Determination of boron in serum, plasma and urine by inductively coupled plasma mass spectrometry (ICP-MS). Use of mannitol-ammonia as diluent and for eliminating memory effect

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Full Paper

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A rapid and accurate method has been developed for the determination of boron in serum, plasma and urine by inductively coupled plasma mass spectrometry. The memory effects of B were examined using different diluents/rinse solutions, including water, nitric acid, Triton X-100, ammonia and mannitol in water, in nitric acid and in ammonia. A combination of ammonia with mannitol, as both diluent and flush solution, gave the best precision, the minimum memory effect and the lowest background. A sample dilution of 20-fold was simply made for serum and plasma and 100-fold for urine for determination with a single calibration curve. Beryllium was employed as the internal standard to control matrix effects and to compensate for possible fluctuation and instrument drift. The isotope $^{10}B^+$ was utilised to avoid spectral overlap by the intense $^{12}C^+$ isotope. The final solution of blank, standards and samples contained 0.25% w/v mannitol, 0.1 M ammonia and 20 ng ml^{-1} of Be. Six samples, including human and horse serum, human and horse plasma, and human urine, were analysed to test the reliability of the method. A limit of detection (3σ) of 0.015 ng ml^{-1} was obtained and the recoveries of spiked boron (two spiking levels for each matrix) from the selected samples ranged from 98% to 104%. Much higher concentrations of B in urine ($\approx$1 μg ml^{-1}) were found compared to those in serum and plasma samples (32.8–61.1 ng ml^{-1}).

Introduction

In recent years, boron has been considered as a dynamic trace element which can affect the metabolism or utilisation of many biomolecules involved in life systems. The substances include amino acids, proteins, microminerals, energy substrates (triglycerides, glucose, and so on), reactive oxygen species and oestrogen. Through the effects, boron can affect bone development and the function or composition of brain, skeleton and immune system.[1] Therefore, the demand for reliable determination of trace B in biological samples is growing.[2–8]

Serum, plasma, and urine are the biological fluids most frequently used for diagnostic purposes and some publications have reported the determination of ultra-trace elements in such body fluids with various techniques, such as neutron activation analysis (NAA), inductively coupled plasma atomic emission spectrometry (ICP-AES), ICP mass spectrometry (ICP-MS), electrothermal vaporisation atomic absorption spectrometry (ETV-AAS), ETV-ICP-AES/MS, and so on.[9–14] Among these techniques, ICP-MS has been developed rapidly for the determination of ultra-trace elements in body fluids because of its high sensitivity, good precision and fast and multi-element measurement. However, difficulties have been encountered with the determination of trace B in biological samples by ICP-MS because of the high memory effect.[4–6,9] Different mechanisms for the memory effects of B in ICP spectrometry have been proposed. Sun *et al.*[5] inferred that the memory effect comes from the reaction of B with the sample introduction system, especially the spray chamber, which is usually made of borosilicate glass or quartz. Al-Ammar *et al.*[15] considered that it originates from the tendency of B to volatilize as boric acid from the sample solution layer that covers the inside surface of the spray chamber. Some efforts have been made for eliminating or minimising the memory effect.[4–6,9,15] Smith *et al.*[4] used a direct injection nebuliser instead of a conventional sample introduction system for fast B cleanout. However, a commercial direct injection nebuliser is expensive and is not easy to use in routine analysis. A simple way for minimising the memory effect of B is to rinse the system with a flush solution. Evans and Krahenbuhl[6] suggested using sodium fluoride as the flush solution, but Prost *et al.*[3] indicated that 13 min was required to remove the memory resulting from 0.5 μg ml^{-1} of B. Dilute nitric acid was also employed as the rinse solution for the determination of B.[10] However, in recent studies, we noted that the signal did not return to the original blank level with a 10 min washout time after a 100 ng ml^{-1} of B was introduced for 5 min. Al-Ammar *et al.*[15] reduced the B signals to blank levels in a very short time by injecting a small volume of ammonia solution into the spray chamber simultaneously with the sample solution. Unfortunately, no practical applications were reported. Sun *et al.* minimised the memory effects for the determination of trace B by ICP-AES with ultrasonic nebulisation by adding mannitol to the sample and flush solutions, and excellent results were obtained by applying the method to the determination of trace B in plants, food and animal tissues.[2,5]

Body fluids, especially serum and plasma, contain appreciable organic and inorganic constituents. Therefore, almost all the analytical methods for such fluids involve sample preparation prior to determination. Although some methods based on the destruction of organic materials such as acid digestion with microwave or high-pressure bombs have been reported, a simple dilution with a suitable diluent is preferable for most workers. This is more important for B because its concentration in serum and plasma is very low and contamination and loss could happen with an acid digestion procedure.[9,10] For dilution procedures, a basic medium is preferred for serum and

plasma (especially for plasma samples) to avoid flocculation, which could be observed with an acidic diluent. The dilution factor generally depends on the concentrations of the analytes, the instrumental sensitivity and the matrices of the samples. In this paper, a simple dilution procedure is described for the accurate determination of B in serum, plasma and urine samples. A solution of mannitol with ammonia was added to blank, standards and samples, and this functioned not only as a diluent and stabiliser, but also minimised the memory effect. Further, drift and fluctuation were controlled by the use of Be as the internal standard as indicated by Vanloe *et al.*[10]

