ANALYTICAL LETTERS, 28(6), 1033-1053 (1995)

QUANTIFICATION OF MUSCLE TISSUE MAGNESIUM AND POTASSIUM USING ATOMIC ABSORPTION SPECTROMETRY

KEYWORDS

Determination of magnesium, potassium, cardiac muscle, skeletal muscle, muscle tissue, atomic absorption spectrometry, AAS, Mg^{2+}, K^{+}.

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ABSTRACT

Flame atomic absorption spectrometry (AAS) has been used to measure magnesium (Mg^{2+}) and potassium (K^{+}) contents of wistar rat myocardium and skeletal muscle. Ten to twenty mg of tissue was homogenized in 10 mM hydrochloric acid, centrifuged, and the clear supernatant was diluted three-fold in deionized distilled water before determination of Mg^{2+} and K^{+} concentrations. The calibration curves of both ions were linear from 0.25 to 2.0 mg/L for Mg^{2+} and from 1.5

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to 15.0 mg/L for K^+. The mean values (±SD) of Mg^{2+} and K^+ contents for the analyzed cardiac muscle were 371.97±12.16 mg/Kg of wet tissue with a coefficient of variation (CV) of 3.3%, and 4571.02±359.74 mg/Kg with CV of 7.9%, respectively. Those for various skeletal muscles varied between 323.35-420.60 mg/Kg Mg^{2+}, with CV ranging from 3.2% to 8.7%, and 4883.84-8156.68 mg/Kg K^+, with CV ranging from 4.4% to 10.1%. The within-day CV were 2.8% to 3.8% for Mg^{2+} and 3.2% to 5.9% for K^+, whereas day-to-day CV were 6.2% to 10.2% for Mg^{2+} and 8.7% to 10.5% for K^+. Recoveries were 92.7% to 104.9% for Mg^{2+}, and 93.2% to 106.1% for K^+. Values for Mg^{2+} obtained by the proposed method were significantly different from those obtained by a published tissue digestion method, those for K^+ were not significantly different.

INTRODUCTION

There has been considerable interest recently concerning the change of body electrolytes due to different disease states as well as those occurring during drug treatment[1-5].

The importance of magnesium ion as a component of the cytoplasm of all living cells and transporters has been recognized long time ago[6,7]. It acts as a cofactor in several enzyme systems, hence, influencing a wide range of cellular functions such as transmembrane transport of other ions, glycolysis, respiration, and

muscle contraction[8-11]. It is well established that magnesium and potassium are interrelated, and Mg^{2+} deficiency is also often followed by K^+ deficiency[12-16]. The loss of Mg^{2+} from the body is known to prevent the cell from maintaining its high intracellular potassium concentration[17], and it encourages the renal loss of potassium[18].

Magnesium and potassium are mainly intracellular ions[19] and, consequently, analysis of intracellular Mg^{2+} or K^+ may be useful to confirm a suspected deficiency. A good estimate of the intracellular concentration of Mg^{2+} is achieved by analysis of magnesium in striated muscle[12]. Potassium may also be analyzed, as its metabolism is closely related to that of magnesium[12-15].

Most of Mg^{2+} and K^+ disorders are presently diagnosed based on plasma levels, which are not reliable indicators of true body Mg^{2+} and K^+ stores[20]. Although studies showed that knowledge of electrolytes concentration in erythrocytes[1,4,21] is useful in such conditions, it is felt necessary to supplement, where possible, with monitoring of their level in muscle cells. Most published methods for the evaluation of tissue Mg^{2+} and K^+ status are time-consuming and difficult[16,22-24].

In the present investigation we adopted a modification of the procedure of Al-Khamis *et al.*[25]

for quantification of muscle Mg^{2+} and K^{+} contents. Analytical validation of the proposed procedure is discussed and compared with the results obtained using an established tissue digestion method[24].

EXPERIMENTAL

Instrumentation

A Tissue homogenizing system (Cole-Parmer, USA) was used. It consists of a stirrer motor with variable-speed (500 to 11000 rpm), fan-cooled and 1/15 hp universal motor including an adjustable chuck and mounting rod; appropriate straight teflon pestle (diameter 0.308")/straight glass tube (volume 2 mL) combination; footswitch, for free hands to alternate samples or adjust motor speed; mounted on a laboratory stand, having an aluminum rod and horseshoe-shaped base and rubber suction-cup feet to stabilize the stand during homogenization.

