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Short communication

Superiority of nitric acid for deproteinization in the determination of trace lithium in serum by graphite furnace atomic absorption spectrometry

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ABSTRACT

Physiological level of trace lithium in human serum was determined by graphite furnace atomic absorption spectrometry (GFAAS). 3.5% HNO_3 (v/v) was employed as a protein precipitant for sample treatment and at the same time verified as a very effective chemical modifier to eliminate the interference of chloride. The analytical conditions for lithium determination in serum were investigated and the optimal pyrolysis and atomization temperatures were 800 °C and 2700 °C. The accuracy and precision of the method were tested by determining lithium in a RANDOX HN1530 assayed human multi-sera and a pooled human serum. The result was in good agreement with the target value and CV of the pooled human serum was 4.74% ($n = 10$). The characteristic mass, the limit of detection (LOD) of the proposed method were 0.8 pg and 0.01 μmol/L, respectively. Median ± S.E.M. of serum lithium in 220 Chinese people was 0.25 ± 0.02 μmol/L.

1. Introduction

Recent years the techniques of determining physiological level of trace lithium in biological fluids are received a great attention because lithium is recognized as an important trace element in biological and medical fields. For some years evidence has accumulated that lithium ions are reabsorbed throughout the proximal tubules (pars convolute and pars recta) in the same proportion as sodium and water, but that no lithium reabsorption occurs in the more distal segments of the nephron. As this special character, lithium clearance is considered by biologist as being an indicative of sodium and water reabsorption in proximal tubules of nephron [1–3]. Use of lithium clearance as a measure of the delivery of water and sodium from the proximal tubules opens up the prospect of new investigations within physiology, pathophysiology, and pharmacology. Many studies use lithium clearance to investigate renal function [3] and some relative diseases of sodium and water reabsorption in proximal tubules of nephron, such as hypertension [2,4], diabetes [5], etc. Concentration of lithium in blood is necessary to be measured for the calculation of lithium clearance. Determination of trace lithium in serum has been carried out by using some sensitive techniques such as graphite furnace atomic absorption spectrometry (GFAAS) [2,6–13], inductively coupled plasma atomic emission spectrometry (ICP-AES) [14,15], inductively coupled plasma mass spectrometry (ICP-MS) [13,16], capillary electrophoresis (CE) [17], ionic chromatography (IC) [18], ion-selective electrode (ISE) [19], etc. Among them, CE, IC and ISE are not sensitive enough to detect physiological level of trace lithium in serum and are normally used for monitoring therapeutic concentration of lithium in serum of patients being treated with lithium-containing drugs. GFAAS is the preferred technique since it requires low sample volume, provides adequate sensitivity and low limit of detection. However, chloride has been reported to have serious interference in GFAAS analysis of lithium [9,20]. Specific methods were used in order to cope with the interference. Some authors recommended the use of chemical modifiers such as ammonium nitrate [7,21] or in combination with phosphate [6]. Others reported using tantalum-coated tubes [9,22] or coated tubes in combination with chemical modifiers [7,11], or tubes lined with tantalum foil [23,24].

Serum samples need be pretreated prior to the determination of lithium by GFAAS. The common way is deproteinization by using trichloroacetic acid (TCA) [8,10,13] or HNO_3 [22]. Though both of these two chemicals are good at protein precipitation, the effects are quite different when they exist in the deproteinized serum in the determination of lithium by GFAAS. TCA is a chlorine-containing reagent, which would cause serious negative interference due to the formation of volatile lithium monochloride, LiCl [7]. On the contrary, HNO_3 is good at eliminating the interference of chloride by reacting with Cl^- to form volatile HCl, which is volatilized from the hot atmosphere during the dry step in GFAAS [20]. So it is

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Table 1
Time temperature program for the determination of lithium in serum.

Step	Temperature (°C)	Hold (s)	Ramp (°C/s)	Argon flow rate (mL/min)	Read
Dry	100	30	20	200	
Dry	150	10	25	200	
Ash	800	20	200	200	
Atomization	2700	3	0	0	√
Clean	2800	3	0	200	

possible to simply use HNO_3 for the dual purpose of precipitating serum proteins and counteracting the interference of chloride in the determination of trace lithium in serum by GFAAS. The possibility has been demonstrated in our present work with great satisfaction. This method is simple and accurate. It can be used for both routine use and research investigations.

