Trace Elements Detection in Urine by Atomic Absorption Spectroscopy

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Summary: A technique has been developed to minimize matrix effects in urine for the estimation of trace elements by atomic absorption spectrophotometer using Flame or electro thermal atomization. The matrix and pH effects are known to change the absorption. This study may be useful for the determination of trace elements in the absence of standard/certified reference materials of urine. Our interest was the determination of trace elements in urine. For this purpose a standard urine solution was made up for the estimation of elements. At least 3-5 standard calibration curves were taken for each element. Percentage recoveries were also determined and found satisfactory. The following percentage recoveries for different elements were found; Ca (101.5 %), Mg (102.9 %), Fe (101.6 %), Zn (102.8 %), Cu (99.05 %). Cr (100.6 %) and for Ni (105.3 %).

The same elements in aqueous standards were also determined against the above calibration curves to see if they could be used instead of the stock standards. The observed concentration of aqueous standards was found to be different from actual values except copper, e.g. 4.0 μg/ ml Ca was found to be 4.67, 0.30 μg/ ml Mg was 0.32, 0.40 μg/ ml Fe was 0.47, 0.50 μg/ ml Zn was 0.52, 40 ng/ mg Cr was 39.15 and 80 ng/ ml Ni was found 98.76. No difference between the actual and observed values of copper was found. Values of observed aqueous standards may be taken as standard concentration against which respective elements can be estimated.

Introduction

Trace elements play an important role in the metabolic activities of human body [1-10]. Several trace elements are cofactors for enzymes without which enzymatically controlled reaction chains cannot occur [11, 12]. Because of the importance of trace elements in metabolic process their accurate determination in various body fluids and tissues is very important [10]. Although Atomic Absorption Spectroscopy is a very precise, accurate and sensitive analytical technique, matrix variation may abscure the reliability of trace element determination by this method [3]. Difference in pH, viscosity, density as well as presence of interfering substances affect the analysis of samples. These effects can be very large in the analysis of blood and urine. It is therefore essential that the matrix of standard and analyte should be comparable.

Sample preparation is a major concern in flame atomization atomic absorption spectrophotometer (FAAAS) as it basically governs the analytical performance characteristics. The major FAAS sample preparation methods are dilution, deprotenization, acid extraction, chelation-solvent extraction and destruction of organic matter by dry ashing or wet digestion. The purpose for sample treatment is to decrease viscosity and to prevent clogging of the burner which may result in losses of analyte. Dilution methods are used to over come the problem of matrix *i.e.* viscosity and presence of interfering substances in the matrix of the sample [3]. When dilution procedure is inadequate the solvent extraction method is used. Destruction of organic matter by wet digestion and/or dry ashing is also of common practice. This procedure converts trace elements bound to organic structures into inorganic or ionic form in solution. The normal levels of many trace elements in biological samples have steadily improved due to elimination of contaminants [13] and interfering substances [2, 5].

For GFAAS several sample preparation methods including direct injection, use of diluents, complete oxidation by dry ashing or wet digestion are commonly used. These procedures prevent the loss of analyte which could result from coating of the graphite tube.

In the present work the method for calibrating a laboratory made standard of urine is

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Table-1: Standard additions and their recoveries in urine.

Elements	Technique	Std. Addition	Mean Recoveries (%)	Mean of Means	Standard Deviation	C.V (%)	RSD
Calcium μg ml^{-1}	F.A	0.500, 1.00, 2.00 3.00, 4.00	0.104×10^3, 0.101×10^3, 0.101×10^3, 0.101×10^3, 0.101×10^3	102%	1.39	1.37	0.0100
Magnesium μg ml^{-1}	F.A	0.050, 0.100, 0.150 0.200, 0.300	0.102×10^3, 0.105×10^3, 0.102×10^3	103%	1.57	1.53	0.0200
Iron μg ml^{-1}	F.A	0.0500, 0.100, 0.200 0.400, 0.500	0.100×10^3, 0.100×10^3, 0.105×10^3	102%	2.51	2.47	0.0200
Zinc μg ml^{-1}	F.A	0.0500, 0.100, 0.200 0.30, 0.50	0.104×10^3, 0.103×10^3, 0.101×10^3	103%	1.54	1.50	0.0100
Copper μg ml^{-1}	F.A	0.0200, 0.0400, 0.0600 0.0800, 0.100	0.098×10^3, 0.100×10^3, 0.099×10^3	99.1%	0.850	0.85 0	0.0100
Chromium ng ml^{-1}	G.A	10.0, 20.0, 40.0, 60.0, 80.0	0.099×10^3, 0.102×10^3, 0.101×10^3	101%	1.67	1.66	0.0200
Nickel ng ml^{-1}	G.A	10.0, 20.0, 40.0, 60.0, 80.0	0.101×10^3, 0.107×10^3, 0.108×10^3	105%	4.18	3.97	0.0400

F.A. Flame Atomization G.A Graphite Tube Atomization (Electro thermal Atomization)

described. This procedure provides good, accurate and precise results. Most important is that this procedure can be applied when certified reference material/standard reference material are not available. Present study also shows the effects of conventional aqueous standard on body fluids determination. This stock standard can also be stored for a long time whereas the same cannot be done with urine.

