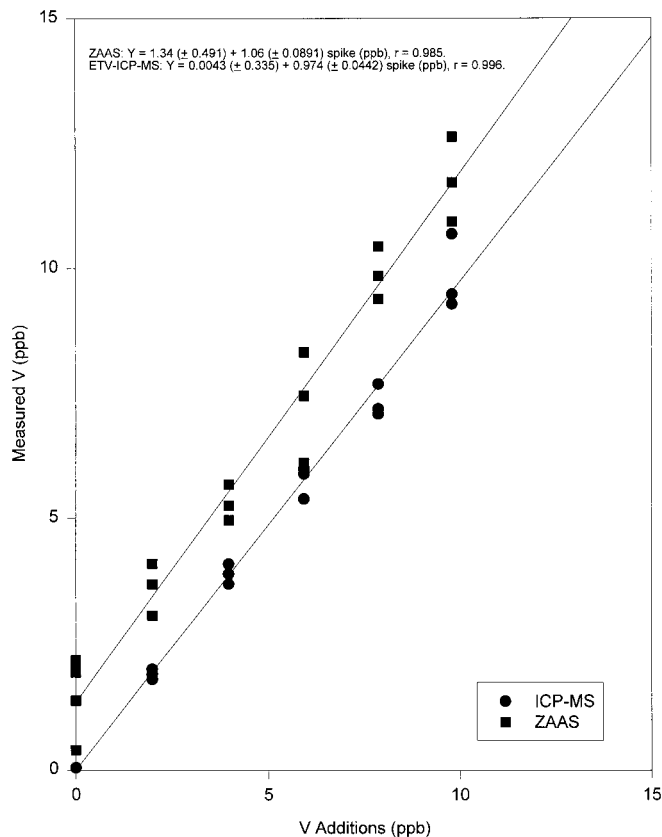
As mentioned above, a regression relation can be developed for Al between the measured values determined by

ETV-ICP-MS and ZAAS because a certified reference material is available for verification. The NIST SRM 1598 (inorganic constituents in bovine serum, certified for  $3.81 \pm 0.9$  ppb of Al) was analyzed by both methods, the results of which were within the certified range of 3.7 and 4.0 ppb for ETV-ICP-MS and ZAAS, respectively. In the regression analysis for Al using the two measurement techniques (Fig. 1), the 95% confidence interval of the y intercept includes 0 and the 95% confidence interval of the slope includes 1. This demonstrates the equivalence of ZAAS and ETV-ICP-MS for Al determination.

Validation of the methods for titanium determination using reference materials is not possible because no such materials are available. Therefore, the comparison and validation of the ZAAS and ETV-ICP-MS are accomplished by analyzing the correlation of the measured concentration of each method against the spiked concentration in the serum sample. The highest spike is excluded in the ETV-ICP-MS data analysis because it is over the linear range of the method. The 95% confidence interval of the slopes for both methods overlap the desired value of 1 (Fig. 2). The intercept of the curves cannot be substantiated because



**Figure 2.** Regression equations for titanium comparing measured vs. spiked concentration for Zeeman background corrected atomic absorption spectrometry (ZAAS) and electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS). The 95% confidence interval of the slope and y intercept are given in parentheses. Note that in all equations the slope is within 5% of the desired value of 1.0 and the 95% confidence intervals bracket 1.0.



**Figure 3.** Regression equations for vanadium comparing measured vs. spiked concentration for Zeeman background corrected atomic absorption spectrometry (ZAAS) and electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS). The 95% confidence interval of the slope and y intercept are given in parentheses. Note that in all equations the slope is within 5% of the desired value of 1.0 and the 95% confidence intervals bracket 1.0.

the concentration of Ti in the base serum is unknown. However, the intercept ranges of the two methods overlap. Because the two methods are independent, the common intercept suggests a true concentration of Ti in the base serum, which in turn supports the validity of the method. It is possible, although unlikely, that the intercepts for both methods are biased in the same direction by the same magnitude. Should that be the case, the data collected with ZAAS will still be comparable to that from ETV-ICP-MS. ETV-ICP-MS is also more precise, as evidenced by the smaller uncertainty associated with the slopes of the two techniques. ETV-ICP-MS produces slightly lower measured values than the ZAAS method.

The data analysis method for Ti is also applied to V due to the lack of a certified material for the element. The data for the regression analysis of the measured concentration versus prepared concentration in the serum are shown in Figure 3 for both techniques. Again the 95% confidence interval of the slopes overlaps the desired value of 1 for each method. Nevertheless, the intercept ranges of the two regression curves do not overlap. The intercept of the ETV-ICP-MS curve is believed to be more accurate because the

normal concentration of V in serum is believed to be at sub parts per billion levels.<sup>8</sup> In addition, the NIST SRM 1598 was analyzed by ETV-ICP-MS and the value was found to be <0.1 ppb, while the NIST information value (noncertified) is 0.062 ppb. Furthermore, the uncertainties in the slopes for each method suggest that ETV-ICP-MS is the more precise method.

An additional comparison can be gained from examining the time required to perform these two sets of studies: ZAAS, nine analytic shifts (nominally 8–9 h each); ICP-MS, three analytic shifts.

## CONCLUSIONS

The results for this comparison study were very encouraging. The far lower detection limits for Ti and V by ETV-ICP-MS offers a distinct advantage in that far fewer specimens will be reported as undetected. Despite the significantly higher capital cost of ETV-ICP-MS equipment, the much reduced labor cost and higher throughput provided by the more rapid simultaneous elemental analysis is a strong argument for its use. Results obtained in this study for Ti and Al suggest that we will be able to safely compare older data obtained by ZAAS with those from newer studies obtained by ICP-MS.

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## REFERENCES

1. Jacobs JJ, Skipor AK, Black J, Tuttle MC, Urban RM, Galante JO. Metal release and excretion in patients with titanium-base alloy total hip replacement components. *J Bone Joint Surg* 1991;73A:1475–1486.
2. Yu L, Koirtiyohann SR, Rueppel ML, Skipor AK, Jacobs JJ. Simultaneous determination of aluminum, titanium and vanadium in serum by electrothermal vaporization-inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* 1997;12:69–74.
3. Skipor AK, Jacobs JJ, Schavocky J, Black J, Galante JO. The determination of titanium in human serum by Zeeman electrothermal AAS. *Atom Spectrosc* 1994;15(3):131–134.
4. Vela NP, Olson LK, Caruso JA. Elemental speciation with plasma mass spectroscopy. *Anal Chem* 1993;65(13):585A–597A.
5. Yoshinaga J. Inductively coupled plasma atomic emission spectroscopy and ICP mass spectrometry. *Nippon Rinsho* 1996;54(1):202–206.
6. Vanhoe H. A review of the capabilities of ICP-MS for trace element analysis in body fluids and tissues. *J Trace Elements Electrolytes Health Dis* 1993;7(3):131–139.
7. Miller JC, Miller JN. *Statistics for Analytical Chemistry*. New York: Wiley; 1988.
8. Versieck J, Cornelis R. *Trace Elements in Human Plasma or Serum*. Boca Raton, FL: CRC Press; 1989.