Determination of Trace Lithium in Human Urine by Electrothermal Atomic Absorption Spectrometry Using Nitric Acid as a Chemical Modifier to Eliminate the Interference of Chloride

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Electrothermal atomic absorption spectrometry (ETAAS) is considered the most common and advanced technique to determine trace lithium in biological fluids. However, chloride existing in samples has been reported to create serious interferences. Nitric acid was verified as a chemical modifier to eliminate the interference of chloride in determining trace lithium in urine samples and the possible mechanism was also elucidated. The influence of chloride was completely eliminated by using 0.5% (v/v) HNO₃ as a chemical modifier. Confidence interval analysis on the difference for the slopes of linear regression curves indicated no significant difference between the slopes of aqueous and of urine-matched standard curves with and without 30 mmol/L NaCl in the presence of 0.5% (v/v) HNO₃ ($P = 0.146$). Thus the direct standardization with an aqueous calibration curve could be used instead of the standard-addition method. We conclude that the developed method is accurate and easily applicable for both routine use and research investigations.

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Introduction

Because of the biological and biochemical importance, the concentration of physiological levels of trace lithium in body fluids is used as a biological indicator of human health and disease; its determination has thus received great attention from biological and medical researchers. Determination of trace lithium in biological fluids has been carried out by using some sensitive methods, such as atomic emission spectrometry (AES),¹⁻³ inductively coupled plasma atomic emission spectrometry (ICP-AES),^{4,6} inductively coupled plasma mass spectrometry (ICP-MS),⁷⁻⁹ isotope dilution mass spectrometry (IDMS)^{10,11} and electrothermal atomic absorption spectrometry (ETAAS).^{9,12-23} Among these techniques, ETAAS has been shown to be very attractive, since it requires low sample volume and provides adequate sensitivity, low detection limits and the possibility of measurements after just a simple dilution step (no need for sample mineralization). However, chloride has been reported to create serious interferences in ETAAS analyses of lithium.¹⁸ Specific methods were used in order to cope with such interference. Some authors recommended the use of chemical modifiers such as ammonium nitrate^{19,21} or phosphate.²³ Others reported using tantalum-coated tubes¹⁸ or coated tubes in combination with chemical modifiers (tantalum carbide-coated graphite tubes-ammonium nitrate,²² tungstate-coated graphite tubes-ammonium sulfate),¹⁴ or tubes lined with tantalum foil.^{17,20} Magnin *et al.*¹⁵ reported that, even if one uses pyrolytically-coated graphite tubes in combination with ammonium nitrate, the method still could not completely abolish the interferences caused by the matrix, notably in urine samples.

So they used solid-phase extraction on strongly acidic cation exchange cartridges to separate the interferents from the urine matrix. The method of extraction to separate lithium from urine matrix was also used by Steinhäuslin *et al.*¹⁹ It was quite time-consuming and not convenient.

In the present study, the effectiveness of nitric acid as a chemical modifier to eliminate the interference of chloride has been investigated for the determination of trace lithium in urine, which contains a large amount of chloride.

Experimental

Reagents and chemicals

All chemicals employed were from Sinopharm Chemical Reagent Co., Ltd. They were analytical reagent grade unless otherwise specified. All solutions were prepared with high purity distilled water. A stock standard lithium solution (100 mmol/L) was made by weighing 0.369 g of extra pure (99.99%) Li₂CO₃ into a 100-ml volumetric flask; the salt was dissolved with a few drops of HCl and the contents were made up to volume with distilled water. The working standard solutions were prepared before use by diluting the stock standard solution with 0.5% (v/v) HNO₃. Concentrated nitric acid (guaranteed reagent) was diluted with distilled water to 0.5% (v/v) in the final concentration as a chemical modifier. Then, 1 mol/L of spectrum pure grade NaCl was prepared for the interferential test and final concentrations were used for the consideration of the concentrations of chlorine existing in urine after 10 times dilution. 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.

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Table 1 Time-temperature program for the determination of lithium in urine

Step	Furnace temperature/°C	Ramp/°C s ⁻¹	Hold/s	Argon flow rate/ml min ⁻¹
1	100	10	30	200
2	150	100	10	200
3	800	150	20	200
4	2700	0	3	0
5	2800	0	3	200

Apparatus

All measurements were carried out using a Thermo S2 atomic absorption spectrometer (England) equipped with a GFS 97 graphite furnace (England) and a GFS 97 furnace autosampler (England). The light source was a lithium hollow cathode lamp (Thermo, UK) operating at 7 mA current at the 670.8 nm resonance line. The bandwidth of the spectrometer used was 0.2 nm (half height). Extended lifetime cuvettes (Thermo Elemental, part No. 9423 393 95041, Germany) were used for all of the experiments. The furnace temperature program used is given in Table 1. Argon was used as purge gas. All pipettes used were Finnpiettes (Thermo Electron) equipped with disposable polypropylene pipette tips.