Results and Discussion

In urine, the concentration of elements under study in general was low so after complete oxidation and evaporation the residue was dissolved in distilled water. It has been reported that acid strength effects analyte concentration [14], therefore the conc. of nitric acid was kept nearly identical in all the standard addition solutions, *i.e.* 1 %. The conc. of acid was also kept the same in blank. The acid concentration in standard and sample solutions was not raised any more in order to prevent corrosion of the conventional neubulizer fitted with a stainless steel capillary tube.

Trace elements in stock solutions were estimated by standard addition technique as shown in Table-4.

Table-1 shows that each calibration curve was made by taking five standard solutions to ensure the linearity of the graph. Ca, Fe, Cu, Zn & Mg as shown by the table were estimated by flame atomization while Cr and Ni on electro thermal atomization. Each calibration curve was taken at least 3 times on different days by using freshly made up set of standard solutions as shown in Table-2.

Table-2: Analytical data for the standard urine solution.

Element	No. of Runs	Mean Conc.	Range	S.D	C.V %	RSD
Calcium mg day^{-1}	5	222	221---225	1.77	0.790	0.00800
Magnesium mg day^{-1}	3	108	107---109	1.54	1.42	0.0100
Iron mg day^{-1}	3	0.220	0.210---0.220	0.0100	4.55	0.0500
Zinc mg day^{-1}	3	1.11	0.990---1.18	0.100	9.40	0.0900
Copper mg day^{-1}	3	0.0700	0.0600---0.0700	0.00600	8.25	0.0800
Chromium ng day^{-1}	3	10.3	9.78---11.1	0.690	6.70	0.0700
Nickel ng day^{-1}	3	234	232---237	2.93	1.25	0.0100

S.D Standard Deviation
C.V Coefficient of Variance
RSD Relative Standard Deviation

This table also shows the analytical data for the standard urine solution. The range of coefficient of variance was from 0.79 % for calcium to 9.40 % for zinc.

Coefficient of variance (C.V.) proves that percentage recovery was satisfactory. It was 101.5 % for Ca, 102.90 % for mg, 101.60 % for Fe, 102.80 % for Zn, 99.05 % for Cu, 100.60 % for Cr and 105.30 % for Ni. Percentage recoveries prove the accuracy of analytical results. Plots of absorbance against concentration were linear in each case as shown in fig 1 to 7. Values of standard deviations indicate that the estimation of elements was accurate and precise.

The estimated values of aqueous standard except copper are substantially different from the actual values (Table-3). It indicates that considerable analytical errors may occur if the instrument is calibrated with conventional standards in water or dilute acids. Observed concentration of calcium, magnesium, iron, zinc, and nickel were found slightly higher than their actual aqueous concentration. Observed value of aqueous solution would minimize the effect of differences of matrix.

Table-3: Apparent concentration of aqueous standards in 1 % nitric acid against standard addition curves.

Element	Known Conc. of Standard Solution (A)	Range	Apparent Mean (B)	% Variation (C)	Potential Analytical Results (D=A/B×100)
Calcium	4.00 $\mu g\ ml^{-1}$	4.57---4.73	4.67 $\mu g\ ml^{-1}$	+16.8%	85.7%
Magnesium	0.300 $\mu g\ ml^{-1}$	0.300---0.330	0.320 $\mu g\ ml^{-1}$	0.00%	93.8%
Iron	0.400 $\mu g\ ml^{-1}$	0.460---0.490	0.470 $\mu g\ ml^{-1}$	+17.5 %	85.1%
Zinc	0.500 $\mu g\ ml^{-1}$	0.500---0.530	0.520 $\mu g\ ml^{-1}$	+4.00%	96.2%
Copper	0.100 $\mu g\ ml^{-1}$	0.100---0.110	0.100 $\mu g\ ml^{-1}$	0.00%	0.100×10^{3} %
Chromium	40.0 ng ml^{-1}	38.4---39.9	39.2 ng ml^{-1}	-2.13%	102%
Nickel	80.0 ng ml^{-1}	$96.1---0.100\times 10^{3}$	98.8 ng ml^{-1}	+23.5%	81.0%

Experimental

Reagents

Double distilled water was used during all studies which were tested for zero response by Atomic Absorption Spectroscopy for each element under estimation.

All glassware, plastic bottles and nozzles of micropipettes were soaked for at least 24hrs in 20 %, HNO_3 to ensure metal free surface [15]. They were then washed well at least six times with pure distilled water. Plastic bottles and nozzles were dried b/w 70° -80°C while glass wares were dried at 105°C. Concentrated nitric acid (BDH, Poole Dorest, UK), Lanthanum chloride (Winlab, Middle Sex, UK) and Hydrogen per oxide (Merck, Darmstadt, Germany) were used during all studies. Working standard of each element used for standard addition technique was diluted from respective stock solution of 1000 ppm (Merck Darmstadt, Germany).

