# TECHNIQUES USED IN TRACE ELEMENT ANALYSIS OF URINARY CALCULI BY ATOMIC ABSORPTION SPECTROSCOPY

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Atomic absorption spectroscopy (AAS) was found to be a suitable method for the estimation of iron, copper, zinc, magnesium, aluminium and lead in urinary calculi; graphite furnace electrothermal atomisation was required for lead and flame atomisation for the others. A standard urinary stone solution was made up and calibrated for each element using the method of standard additions. In all case, absorption varied linearly with concentration; reproducibility, accuracy and recovery were satisfactory for all elements. Conventional atomic absorption standards in 1% nitric acid solution had low apparent analyte concentrtions against the standard stone solution. If those were used as primary standards it was found that apprent values as a percentage of the true values would be: Fe, 108.5; Cu, 103.1; Zn, 106.4; Mg, 125.0; Al, 103.6; Pb, 102.2%. Also, apparent concentrations of magnesium, zinc and copper in dilute nitric acid solution varied significantly with acid strength. It was concluded that in order to minimise matrix and pH effects, urinary stone analysis should only be done against standard urinary stone solution.

*Key words:* Techniques, A. A. S. Methods, Urinary calculi.

## Introduction

A great deal of interest has developed world-wide in the role played by trace elements in metabolic processes in humans and animals, both in health and disease. This is evident by the enormous growth in published material on these subjects over the past twenty years and the number of conferences and meetings held annually. There are over 40 elements present in the human body of which at least 14 are known to be essential trace elements, being present to the extent of less than 50 mg in the whole body and which are essential in the diet for health and well-being.

The causes and initiation processes of urinary stone formation (urolithiasis) are obscure in the great majority of cases but evidence is accumulating that trace elements play a role in mineralisation inspite of their low concentrations in blood, urine and in the calculi themselves [1-10].

From the point of view of accuracy, speed and cost per test, the estimation of trace elements by atomic absorption spectroscopy is the most convenient, although the cost of the instrument itself is high. A solution of the sample to be analysed is either aspirated or injected into the instrument depending on whether the flame atomisation or the graphite furnace electrothermal mode is used. Analysis by the former is very much quicker but is often restricted by the relatively high detection limit.

In the measurement of trace element concentrations, we are often in the realm of estimating parts per billion (ppb), a scrupulously dust and contamination-free environment must

be used and great care taken to prevent the contamination solutions.

Unfortunately, good results are not obtained by comparing the absorption at the appropriate wavelength for the analyte with that of a standard solution in either water or in dilute acid when handling body fluids, as is usully the case for inorganic material. Differences in pH and viscosity often have profound effects as well as the presence of interfering substances in the matrix of the sample. These effects can be very large in the analysis of blood and urine samples and here we have found them to be significant in the analysis of urinary stone solutions.

Owing to the general lack of standards in body fluids, reports concerning attempts to overcome the viscocity problem in blood analysis, for example, have been made so that aqueous standards can be used. Some researchers have added glycerol to aqueous standards to increase the viscosity to that of serum or plasma [11]. Deproteinisation of blood, serum or plasma to decrease viscosity and to prevent clogging of the burner or coating of the graphite tube may result in losses of analyte of up to 20% by co-precipitation [12]. One of us (W.W.T.M.) using a Pye-Unicam SP 2900 atomic absorption spectrophotometer by flame aspiration found errors which give from 77.9% of the true value for copper to 188.4% for a lead in blood by calibrating the instrument with aqueous standards [13].

In spite of this and other well documented evidence, results are still appearing and are being presented at conferences which are spurious due to ignorance of these facts. Over the years, the normal levels of many trace elements in blood,

plasma, serum and urine have steadily decreased due to increased awareness of contamination and of the above effects.

In the present work, we describe the method for calibrating a laboratory-made standard urinary stone solution, the evidence that our analytical techniques are good, the effects of using conventional dilute acid standards and of different acid strengths on analytical results.

## Materials and Methods

*Water.* All water used was doubly deionised distilled stored in a previously acid-soaked and well washed polythene container. It was tested for zero response by atomic absorption spectroscopy for each element under investigation.

*Glassware and pipettes.* Nichiryo digital micropipette model 5000 DG and a juster TM 1100DG were used. Tips and all glassware were soaked for at least 24 hrs in 20% $HNO_3$ to ensure metal-free surfaces. They were then washed atleast six times in pure water before oven drying.

*Chemicals.* "Spectrosol" grade reagents and acids from B.D.H., Poole, U.K. were used.