The instrument used for analysis of Mg^{2+} and K^{+} is a Varian flame double-beam atomic absorption spectrophotometer model AA775 (USA). Table I summarizes the instrument parameters used for the analysis of Mg^{2+} and K^{+}.

A Beckman model J-6B centrifuge (USA), and 1-mL and 5-mL "Transferpette" plunger-operated pipettors (Brand GMBH, Germany) were used.

TABLE I

Instrumental Parameters for Analysis of Mg^{2+} and K^{+} in Tissue Extracts

Parameter	Mg^{2+}	K^{+}
Light source (lamp)	Hollow cathode	Hollow cathode
Wavelength, nm	202.5	769.9
Max.lamp current	3.5 mA	5.0 mA
Flame:Bandpass, nm Flame type	1.0 Air/Acetylene	1.0 Air/Acetylene
Read-out mode	Absorbance	Absorbance

Reagents

All reagents were prepared in deionized, distilled water from reagent-grade materials, unless otherwise stated.

Acid-washed glassware was used throughout the study. Plasticware was used whenever possible. Blanks were analyzed throughout the study for quality assurance of the repeatability of the analyses.

Concentrations of magnesium and potassium were expressed in mg/Kg of wet tissue.

Magnesium (ion standard solution), from Fisons scientific equipment (Loughborough, England): magnesium

solution 1000 ppm (mg/L) in approximately 1M Nitric acid.

Potassium (ion standard solution), from Fisons (Loughborough, England): potassium solution 1000 ppm (mg/L) in approximately 1M Nitric acid.

0.5 M Hydrochloric acid (as stock) : Approximately 5 M solution (a 50 mL bottle, BDH, England) of HCl was diluted with water to make 500 mL.

10 mM Hydrochloric acid (as diluent) : was prepared by mixing 20 mL of 0.5 M HCL with 980 mL water.

Preparation of working standard solutions for muscle tissue measurements: Five standard solutions (with both Mg^{2+} and K^{+}) containing 0.25, 0.5, 1.0, 1.5, and 2.0 mg/L of Mg^{2+}, and 2.5, 5.0, 7.5, 10.0, and 15.0 mg/L of K^{+}, respectively, were prepared weekly using standard stock solutions diluted with 10 mM hydrochloric acid. The mixed standard solutions were kept in refrigerator at 4°C. Each series was sprayed at the beginning and end of each set of determination. Ten mM HCl was passed through the flame at intervals for autozeroing of the instrument. Calibration curves were obtained by plotting absorbance versus concentration (in mg/L).

Autocalibrator

A standard solution containing 1.5 mg/L Mg^{2+} and 7.5 ppm K^{+} was employed as midpoint autocalibration standard after each set of samples readings. It was planned that a new standard curve should be generated

if the midpoint autocalibration differed by more than 5%. However, we did not encounter any deviation of more than 5%.

TISSUE SAMPLING

Adult male white Wistar-Lewis rats weighing 200-250 g (from Experimental Animal Care Centre, College of Pharmacy, KSU, Riyadh) were used. They had not been subjected to experimentation. They were maintained on tap water and hard food pellets *ad lib*. Cardiac and skeletal muscle tissues were obtained immediately after sacrificing the animal. Obtained tissue samples were placed in polystyrene petri dishes and carefully dissected free from all visible fat and connective tissue. Blood traces were removed by brief plotting on filter paper. The weighed specimens were then placed in preweighed capped polystyrene plastic tubes, to minimize loss of the moisture content. They were stored in refrigerator at 4°C pending analysis of Mg^{2+} and K^{+} contents.