Apparatus

Atomic Absorption Spectrophotometer (Hitachi Model Z-8000) equipped with Zeeman background corrector and a data processor was used. Flame atomization was used for Fe, Cu, Zn, Mg, and Ca while electro thermal atomization was used for Cr & Ni. The parameters were set according to the instrument manufactures instruction as shown in Tables-4 & 5A-5C.

Preparation of Stock Solution and Aqueous Standard

The general procedure used, was followed as suggested in ref. [1, 2, 16]. The final volume was kept half of volume of the original urine.

Urine sample was treated with pure nitric acid (BDH, Poole Dorest, UK), perchloric acid (Merck, 64271, Darmstadt, Germany) and hydrogen peroxide (Merck, 64271, Darmstadt, Germany) in the ratio of 10:2.5:1 respectively. This solution was evaporated to dryness. The residue obtained was treated with nitric acid alone [16]. The final solution was made up to mark in the volumetric flask. The choice of size of volumetric flask was depended on

Table-4: Instrumental conditions for flame atomization.

Conditions	Element				
	Calcium	Magnesium	Iron	Zinc	Copper
Lamp Current (mA)	7.5	7.5	10.0	10.0	7.5
Wave Length (nm)	422.7	285.2	248.3	213.8	324.8
Slit width (nm)	0.4	1.3	0.2	1.3	1.3
Oxidant	Air	Air	Air	Air	Air
Oxidant Pressure (Kg cm^{-2})	1.6	1.6	1.6	1.6	1.6
Fuel	C_2H_2	C_2H_2	C_2H_2	C_2H_2	C_2H_2
Fuel Pressure (Kg cm^{-2})	0.35	0.20	0.3	0.2	0.3
Burner Height (mm)	10.0	7.5	7.5	7.5	7.5
Calculation mode	Integration				
Calculation time (sec)	1.0	1.0	1.0	1.0	1.0

Table-5a: Electro thermal atomization.

Conditions	Element	
	Chromium	Nickel
Lamp Current	7.5	10.0
Wave length	359.3	232.0
Slit width	1.3	0.2
Cuvette	Graphite tube	Graphite tube
Carrier gas (Ar)(ml min^{-1})	200	200
Interrupted gas(ml min^{-1})	30	30
Sample volume (μl)	20	20
Calculation mode	Integration	Integration
Calculation Time	10.0	10.0

Table-5b: Temperature program for chromium.

No.	Stage	Temperature °C Start	End	Time (Sec)
1	Drying	80	120	30.0
2	Ash	700	700	30.0
3	Atomization	2900	2900	10.0
4	Cleaning	3000	3000	3.0

Table-5c: Temperature program for nickel.

No.	Stage	Temperature °C Start	End	Time (Sec)
1	Drying	80	120	30.0
2	Ash	700	700	30.0
3	Atomization	2700	2700	10.0
4	Cleaning	2800	2800	3.0

the concentration of elements under study. Appropriate dilutions were made up if required, such as to keep the concentration of each element within the linear range of absorbance and in above the detection limits (Table-6).

Table-6: Detection limit of elements of AAS for flame and graphite furnace.

Element	Mode of Atomization	Detection Limit
Calcium	Flame	0.003mg L^{-1}
Magnesium	Flame	0.005mg L^{-1}
Iron	Flame	0.02mg L^{-1}
Zinc	Flame	0.005mg L^{-1}
Copper	Flame	0.01mg L^{-1}
Chromium	GF	1.0ng L^{-1}
Lead	GF	0.05ng L^{-1}
Nickel	GF	1.0ng L^{-1}

For the estimation of calcium in urine, $LaCl_3$ was used in final solution. Standard solutions were also prepared in 1 % lanthanum solution [17].

Aqueous standards were made by dilution of Merck stock standards of 1000 ppm (Darmstadt, Germany).

Standardization of Stock Solution

Standard addition technique was used for the estimation of elements. A set of different concentrations of each element was made so that all concentrations remained with in the linear range of absorbance. Zero of each set (without adding standard) was also prepared. Standard calibration curve of each element was prepared on Atomic Absorption Spectrophotometer using flame or furnace. Recovery of each concentration was found out and mean recovery of each graph was calculated.

Apparent concentration of each element in aqueous standard was also determined against its respective calibration graph.

Conclusion

The precision of the most of the concentrations are quite good. The linearity is also excellent over the concentration range of interest. The detection limits for all elements are below the range of concentration. It is therefore, concluded that in order to minimize matrix and pH effects, urine analysis should only be done against their respective standard stock solutions. Apparent aqueous standard concentrations can also be used for the estimation of elements in urine. But there is still need of certified reference material that would help in the evaluation of accuracy for the method when applied to their respective samples.

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