2. Materials and methods

2.1. Reagents and chemicals

All chemicals employed were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and had analytical reagent grade unless otherwise specified. All solutions were prepared with high purity distilled water. A 100 mmol/L Li stock standard solution was used for making standard working solutions by dilution before use. Final concentration of 3.5% HNO_3 (v/v) (guaranteed reagent) was used for precipitating protein and eliminating the interference of chloride. High purity distilled water with an absorbance about 0.0050 (the same as for an air injection) was obtained through a distilled water system made of quartz. A RANDOX HN1530 assayed human multi-sera (RANDOX Laboratories Ltd., United Kingdom) was purchased from Shanghai Zhi Cheng Bio-tech Co., Ltd. for testifying the accuracy of the method.

2.2. Instrumentation

All measurements were carried out by using a Thermo S2 atomic absorption spectrometer (England) equipped with a GFS 97 graphite furnace and autosampler (England). The light source was a lithium hollow cathode lamp (Thermo, United Kingdom) operating at 7 mA current at 670.8 nm resonance line. The bandwidth of the spectrometer was set at 0.2 nm (half height). Extended Lifetime Cuvettes (Thermo Elemental, part no. 9423 393 95041, Germany) were employed for all of the experiments. Atomization was performed from the wall and peak height was used for calculations. The furnace temperature program is given in Table 1. Argon was used as purge gas. Every sample was determined twice repeatedly. All pipettes used were Finnpipette (Thermo Electron Corporation) equipped with disposable polypropylene pipette tips. Thermo SOLAAR software was applied to control all of the operations.

2.3. Sample collection

Serum samples were from the residents living in Shanghai, China with no apparent contamination with lithium products for at least 2 weeks before blood collection. A pooled serum was made for the preparations of standard-addition working curve and precision test of the method. Serum samples were stored at −20 °C and thawed before use.

2.4. Standard-addition working curve and sample preparation

0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μL of 2.5 μmol/L Li standard solutions were pipetted, respectively in several 0.5-mL Eppendorf tubes. 100 μL pooled serum, 350 μL 5% HNO_3 and suitable amount of H_2O were added to each of them to make the final volume 500 μL for every standard-addition solution. For the sample treatment, we simply mixed 100 μL serum with 400 μL 4.4% HNO_3.

All solutions were vortexed for 3 min and centrifuged 12,000 rpm for 5 min. The supernatants were transferred to the special cups for autosampler. Volumes of 10 μL were injected into the atomizer for analyses. The standard-addition concentrations of lithium were 0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, 0.20, 0.225 and 0.25 μmol/L, respectively. Standard-addition linear least squares method was used for the calculation of the results.

2.5. Statistical analysis

Data were analyzed as mean ± standard deviation (SD) and evaluated statistically by Student's *t*-test or one-way ANOVA. Significance was determined as $P < 0.05$.

2.6. Calculations of recovery and limit of detection

Recovery test was carried out by spiking with known quantities (from 0.025 to 0.25 with interval 0.025 μmol/L) of lithium to serum sample before deproteinization. Calibration curve with the standard-addition method was established enabling to calculate lithium concentrations from the original serum $C_{Li}(O)$ and recovered from the spiked serum $C_{Li}(O+S)$. The equation of recovery $(\%) = [C_{Li}(O+S) - C_{Li}(O)]/C_{Li}(S) \times 100$. $C_{Li}(S)$ was the concentration of spike value.

The limit of detection (LOD) was calculated by using the equation [25,26] $LOD = 3 \times S_{BL}/b$, where S_{BL} was the standard deviation of 10 consecutive measurements of the blank solutions and b was the slope of the calibration curve. Ten consecutive measurements of 3.5% HNO_3 were carried out for the calculation of S_{BL}. Its correlative lithium concentration was calculated by the linear equation of the calibration curve.