*Instrument.* Trace element analysis was carried out on a Hitachi Z-8000 atomic absorption spectrometer equipped with Zeeman background correction and a data processor. Flame atomisation was used for iron, copper, zinc, magnesium and aluminium, and graphite furnace atomisation for lead.

Instrumental conditions.

| Element | Fe | Cu | Zn | Mg | Al |
|---|---|---|---|---|---|
| *Flame atomisation* | | | | | |
| Lamp current (mA) | 10.0 | 7.5 | 10.0 | 7.5 | 10.0 |
| Wavelength (nm) | 248.3 | 324.8 | 213.8 | 285.2 | 309.3 |
| Slit width (nm) | 0.2 | 1.3 | 1.3 | 1.3 | 1.3 |
| Oxidant | Air | Air | Air | Air | $N_2O$ |
| Oxidant pressure (kg/cm²) | 1.60 | 1.60 | 1.60 | 1.60 | 1.40 |
| Fuel | $C_2H_2$ | $C_2H_2$ | $C_2H_2$ | $C_2H_2$ | $C_2H_2$ |
| Fuel pressure(kg/cm²) | 0.30 | 0.30 | 0.20 | 0.20 | 0.40 |
| Burner height (mm) | 5.5 | 7.5 | 7.5 | 7.5 | 10.0 |
| Calculation mode | | | Integration | | |
| Calculation time (sec) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

*Graphite furnace*

| | |
|---|---|
| Element | = Pb |
| Lamp current | = 7.5 mA |
| Wavelength | = 283.3 nm |
| Slit width | = 1.3 nm |
| Cuvette | = Graphite tube |
| Carrier gas (Ar) | = 200 ml/min. |
| Interrupted gas | = 30 ml/min. |
| Sample volume | = 20 µl |

Calculation mode = integration
Calculation time: = 10.0 sec.

Temperature program for lead.

| | | Temperature (°C) | | |
|---|---|---|---|---|
| No. | Stage | Start | End | Time (sec.) |
| 1. | Dry | 80 | 120 | 30.0 |
| 2. | Ash | 400 | 400 | 30.0 |
| 3. | Atomisation | 2000 | 2000 | 10.0 |
| 4. | Cleaning | 2400 | 2400 | 3.0 |

*Preparation of standard urinary stone solution.* Several urinary stones were carefully cleaned with water and oven dried at 110° before grinding to powders in an Agate mortar and pestle. About 10g of the combined powders was dissolved in 15 ml of concentrated nitric acid (Spectrosol) grade, BDH, Poole, U.K.) and heated until all was in solution and any chemical reaction had ceased. The resulting solution was diluted to one litre with pure water.

## Results and Discussion

In order to investigate the role played by trace elements in urolithiasis, it is necessary to estimate their concentrations in a large number of calculi of different types, their concentration in the urine of the patients and then to draw conclusions if possible. Analysis was carried out by atomic absorption spectroscopy. Initially, it was necessary, for reasons discussed in the introduction, to make a standard stone solution and to estimate in this solution the elements of interest. The known concentrations were then used, later to calibrate the instrument for the trace element analysis of the urinary stones under investigation. Also it was necessary to obtain evidence that our analytical procedure was satisfactory; the results are discussed here.

Using the method of standard additions [14] each trace element was estimated five or six times, after suitable dilution, on different days using freshly made up solutions. Lead was estimated using graphite furnace electrothermal atomisation and iron, copper, zinc, magnesium and aluminium by flame aspiration. Initially a phosphate-magnesium nitrate moderator was used in the estimation of lead to prevent premature volatilisation [15] but was later found to be unnesessary. On every occasion a linear plot was obtained of absorbance against element concentration. Extrapolation to zero absorbance gave the concentration in the treated standard stone solution. The plot and extrapolation was automatically done by the spectrometer computer. From the results Table 1, we conclude that the reproducibility of results for each element was satisfactory: coefficients of variation (CV) ranging from 3.7% for lead to 12.1% for magnesium.

The actual standard additions taken for each element are given in Table 2. On having taken any one curve for any element, knowing the concentration at each point which was that of the respective trace element found in the standard stone solution plus the standard addition at that point and taking the found concentration from the plot, the recovery was easily calculated. The mean recovery for each run is given in Table 2 together with the mean of these means for each element. The CVs in general were low, ranging from 2.3% for zinc to 7.6% for magnesium. This confirmed that the reproducibility is satisfactory. The mean of the mean recoveries varied from 95.9% for aluminium to 103.0% for copper; in other words, none were far from 100% and with the low CVs one can say that analytical results are accurate for these elements.