PROCEDURE

The whole muscle was first homogenized and specimens, in the range of 10-20 mg, were accurately weighed to the nearest 0.1 mg. Each weighed sample was further homogenized at room temperature in 2 mL of 10 mM HCl. The homogenization tube and pestle were washed twice with 1 mL of 10 mM HCl. The washings were added to the homogenized tissue suspension and vortexed for

15 sec. The combined extract and washings were centrifuged at 2500 g for 10 min., and 1 mL of the clear supernatant was further diluted with two mL of water, and vortexed for 15 sec. This dilution was used for the analysis of magnesium and potassium contents by atomic absorption spectroscopy. All estimates were made in at least three replicates of each sample. From each muscle 2-5 specimens were taken depending on the obtained whole muscle size.

Reading of results, in mg/L, were made with reference to standard curves, and tissue Mg^{2+} or K^{+} concentration was calculated in mg per Kg of wet muscle tissue.

The following analytical variables were checked for the evaluation of the proposed procedure for determination of tissue Mg^{2+} and K^{+}:

Reproducibility

To assess the "within-day" reproducibility of the proposed method, we assayed fifteen 10-mL HCl extracts of cardiac and vastus medialis muscles of one rat for Mg^{2+} and K^{+} contents three times on the same day (morning, midday and afternoon). The day-to-day reproducibility was similarly evaluated by assaying the fifteen samples, stored in refrigerator at 4°C, daily for fourteen days. The same set of working Mg^{2+} and K^{+} standard solutions were used throughout the study to calibrate the atomic absorption spectrometer.

Recovery

Analytical recoveries of Mg^{2+} and K^{+} in the assay were measured by spiking three specimens of vastus medialis and extensor communis muscles of each of nine rats with known concentrations (low, medium and high) of Mg^{2+} and K^{+}. The added concentrations were 130.0, 230.0, and 700.0 mg/Kg of Mg^{2+} and 1700.0, 4200.0 and 9500.0 mg/Kg of K^{+} and processed as discussed in the procedure. The total number of samples analyzed for each added concentration was 81 measurements. The same number of measurements were also performed for the determination of the mean values of Mg^{2+} and K^{+} contents in muscle specimens without spiking. The total amount of standard added plus the content of the muscle were diluted to be within the range of the calibration curve used.

Recovery was calculated as the ratio of the element determined in the muscle plus the standard to the total sum of the individually analyzed element in muscle alone plus the added standard.

Reliability

This experiment was performed in order to establish the relationship between wet sample weight (in increasing sequence from 5.65 mg to 48.00 mg) and the Mg^{2+} and K^{+} concentrations in the HCl extract of biceps femoris muscle.

Method comparison

The results of analyzing cardiac muscle for Mg^{2+} and K^{+} contents by the proposed method (A) were compared with those using Reinhart *et al* method[24](B), which involved hydrolysis of wet tissue from same muscle specimen using concentrated HNO_3 (65% V/V) at 80°C for four hours and addition of isotonic 150 mM saline, then determining Mg^{2+} and K^{+} contents by flame AAS after appropriate dilution of sample solution with $LaCl_3$ (for Mg^{2+}) and LiCl (for K^{+}) solutions.

Statistical analysis

All values are reported as mean±SD. Least squares linear regression was employed in the calibration curves and reliabilty. Statistical evaluation of differences between the proposed method and the published one[24] was achieved by analyzing the mean concentration of Mg^{2+} and K^{+} using a two tailed, Student's t-test for paired observations. Significance level was set at $P<0.05$. Data analyses were obtained by using Statistical Package for the social sciences (SPSS/PC).

RESULTS AND DISCUSSION

The aim of the present study was to develop a simple and reliable method for rapid quantification of magnesium and potassium in small tissue specimens (down to 5 mg) which may be applied clinically for diagnostic

purposes. Homogenization of tissue with 10 mM HCl was adopted to enhance liberation of the protein-bound electrolytes, their solubility when diluted with deionized water and easy detection by flame AAS.

Standard curves for the assay of Mg^{2+} and K^{+} in the range of 0.25 to 2.0 mg/L and 1.5 to 15.0 mg/L, respectively, were employed. Each point in the curve was based on thirty determinations. Least squares linear regression of the data showed good linearity between absorbance and the concentration at the above-mentioned range with the mean correlation coefficient (r) greater than 0.998 (TABLE II). Standard curves were constructed over ten weeks period to determine the variability of the slope and intercept. The results showed little day-to-day variability in the slope and intercept. The coefficient of variation for the slopes were 7.3% and 10.3% for Mg^{2+} and K^{+}, respectively, which indicated good reproducibility of the method.