3. Results and discussion

3.1. Effect of HNO_3 to eliminate NaCl background

Background signals were measured at the wavelength 283.3 nm by using a deuterium lamp. HCl, H_2SO_4 and H_3PO_4 were also tested besides HNO_3 and an H_2O solution was used for comparison. Every testing solution containing 50 mmol/L NaCl. The results are shown in Fig. 1A. Compared to H_2O solution, HNO_3 was found to be the most effective to reduce NaCl background. HCl had no use and the background was even lifted a little bit. H_3PO_4 and H_2SO_4 changed the time of the background peaks, which were delayed about 0.15 s, due to the formation of thermostable Na_3PO_4 and Na_2SO_4.

Next we examined the dose-effect of HNO_3 on eliminating chlorine in GFAAS. Fig. 1B shows the background profiles of 50 mmol/L NaCl in the presence of 0, 0.5, 1, 1.5, 2, and 2.5% HNO_3, respectively. The results showed that NaCl background was completely eliminated when HNO_3 concentrations were ≥1%.

To determine whether HNO_3 was effective on removing chlorine existed in serum matrix before atomization in GFAAS, we measured the background of serum deproteinized by either 3.5% HNO_3 or 3.5% TCA (Fig. 1C). The results showed that the background of serum in 3.5% HNO_3 was much lower than in 3.5% TCA. 3.5% HNO_3 reduced the background of serum to a very low extent, but could not completely eliminate it. This kind of background was induced by serum matrix, which contained a large amount of different ions besides Cl^-, during the atomization step.

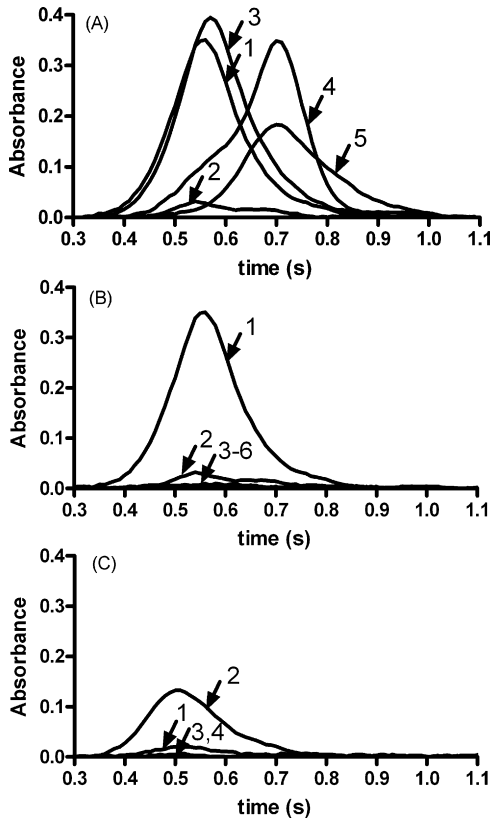

Fig. 1. Backgrounds of NaCl and serum in various media. Background signals caused by 50 mmol/L NaCl (A and B) and serum (C) were measured at the wavelength 283.3 nm by using a deuterium lamp in various solutions. (A) 1 = H_2O; 2 = 80 mmol/L HNO_3; 3 = 80 mmol/L HCl; 4 = 27 mmol/L H_3PO_4; 5 = 40 mmol/L H_2SO_4. (B) 1, 2, 3, 4, 5, 6 = 0, 0.5, 1, 1.5, 2, 2.5% HNO_3, respectively. (C) 1 = deproteinized serum (100 μL serum + 400 μL 4.4% HNO_3); 2 = deproteinized serum (100 μL serum + 400 μL 4.4% TCA); 3, 4 = 3.5% HNO_3, 3.5% TCA.

3.2. Selection of protein precipitant

A serum sample was deproteinized by 3.5% HNO_3, 3.5% TCA or 2.4% $HClO_4$ (another popular protein precipitant used in biological field), respectively. Supernatant of each sample was clear after centrifuging. These supernatants were then introduced into graphite tube to determine lithium at the wavelength 670.8 nm by GFAAS. Lithium signals were greatly reduced in either 3.5% TCA or 2.4% $HClO_4$ compared to 3.5% HNO_3 solution (Fig. 2). This experiment demonstrated that chlorine was the interferent for both of TCA and $HClO_4$ were chlorine-containing reagents. We found that chlorine