Analytical procedure

Urine samples were diluted 1:10 by 0.55% HNO₃ (v/v) in the special cups for autosampler. Five microliters of this solution were injected into the graphite tube directly. The atomic absorption was measured under the ETAAS operating conditions as given in the description of the apparatus and in Table 1. Two drying steps with different temperatures were used in order to avoid possible sputtering. The concentrations of lithium in urine samples were obtained by a general standard curve method. Absorbances were measured using peak height for calibration because of the higher sensitivity.

Statistical analysis

Data were analyzed as mean $\pm$ standard deviation (SD) and evaluated statistically by the student's *t*-test. GraphPad Prism software was used to test whether slopes of linear regression curves were significantly different. Significance was determined as $P < 0.05$.

Results and Discussion

Pyrolysis and atomization temperature optimization

To establish an optimal temperature program for the determination of lithium in urine by ETAAS, we used 0.5 μ mol/L Li and urine (diluted 1:10) as the specimens for observing the pyrolysis and atomization temperature for the graphite furnace heating program. As 0.5% HNO₃ is the most common medium used in atomic absorption analysis, we also observed Li pyrolysis and atomization temperature curves in this medium as well as in H₂O. The initial atomization temperature (2700°C) was used for the pyrolysis curves according to the manual of the instrument. One hundred degree intervals were used for both pyrolysis and atomization temperature curves. For observing the thermal stability of lithium compound, we used temperatures from 500 to 1400°C. The optimum atomization temperatures were determined by varying the temperature between 1800 and 2800°C. The results are shown in Fig. 1A. Pyrolysis curves are

Fig. 1 Pyrolysis and atomization temperature curves of lithium in different kinds of media. 0.5 μ mol/L Li and natural urine (diluted 1:10) in either H₂O or 0.5% HNO₃ solutions were determined by ETAAS. The atomization temperature was 2700°C for the pyrolysis curves and ash temperature was 800°C for the atomization curves. Panel A shows the pyrolysis and atomization temperature curves of lithium in different kinds of media. Arrows and dashed lines point out optimal temperatures for ash and atomization, respectively. Results are expressed as mean $\pm$ SD of duplicate samples: filled symbol, 0.5 μ mol/L Li; opened symbol, urine (diluted 1:10). Data of panels B and C were collected from panel A at the ash temperature of 800°C and atomization temperature of 2700°C. Results are expressed as mean $\pm$ SD of quadruplicate samples.

on the left part and atomization curves are on the right part. The optimal ash and atomization temperature ranges are pointed out by arrows and dashed lines. Eight hundreds and 2700°C were selected as the ash and atomization temperatures, respectively, according to the data from the pyrolysis and atomization temperature curves for determining urinary lithium in our experiments. We noticed that signals in 0.5% HNO₃ were much stronger than those in pure H₂O, especially for urine samples (Figs. 1B and 1C). This was because chlorine existing in urine interfered with Li signals, while this kind of interference was suppressed by 0.5% HNO₃.²⁴ Besides HNO₃ had some ability to enhance the sensitivity of Li as the absorbance of H₂O and 0.5% HNO₃ blanks were the same (data not shown). This was because NO₃⁻ increased the numbers of free Li atoms during the atomization step.

Interference of chloride

To observe the interference of chloride, we compared standard curves of 0, 0.1, 0.2, 0.3, 0.4, 0.5 μ mol/L Li in H₂O and urine (diluted 1:10) with and without 30 mmol/L NaCl (Fig. 2A). The slopes of the standard curves with 30 mmol/L NaCl were much lower than the related curves that were measured without adding extra NaCl. Perhaps this was due to the vaporization of LiCl during the pyrolysis step.²⁵ The slope of the Li standard curve with 30 mmol/L NaCl was only 0.00536; this value was much lower than the slope of the NaCl-free Li standard curve (0.238). In urine, there is a large amount of ammonium which will react