The calibration equations were used for the calculation of Mg^{2+} and K^{+} contents of the muscle extracts.

The results of total Mg^{2+} and K^{+} contents of various analyzed muscles, as obtained by the proposed method, are given in Tables III and IV, respectively. We could not find reference values for rat tissue Mg^{2+}

TABLE II

Least Squares Regression Analysis Data of Calibration Curves for Mg^{2+} and K^{+}.

Element	Concentration Range (mg/Kg)	Intercept	Slope	r	CV,%
Mg^{2+}	0.25- 2.00	0.023	0.185	0.999	7.3
K^{+}	2.50-15.00	0.035	0.049	0.999	10.3

TABLE III

Values of Mg^{2+} in mg per Kg of Wet Weight of Muscle Sample.

Muscle Sample	n*	mean±SD Mg^{2+} (mg/Kg)	CV,%
Cardiac	99	371.97±12.16	3.3
Vastus medialis	82	393.85±26.74	6.8
Caudofemoralis	88	345.23±21.88	6.3
Biceps femoris	155	381.70±34.04	8.9
Extensor communis	66	340.37±14.59	4.3
Quadriceps extensor	77	364.68±17.02	4.7

* n = Number of observations.

and K^{+} contents in the literature. Those reported were for free Mg^{2+} in myocardium, ranging widely from 0.4 to 3.5 mM[23,26-30] (9.72 to 85.09 mg/L). Ion-selective microelectrode measurements of free Mg^{2+} in cardiac tissue vary from 0.4[26,27] (9.72 mg/L) to 3.0-3.5 mM[28] (72.92-85.09 mg/L). Therefore the values shown in

TABLE IV

Values of K^+ in mg per Kg of Wet Weight of Muscle Sample

Muscle Sample	n*	mean±SD K^+ (mg/Kg)	CV,%
Cardiac	104	4571.02±359.74	7.9
Vastus medialis	91	7405.92±750.76	10.1
Caudofemoralis	98	6377.54±324.55	5.1
Biceps femoris	147	6049.08±434.03	7.2
Extensor communis	67	6467.47±281.53	4.4
Quadriceps extensor	78	5298.32±414.48	7.8

* n = Number of observations.

Tables III and IV represent averages of analysis of samples from twelve adult wistar rats. The variations in results (CV for Mg^{2+} ranged 3.3%-8.9%, and 4.4%-10.1% for K^+) may be due to errors inherent in the whole procedure and atomic absorption spectrometry. Variability in the values of element contents of individual rats may play a role also. Nevertheless, these variations are not far from the limit of the calibration curves.

As shown in Table V, the within-day coefficients of variation were 4.0% (cardiac) and 2.6% (vastus medialis) for Mg^{2+}, those of K^+ were 3.2% and 5.9%, respectively. The day-to-day coefficients of variation for the same specimens were 6.0% and 10.3% for Mg^{2+},

TABLE V

Within-Day and Day-To-Day Reproducibility Data For Muscle Mg^{2+} and K^{+} in mg/Kg Wet Tissue

Type of Muscle	Within-day		Day-to-day	
	Mean±SD (mg/Kg)	CV,%	Mean±SD (mg/Kg)	CV,%
Cardiac:				
Mg^{2+}	362.25± 14.59	4.0	367.11± 21.88	6.0
K^{+}	4774.35±152.50	3.2	4445.90±383.20	8.6
Vastus medialis				
Mg^{2+}	371.97± 9.72	2.6	376.84± 38.90	10.3
K^{+}	7707.00±453.58	5.9	7229.96±758.58	10.5

and 8.6% and 10.5% for K^{+}, respectively. These values indicate that the reproducibility of the proposed method was acceptable for the samples studied.