A spectroscopically pure solution of each analyte in 1% nitric acid was treated as a sample on each standard additions plot. "Spectrosol" grade (BDH, Poole, U.K.) solution were used. These are the conventional standards used normally in atomic absorption spectroscopy. Almost all the individual found concentration were lower than the known value. The CVs were low, indicating good reproducibility, but the means of the found concentrations of each element varied from 2.2% lowers than the true value for lead to 20.0% for magnesium

(Table 3). For example the instrument were calibrated with a magnesium standard made up and assumed to be 0.3 ppm in 1% nitric acid, analytical results would be 125.0% of the true values. For correct results, the value 0.24 ppm should be used.

It is well documented that for inductively coupled plasma spectroscopy the apparent analyte concentrations vary with acid strength [16]. Solutions of known concentration of magnesium (0.3 ppm), zinc (0.5 ppm) and copper (100 ppb) were made up in pure water and in solutions containing various strengths of nitric acid. For each element the instrument was calibrated with the solution in pure water and the concentrations were found to vary with acid strength. Results are in Fig.1 and it may be concluded that the variations are significant.

Thus considerable analytical errors may occur if the instrument is calibrated with conventional standards in water or dilute acid using "weighed out" concentrations. Calibration with a standard stone solution of known trace element concentration would minimize the effects of differences of matrix and pH, providing that the solutions of the urinary stones under test are made up in the same way as the standard stone solution.

TABLE 1. ANALYTICAL DATA FOR THE STANDARD STONE SOLUTION.

| Element | Dilution | No of runs | Mean conc. (ng/ml) | Range (mg/ml) | S.D. (ng/ml) | C.V. (%) |
|---------|----------|------------|---------------------|---------------|--------------|----------|
| Fe | 1 : 1.33 | 5 | 427 | 400-467 | 21.3 | 5.0 |
| Cu | 1; 1.11 | 5 | 67 | 55-78 | 7.8 | 11.6 |
| Zn | 1; 2.00 | 5 | 70 | 60-76 | 6.0 | 8.6 |
| Mg | 1; 25 | 6 | 3500 | 3000-4000 | 425 | 12.1 |
| Al | 1; 10 | 6 | 2700 | 2500-2900 | 170 | 6.3 |
| Pb | 1; 2.00 | 5 | 132 | 124-136 | 4.9 | 3.7 |

TABLE 2. STANDARD ADDITIONS AND RECOVERY DATA.

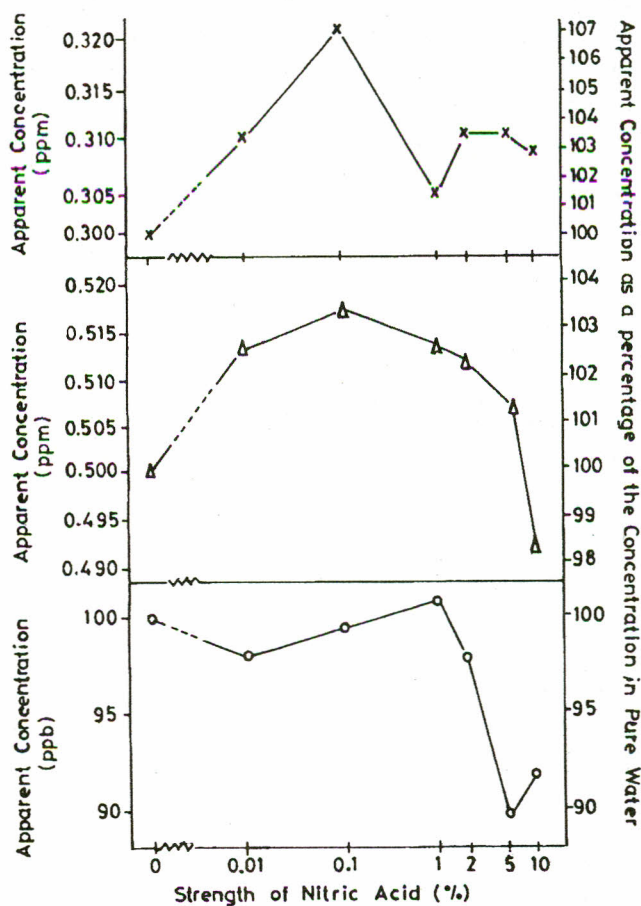| Element | Standard additions (ppm) | Means recoveries (%) | Mean of means (%) | S.D. (%) | C.V. (%) |
|---------|--------------------------|----------------------|-------------------|----------|----------|
| Fe | 0,2,0.5,1.0 1.5,2.0 | 96.5,100.0,95.6, 103.2,101.2 | 99.3 | 3.2 | 3.2 |
| Cu | 0.2, 0.4,0.6 0.8, 1.0 | 106.9,103.6,100.4, 104.3, 99.7 | 103.0 | 3.0 | 2.9 |
| Zn | 0.1,0.2,0.3, 0.4,0.5 | 98.8,103.8,100.5 103.7, 103.7 | 102.1 | 2.3 | 2.3 |
| Mg | 0.05,0.10,0.15 0.20,0.30 | 94.6,92.2,112,6 101.6,94.0,99.4 | 99.1 | 7.5 | 7.6 |
| Al | 0.5,1.0,1.5 2.0,2.5 | 91.6,93.0,95.3, 99.5,96.0,99.6 | 95.9 | 3.3 | 3.4 |
| Pb | 10.20,40,60 80(ppb) | 94.5,96.1,106.0 99.2,103.4 | 99.8 | 4.8 | 4.8 |