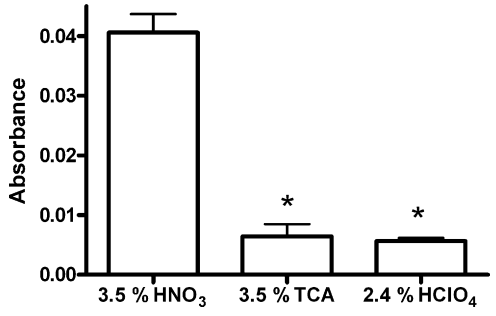

Fig. 2. Lithium signals in different kinds of protein precipitants. Serum was deproteinized by 3.5% HNO_3, 3.5% TCA or 2.4% $HClO_4$. Supernatants were used for lithium determination by GFAAS. Results are expressed as mean ± SD of duplicate samples with two repeated determinations for each sample. *$P < 0.001$ in comparison to 3.5% HNO_3.

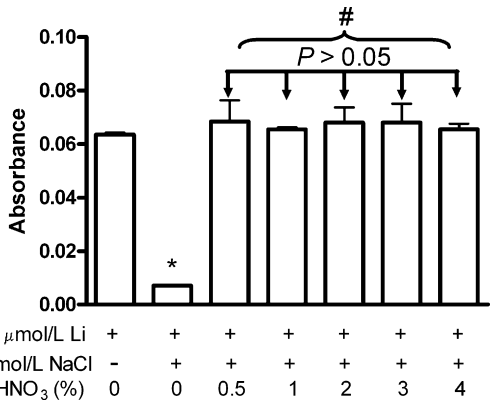

Fig. 3. Effect of HNO_3. Lithium absorbance was determined in different concentrations of HNO_3 in the presence of 30 mmol/L NaCl. *$P < 0.001$ in comparison to 0.2 μmol/L Li in H_2O. #$P < 0.001$ in comparison to 0.2 μmol/L Li in 30 mmol/L NaCl.

even had "memory effect". Lithium signals were depressed after using TCA and $HClO_4$. This kind of "memory effect" could be eliminated by injecting several volumes of 3.5% HNO_3. We would like to indicate that any chlorine-containing reagents should be avoided using in the determination of trace lithium by GFAAS.

3.3. Efficiency of HNO_3 to overcome NaCl interference

Negative interference of chloride is recognized as a major issue in the determination of trace lithium by GFAAS in biological samples. Normal concentration of blood chlorine is about 100 mmol/L. Serum was diluted 5 times during sample treatment process and chlorine existed in sample was reduced to about 20 mmol/L. For the consideration of some individual variations, 30 mmol/L NaCl was used as the interferent. 0–4% HNO_3 were used for observing the effect in the presence of 0.2 μmol/L Li and 30 mmol/L NaCl. A same concentration of Li standard solution prepared by H_2O was used for comparison. The results indicated that lithium signal was greatly suppressed by 30 mmol/L NaCl and this kind of interference was completely eliminated by the addition of 0.5–4% HNO_3. The results of one-way ANOVA with post-Tukey's multiple comparison test showed that lithium absorbance of all paired groups between two different concentrations of HNO_3 were not significant (all P values > 0.05, Fig. 3). So 3.5% HNO_3 is no doubt to fit the need to eliminate the interference of chloride for all serum samples in the determination of lithium by GFAAS.

3.4. Pyrolysis and atomization temperature optimization

Thermal stability and optimal temperature of atomization of lithium were investigated by using a deproteinized serum in 3.5% HNO_3. The pyrolysis and atomization temperature curves were obtained by initially using the recommended atomization temperature and the best pyrolysis temperature. Fig. 4 shows the pyrolysis and atomization temperature curves of lithium in serum. For the considerations of the sensitivity, the effect to eliminate interference, the low atomization temperature, which contributes to increase the graphite tube lifetime, 800 °C and 2700 °C were selected as optimal pyrolysis and atomization temperature, respectively.

3.5. Selection of the measurement signal

Lithium concentrations calculated by peak height and peak area were compared. Paired t-test showed that the difference was not significant ($P = 0.77$, $n = 35$). Measurements were preferred to peak height because of the higher sensitivity.