The accuracy of the procedure was tested by determining analytical recoveries of Mg^{2+} and K^{+} from the HCl tissue extracts at three different concentrations (low, medium, and high). As shown in Tables VI and VII, recoveries of Mg^{2+} ranged from 93.4% to 104.9%, and K^{+} from 92.7% to 106.1%. This was considered acceptable when variations due to individual animals, sample handling and instrumentation were taken into account. The results indicate the reliability of the proposed method in allowing the detection of

TABLE VI

Analytical Recoveries of Mg^{2+} from Vastus Medialis and Extensor Communis Muscle Extracts after Standard Addition

Added Conc. (mg/kg)	Tissue conc. mean±SD (mg/Kg)*	Measured Conc. (Tissue plus Standard) mean±SD (mg/Kg)*	Analytical Recovery mean±SD (%)	Recovery Range, (%)
	Vastus			
130.0	402.45	508.56±49.76	95.5±9.4	83.8-111.5
230.0	±32.48	657.81±48.62	104.0±7.7	97.0-118.8
700.0		1030.80±94.79	93.4±8.6	81.8-108.1
	Extensor			
130.0	360.70	485.19±44.88	98.9±9.2	89.2-116.3
230.0	±30.78	619.40±62.59	104.9±10.6	87.5-116.1
700.0		1078.89±106.4	101.7±10.0	85.2-111.7

* Mean of 81 observations. Mg/Kg = mg of Mg^{2+} per Kg of wet muscle tissue.

reduced levels of Mg^{2+} and K^{+} in the tissue to the concentrations of 315.0 and 5280.0 mg/Kg, respectively.

Acceptable linearity between wet weight of biceps femoris muscle and the Mg^{2+} and K^{+} concentrations of HCl extracts was demonstrated as shown in Fig. 1, with correlations of 0.998 for Mg^{2+} and 0.996 for K^{+}. This linear correlation is a good indication of the reliability of the proposed method for the determination of these electrolytes over the range of sample sizes used.

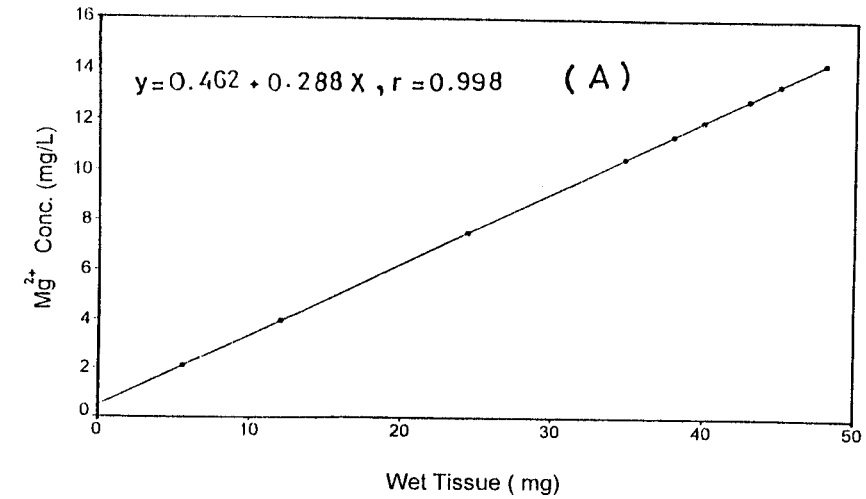
TABLE VII

Analytical Recoveries of K^+ From Vastus Medialis and Extensor Communis Muscle Extracts after Standard Addition

Added Conc. (mg/kg)	Tissue Conc. mean±SD (mg/kg)*	Measured Conc. (Tissue plus Standard) mean±SD (mg/Kg)*	Analytical Recovery mean±SD (%)	Recovery Range, (%)
	Vastus			
1700.0	7479.70	9376.07± 764.03	102.1± 8.3	85.8-111.5
4200.0	±772.68	11682.54±1277.70	100.0±10.9	88.1-128.2
9500.0		18012.82±2126.63	106.1±12.5	94.1-127.7
	Extensor			
1700.0	6186.32	7312.82± 702.36	92.7±8.9	83.3-108.0
4200.0	±561.53	10276.50±1001.93	98.9±9.6	90.6-113.1
9500.0		16083.76±1426.65	102.5±9.1	92.7-116.1

* Mean of 81 observations. Mg/Kg = mg of K^+ per kg of wet muscle tissue.