Experimental

Instrumentation

A Hewlett-Packard HP 4500 ICP mass spectrometer (Yokogawa Analytical Systems, Tokyo, Japan) was employed for all analytical determinations. The ICP mass spectrometer was equipped with a nickel sampler core (1.0 mm orifice) and a nickel skimmer (0.4 mm orifice). The instrumental parameters were optimised with a solution containing 100 ng ml^{-1} of Li, Y, Ce and Tl. A peristaltic pump, a V-groove Babington type nebuliser, and a double pass spray chamber were employed for sample introduction. The main operating conditions are listed in Table 1.

Reagents

High purity water (specific resistance: 18.2 MΩ cm^{-1}) used for the preparation of all blank, standard and sample solutions was obtained from a Millipore water purification system (Millipore, Molsheim, France). The stock solutions of B (1000 μg ml^{-1} of B in 0.3 M ammonia) and Be (10 000 μg ml^{-1} of Be in 0.5 M HCl), ammonia (21%, w/w, UpA grade) and nitric acid (69%, w/w, UpA grade) were purchased from Romil Ltd. (Cambridge, UK) and high purity D-mannitol (99+%) was purchased from Sigma-Aldrich (Steinheim, Germany). Various calibrated polyethylene tubes and containers (Sarstedt Aktiengesellschaft, Germany) were used throughout for solution preparation to avoid possible boron contamination. The calibration blank and a series of working standards of B contained 0.25% w/v mannitol, 0.1 M ammonia and 20 μg l^{-1} Be.

Sample preparation

Polyethylene tubes or flasks were employed throughout for the preparation of the samples. Serum and plasma samples were obtained from the Royal Hallamshire Hospital (Sheffield, UK) and were stored at −25 °C prior to analysis. Urine samples were collected from two of the authors.

After defrosting and homogenisation, 0.5 ml of serum or plasma sample was accurately transferred with a calibrated pipette into a 15 ml polyethylene calibrated tube. To each tube, 1 ml of ammonia (1.0 M), 1 ml of mannitol solution (2.5%, w/v) and 1 ml of Be solution (200 ng ml^{-1}) were added. For the spiking tests, 1 ml of B solution (10 ng ml^{-1} or 25 ng ml^{-1}) was also added. The sample was finally brought to 10 ml with de-ionised water. A reagent blank was prepared with the same procedure.

For urine, 0.5 ml of the sample was accurately transferred into a 50 ml polyethylene flask to which 5 ml of ammonia (1.0 M), 5 ml of mannitol solution (2.5%, w/v) and 5 ml of Be solution (200 ng ml^{-1}) were added. For the spiking tests, 5 ml of B solution (50 ng ml^{-1} or 100 ng ml^{-1}) were also added. The sample was finally brought to volume with de-ionised water. A reagent blank was prepared using the same procedure.

The final sample solutions for analysis contained 0.25% of mannitol (w/v), 0.1 M ammonia, 20 ng ml^{-1} of Be and different levels of B (0, 1 and 2.5 ng ml^{-1} for serum and plasma samples; 0, 5 and 10 ng ml^{-1} for urine samples) as spike. All solutions were prepared in duplicate.

Table 1 Operating conditions for ICP-MS (HP 4500)

Forward power/W	1300
Reflected power/W	<3
Coolant gas/l min^{-1}	16
Auxiliary gas/l min^{-1}	1
Carrier gas/l min^{-1}	1.2
Sample depth/mm	7.3
Integration time/s	1 for $m/z=10$ (B) 0.1 for $m/z=9$ (Be)
Solution uptake rate/ml min^{-1}	0.5
Data acquisition modes	Time resolved analysis and spectrum
Spray chamber temperature/°C	2

Analytical determination

Prior to analysis, the possibility of spectral interference was evaluated. As already mentioned by Smith *et al.*,[4] the intense peak from $^{12}C^+$ could compromise determination based on the $^{11}B^+$ isotope due to the added mannitol and the high content of sample matrices (especially for serum and plasma samples). To evaluate the spectral interference, the $^{12}C^+$ signals at $^{10}B^+$ and $^{11}B^+$ were measured with different concentrations of mannitol in 0.1 M ammonia, but without the addition of B. The results are shown in Fig. 1. It can be seen that the signal of $^{11}B^+$ increases with increasing concentration of mannitol. On the other hand, the signal of $^{10}B^+$ was not influenced by C, therefore, $^{10}B^+$ was selected for all analytical determinations. In the current work, Be was employed as the internal standard because of the comparable mass and ionisation potential between ^{10}B and Be. Calibration was made by using aqueous standard solutions of B (0, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50 and 100 ng ml^{-1}). Each standard solution contained 0.25% (w/v) of mannitol, 0.1 M ammonia and 20 ng ml^{-1} of Be. B was determined using operating conditions given in Table 1 with the time resolved analysis (TRA) data acquisition mode in which 3 measurement points, *i.e.*, the centre point and the preceding and succeeding points, were utilised. Blank, standards and samples were aspirated for 1 min before the ion signals were collected. A flush solution, including 0.25% w/v mannitol and 0.1 M ammonia, was introduced for 1 min after each standard or sample. The concentration of B in each sample was calculated from the average ratio of B counts/Be counts of six determinations.