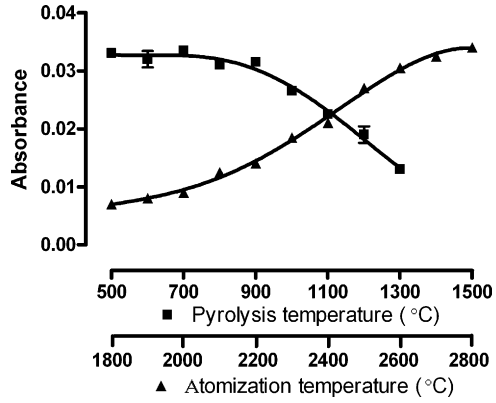

Fig. 4. Pyrolysis and atomization temperature curves. Serum lithium in 3.5% HNO_3 was determined by GFAAS. The atomization temperature was 2700 °C for the pyrolysis curve and pyrolysis temperature 800 °C for the atomization curve.

3.6. Characteristic mass and limit of detection

The linear equation of Li calibration curve was $Y = 0.4498X - 0.0006977$. The characteristic mass was 0.8 pg that produced an absorbance signal of 0.0044. Standard deviation of blank was 0.00057 calculated by the absorbance of 10 consecutive measurements of 3.5% HNO_3. The correlative lithium concentration of $3 \times S_{BL}$ was 0.0053 μmol/L. LOD was 0.01 μmol/L calculated by the equation in Section 2.6.

3.7. Accuracy, precision and the applied linear range of the calibration curve

The accuracy of the present method was not easily established, since no suitable certified reference material of serum was available. However, as the analytical method developed dealt with the elimination of interference of chloride towards lithium determination in GFAAS analysis, using HNO_3 modifier, a RANDOX HN1530 assayed human multi-sera was diluted 1000 times with 100 mmol/L NaCl for testifying the method. Five diluted samples were treated equally according to Section 2.4. Lithium concentrations were calculated by an aqueous standard calibration curve because after 1000-fold dilution, the property of the sample matrix was close to aqueous solution. The detected lithium concentration was 1.04 ± 0.03 mmol/L which was in good agreement with the target value (0.98 ± 0.12 mmol/L, flame photometry).

Recovery test was carried out according to the experiment and calculation in Section 2.6. The average recovery was $99.34 \pm 2.31\%$ (94.5–102.1%, $n = 10$).

For testing precision of the method, 10 specimens were taken from a pooled human serum and deproteinized separately according to Section 2.4. Lithium concentrations were determined consecutively by GFAAS. The coefficient of variation (CV) was 4.74% which was acceptable in analytical chemistry.

The correlation coefficient of the calibration curve was 0.999 in the lithium standard-addition concentration range of 0–0.25 μmol/L. As serum samples were diluted 5 times during the deproteinization process, this concentration range equivalent to the corresponding concentrations of the results were 0–1.25 μmol/L. The concentration of lithium in the pooled serum, which was used for the preparation of the standard-addition working solutions in our experiment, was about 0.4 μmol/L. So the highest point of standard-addition equivalent to the concentration of the result was 1.65 μmol/L, that covered all of our measurements without any exception.

Our experimental results showed that the confidence interval analysis on the difference for the slopes of linear regression curves was significant difference between slopes of aqueous and serum-matched standard curves ($P < 0.05$, data not shown). Physical issues such as volume change after deproteinization, different viscosities between aqueous and standard-addition solutions, might be the major reasons besides some other possible chemical interference. These reasons would influence the slopes of the standard curves. So standard-addition method was still recommended to compensate these physical and possible chemical interferences though the negative interference of chloride was eliminated by HNO_3.

Table 2
Serum lithium concentrations of 220 Chinese people (μmol/L).

Gender	Median	S.E.M.	*n*	Minimum	Maximum
Male	0.23	0.026	138	0.06	1.54
Female	0.26	0.030	82	0.07	0.52
Total	0.25	0.020	220	0.06	1.54

3.8. Application

The recommended method was applied to the determination of lithium in human serum. Standard-addition linear least squares method was used to find out the lithium concentrations for the samples. Results are shown in Table 2. *t*-Test showed that the difference of lithium concentrations between male and female was not significant ($P = 0.872$).

4. Conclusions

Determination of physiological level of trace lithium in human serum could be carried out by the described GFAAS method, where minimal sample preparation was required. Application of HNO_3 provided the reagent for both protein precipitation and elimination of the interference of chloride.

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