Fig. 1. Variation in the apparent concentration of X Magnesium, Δ Zinc and O Copper in various strengths of nitric acid solution.

TABLE 3. APPARENT CONCENTRATIONS OF STANDARDS IN 1% NITRIC ACID FROM THE STANDARD ADDITION CURVES.

| Element | Known conc. of std. soln. (A) | Apparent mean conc. (B) | Range (ppm) | S.D. (ppm) | C.V. (%) | Percentage error (C) | Potential analytical results (D) |
|---|---|---|---|---|---|---|---|
| Fe | 5.0 ppm | 4.61 ppm | 4.49 - 4.71 | 0.08 | 1.74 | -7.80% | 108.5% |
| Cu | 1.0 ppm | 0.97 ppm | 0.87 - 1.06 | 0.09 | 9.28 | -3.00% | 103.1% |
| Zn | 0.5 ppm | 0.47 ppm | 0.46 - 0.49 | 0.01 | 2.68 | -6.00% | 106.4% |
| Mg | 0.3 ppm | 0.24 ppm | 0.21 - 0.26 | 0.02 | 8.90 | -20.00% | 125.0% |
| Al | 2.0 ppm | 1.93 ppm | 1.84 - 1.98 | 0.07 | 3.66 | -0.50% | 103.6% |
| Pb | 80 ppb | 78.26 ppb | 76.58 - 80.48 ppb | 1.63 pbb | 2.09 | -2.20% | 102.2% |

C = (B/A100) - 100%, D = A/B x 100%. This is the percentage of the true result which will be obtained if an unknown is estimated after celebrating the spectrometer with the standard in 1% nitric acid using its true valuer (A). Using the apparent value (B) would give the correct result.

## References

1. A. E. Sobel, S. Nobel and A. Hanok, Proc. Soc. Exptl. Biol Med., **72**, 68 (1979).

2. J. L. Meyer and E. F. Angino, Invest. Urol.,**14**, 347 (1977).

3. W. F. Neuman, T. Y. Toribara and B.J. Mulryan, Arch. Biochem., **98**, 384 (1962).

4. J. F. Desmars and R. Tawarshi, Biochem. Biophys. Acta; **131**, 256 (1973).

5. D. J. Sutor, Br. J. Urol.,**41**, 171 (1969).

6. A. A. Lovinson, M. Nosal and M. Davidman *et al*. Invest. Urol.,**15**, 270 (1978).

7. W. C. Thomas, (Jnr.), Proc. Soc. Exptl. Biol. Med., **170**, 321 (1982).

8. G. Hammarsten, C. R. Trav. Lab. Carlsberg, **17**, 1 (1929).

9. D.R. Simpson, Am. Mineral., **53**, 432 (1968).

10. W. F.Neuman and M. W. Neuman, Chem. Revs.,**53**, (1953).

11. K. B. Sprenger and H. E. Franz, Clin. Chem., **29**, 1522 (1985).

12. T.R. C. Boyd and S. W. N. Wu, Clin Chem. Acta, **88**, 49 (1978).

13. S. Haider and W. W. T. Manser, J. Pak. Med. Assn.,**39**, 17 (1989).

14. W. W. T. Manser and M. A. Khan, J.Pak. Med. Assn.,**39**, 43 (1989).

15. W. Slavin, Sci. Tot. Environ.,**71**, 17 (1988).

16. M. Thompson and J.N. Walsh, *A Handbook of Inductively Coupled Plasma Spectrometry* (Blackie, Glasgow & London, 1983), pp.31.