Tables VIII and IX show results of comparing cardiac muscle K^+ and Mg^{2+} analyses by the proposed method (A) and a published tissue digestion with concentrated HNO_3 assay procedure[24] (method B) as run in our laboratory. K^+ values did not show statistically significant difference between the two methods, ($P=0.2$, Table VIII). The paired test of comparison showed a significant difference ($P<0.001$, Table IX) between the two methods in terms of Mg^{2+} results. However, our procedure is simpler and showed less scattered range than that of the reported method.



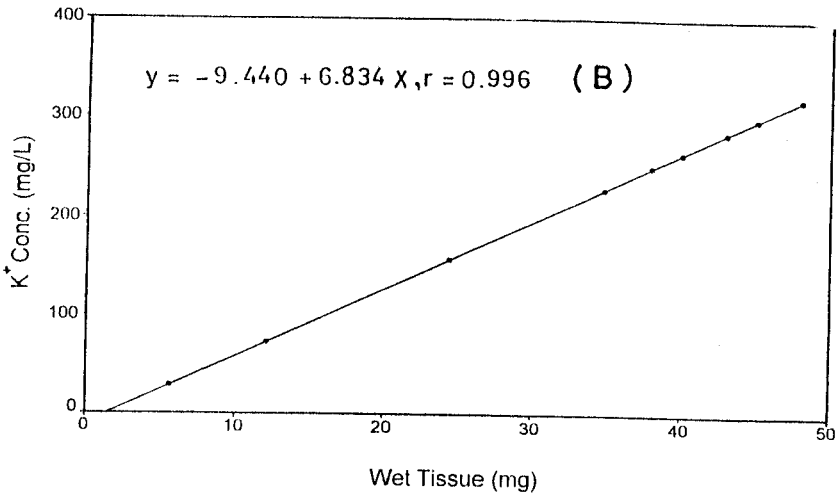


FIG. 1: Relationship between the wet weight of biceps femoris muscle and the magnesium (A) and potassium (B) concentrations of HCl extracts.

Table VIII
Comparison of the Mean Cardiac Muscle K^+ Concentration, in mg/Kg, obtained by the Proposed Method (A) and Tissue Digestion (B)

Variables	(A)	(B)
Mean (n=20)*	4367.69	4414.62
Range	4195.64-4602.31	4140.90-4664.87
SD	78.20	136.86
CV, %	1.8	3.1
P = 0.2		

* Number of measurements of 5 digested specimens with four replicates of each.

Table IX
Comparison of the Mean Cardiac Muscle Mg^{2+} Concentration (in mg/Kg) Obtained by our Proposed Method (A) and Tissue Digestion (B)

Variables	(A)	(B)
Mean (n=20)*	379.27	393.85
Range	367.11-396.29	379.27-413.30
SD	7.29	9.72
CV, %	1.9	2.5
P<0.001		

* Number of measurements of 5 digested specimen with four replicates of each.

In conclusion, the results of the present study describes a simple and easy procedure for quantification of muscle tissue magnesium and potassium with good analytical performance. The method involves homogenization of the muscle tissue specimen in 10mM HCl, centrifugation of the digested extract, and

three-fold dilution of the clear supernatant with water before determination of Mg^{2+} and K^{+} concetrations using AAS. It is relatively faster as compared to previously reported methods in terms of sample preparation. In addition, a smaller sample size is required, allowing processing of even less than 10 mg of muscle tissue. The reliability of the method is indicated by the reasonable values of CVs. Acceptable reproducibility of the proposed method for the samples studied are shown by the CV of various muscles and day-to-day results. Furthermore, the proposed method is applicable to various muscles in the body. These features, together with basic laboratory equipment and reagents, make the present method suitable for use in routine laboratory.

ACKNOWLEDGEMENT

This study was supported by a grant from the College of Pharmacy Research Center (CPRC-36), KSU, Riyadh. The authors would like to acknowledge the technical assistance of Mr. M. A. Bahjat, and Mr. M. Saleem Feroz for typing the manuscript.

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Received July 25, 1994
Accepted January 18, 1995