Fig. 2 Interference of chloride. Panel A shows the standard curves of 0.1, 0.2, 0.3, 0.4, 0.5 $\mu\text{mol/L}$ Li in H_2O and urine (diluted 1:10) with or without 30 mmol/L NaCl. Panel B shows absorbance of 0.5 $\mu\text{mol/L}$ Li in different kinds of chlorides. The ash and atomization temperatures used were 800 and 2700°C, respectively. Results are expressed as mean $\pm$ SD of duplicate samples. * denotes $P < 0.001$ in comparison with 0.5 $\mu\text{mol/L}$ Li in H_2O .

with chloride to produce ammonium chloride, which has low melting and boiling points. Thus, parts of ammonium chloride were vaporized and removed during the dry and ash steps and this reduced the interference of chloride.

To further validate that the interference was from chlorine, we used chlorides with different cations to test whether other chlorides could reduce the signal. Figure 2B shows the absorbance of 0.5 $\mu\text{mol/L}$ Li in different kinds of chlorides. The interference of NH_4Cl was weaker, as compared with those of NaCl or KCl. This is because parts of NH_4Cl were vaporized during the ash stage as described above.

Effects of different kinds of inorganic acids

Chloride reacts with acids to produce hydrogen chloride, which has both a very low melting point and a very low boiling point. We compared the effect of eliminating the interference of chloride by different kinds of inorganic acids; 0.5 $\mu\text{mol/L}$ Li was used to evaluate the performance of HNO_3 , H_2SO_4 , H_3PO_4 and HCl in the presence of 30 mmol/L NaCl (Fig. 3). The amounts of hydrogen in all acid solutions were the same. All of the inorganic acids showed very good effects of eliminating the interference except HCl; this exception further demonstrated that Cl^- was the interferent.

Superiority of nitric acid compared to ammonium nitrate

NH_4NO_3 was considered a priority of the chemical modifier to eliminate the interference of chloride in the determination of trace lithium in biological samples.^{15,19,21-23} For comparing the discrepancy of the effect between HNO_3 and NH_4NO_3 , we used 10–120 mmol/L HNO_3 and NH_4NO_3 to determine the minimum effective dose in the presence of 0.5 $\mu\text{mol/L}$ Li and 30 mmol/L

Fig. 3 Effect of inorganic acids. HNO_3 , H_2SO_4 , H_3PO_4 and HCl were used to compare the effects of eliminating the interference of chloride. The amounts of hydrogen were equal (80 mmol/L) in different kinds of inorganic acid solutions. Results are expressed as mean $\pm$ SD of duplicate samples. * denotes $P < 0.001$ in comparison with 0.5 $\mu\text{mol/L}$ Li in H_2O ; # denotes $P < 0.001$ in comparison with 0.5 $\mu\text{mol/L}$ Li in 30 mmol/L NaCl.

NaCl. Figure 4A shows the absorbance as functions of the concentrations of HNO_3 and NH_4NO_3 . As could be seen, each of them was effective, but HNO_3 seemed to have a higher potency than NH_4NO_3 . Both 60 mmol/L HNO_3 and 80 mmol/L NH_4NO_3 gave satisfactory absorbances. Then the absorbance reached to flat plateaus with the further increase of the concentration of either HNO_3 or NH_4NO_3 .

The effects of HNO_3 and NH_4NO_3 were also checked by determining the NaCl background in the presence and absence of either of these reagents at the wavelength of 283.3 nm using a deuterium lamp. Testing samples were composed of 80 mmol/L HNO_3 or NH_4NO_3 as chemical modifiers with 30, 50 and 100 mmol/L NaCl as indicators of interferents. Background signals caused by NaCl were considerably completely eliminated (Fig. 4Ba) or dramatically reduced (Figs. 4Bb and 4Bc) in the presence of 80 mmol/L HNO_3 . Figure 4B further demonstrated that HNO_3 was more effective than NH_4NO_3 as a chemical modifier to eliminate the interference of chloride.

Variations of the slopes of lithium standard addition curves from various urine samples (10-fold dilution) were compared in the presence of either 80 mmol/L HNO_3 or 100 mmol/L NH_4NO_3 . Results are shown in Table 2. Confidence interval analysis on the difference for the slopes of linear regression curves indicated significant differences between slopes of different standard addition curves in 100 mmol/L NH_4NO_3 group ($P = 0.013$) with CV of 6.94%; this value was not as high as reported by Magnin *et al.*¹⁵ since the dilution factors of the samples and concentrations of NH_4NO_3 were different. The CV value of 80 mmol/L HNO_3 group was 3.74% and $P = 0.175$, which meant that the differences between slopes of different standard addition curves in this group were not significant.

Our experimental results proved Magnin's observation that the CV values of the slopes of lithium standard addition curves from various urine samples were high when using NH_4NO_3 as the chemical modifier. We would like to indicate that dilution is an easy and effective way to reduce interferents in samples. It would be much more effective to eliminate interferences when dilution was combined with chemical modifiers.

The normal concentration of chlorine in urine is 170–250 mmol/24 h and the normal volume of urine is about 1.5 L/24 h. So the concentration of chlorine in urine is less than 200 mmol/L.

Fig. 4 Effect of nitric acid and ammonium nitrate. In panel A, 10–120 mmol/L HNO₃ and NH₄NO₃ were used to test the effect of eliminating the interference of chloride. 0.5 μ mol/L Li and 30 mmol/L NaCl were present in every specimen. Panel B shows the background signals due to NaCl in the absence and presence of HNO₃ and NH₄NO₃. Concentrations of NaCl in Figs. 4Ba, 4Bb and 4Bc were 30, 50 and 100 mmol/L, respectively. Heating program of the graphite furnace was the same as that shown in Table 1. Background signals were measured by a deuterium lamp at the wavelength of 283.3 nm. 1, H₂O; 2, 80 mmol/L NH₄NO₃; 3, 80 mmol/L HNO₃ solutions.

By 10 times dilution in the process of sample preparation, the final concentration of chlorine in prepared samples should be less than 20 mmol/L. As shown in Fig. 4Ba, 80 mmol/L HNO₃ has the ability to completely eliminate 30 mmol/L chlorine during dry and ash steps; this concentration of nitric acid should be available to eliminate the interference caused by chlorine in all urine samples.

Slopes of lithium standard curves in 0.5% HNO₃

The slopes of the standard curves were compared again. All of the conditions were the same as described in Fig. 2A except for containing 0.5% HNO₃ (Fig. 5). GraphPad Prism software was employed to test whether the slopes of the four standard curves were significantly different and the *P* value was 0.146. The differences were not significant. To further validate that the common standard curve could be used instead of using the standard addition, we tested the slopes of these two standard curves again; the result showed that the *P* value was 0.123. The differences between the slopes were not significant. This means that direct standardization with an aqueous calibration curve could be used for the determination of trace lithium in urine.

Possible mechanism of nitric acid as a chemical modifier and its advantages

The effectiveness of nitric acid in eliminating chloride interference results from the effect of this acid in releasing

Table 2 Slopes of urine-matched standard addition curves^a

Chemical modifier	Mean	SD	CV, %	<i>n</i>	<i>P</i>	Differences between slopes
80 mmol/L HNO ₃	0.206	0.0077	3.74	7	0.175	Not significant
100 mmol/L NH ₄ NO ₃	0.214	0.0148	6.92	7	0.013	Significant

a. Addition of 0, 0.1, 0.2 and 0.3 μ mol/L Li standards, respectively in each 10-fold diluted urine sample.

Fig. 5 Lithium standard curves in 0.5% HNO₃. Lithium standards were prepared in aqueous solutions and urine (diluted 1:10) with or without 30 mmol/L NaCl. All solutions contained 0.5% HNO₃. GraphPad Prism software was used to test whether slopes of linear regression curves were significantly different.

chloride as hydrogen chloride (HCl) from the hot atmosphere. The procedure could be chemically expressed as follows:

M represents metal.

Nitric acid reacts with chloride to form nitrate and volatile hydrogen chloride, which has a very low melting point (−114.8°C) and boiling point (−85°C). The dried hydrogen chloride is very stable and volatilized from the hot atmosphere during the dry step of ETAAS.

Normally 0.5% HNO₃ is widely recommended as a medium for different elements determination by atomic absorption spectrometry. So no extra specific chemical modifiers are needed if 0.5% HNO₃ existed while determining trace lithium in urine by ETAAS.

Application

The developed method was applied to the determination of trace lithium in human urine. Seventy-five samples (including 47 from males and 28 from females) were measured. The minimum, maximum, mean $\pm$ SD were 1.38, 21.4, 6.69 ± 3.74 μ mol/24 h urine for males and 1.57, 16.2, 5.99 ± 3.18 μ mol/24 h urine for females. *t*-test was applied to examine whether the results between males and females differed significantly at the 95% confidence level limit. There was no statistically significant difference (*P* = 0.414).

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