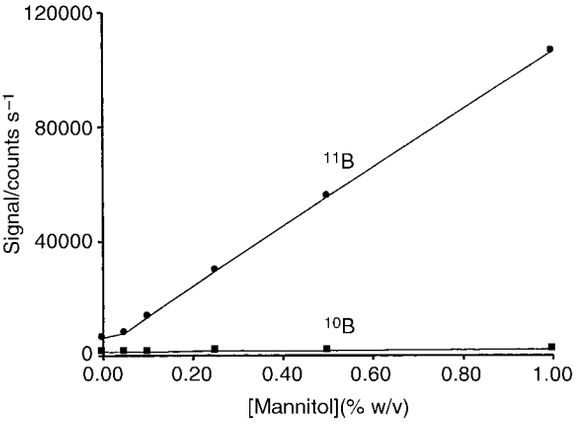

Fig. 1 The apparent signal (counts/s or CPS) of $^{10}B^+$ and $^{11}B^+$ with the existence of different concentrations of mannitol in 0.1 M ammonia.

Results and discussion

Selection of diluent

Seven reagents, including water, nitric acid (0.14 M), Triton X-100 (0.1%, w/v), ammonia (0.1 M), mannitol in water (0.25%, w/v), mannitol (0.25%, w/v) in HNO_3 (0.14 M) and mannitol (0.25%, w/v) in ammonia (0.1 M) were tested and evaluated according to the memory effect, the analytical precision and the background. For each reagent, an equivalent blank and a 100 ng ml^{-1} standard solution were analysed. The ion-time response for $^{11}B^+$ was continuously monitored. First, the signal was collected with the reagent blank for about 5 min, then the B solution with the same reagent was introduced for 5 min, and finally the reagent blank was introduced again as the flush solution for about 10 min.

Fig. 2–4 gives the B ion-time response [counts s^{-1} (CPS)] for the selected reagents. The results show that 0.14 M HNO_3 and H_2O exhibit similar and significant memory effects [Fig. 2(B)]. The memory effect could still be seen with ammonia as the medium and flush solution, though the background was the lowest one compared to the other reagents. In a neutral medium (H_2O), mannitol caused a different influence on the B signal; the ion response did not drop immediately but decayed slowly when the flush solution (0.25% w/v mannitol in H_2O) was introduced. However, this phenomenon did not appear in the acidic (0.14 M HNO_3) or basic (0.1 M ammonia) medium. Significant memory effects could be seen with mannitol in HNO_3 or in H_2O. However, almost no memory effect was found with the mannitol–ammonia combination [Fig. 3(B)]. Lorber *et al.*[16] used Triton X-100 to decrease the memory effect and matrix effects for the determination of uranium in urine and serum by ICP-MS and some other workers[3,12,13,17,18] also employed the same surfactant as the diluent for determination of some trace elements in serum, blood and urine. However, the results in Fig. 4 show that Triton X-100 is not a good diluent for B determination due to the anomalous transient spike and the significant memory effect.

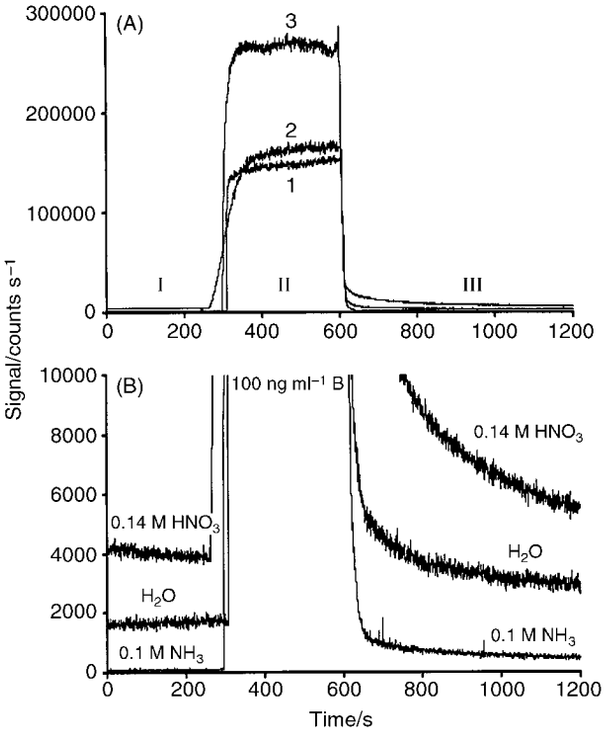

Fig. 2 (A) The time resolved signal (CPS) of $^{10}B^+$: (I) with reagent blanks; (II) 100 ng ml^{-1} of B in different reagents [(1) H_2O, (2) 0.14 M HNO_3, and (3) 0.1 M ammonia]; (III) with reagent blanks as the flush solutions. (B) The enlargement of the lower part of (A).

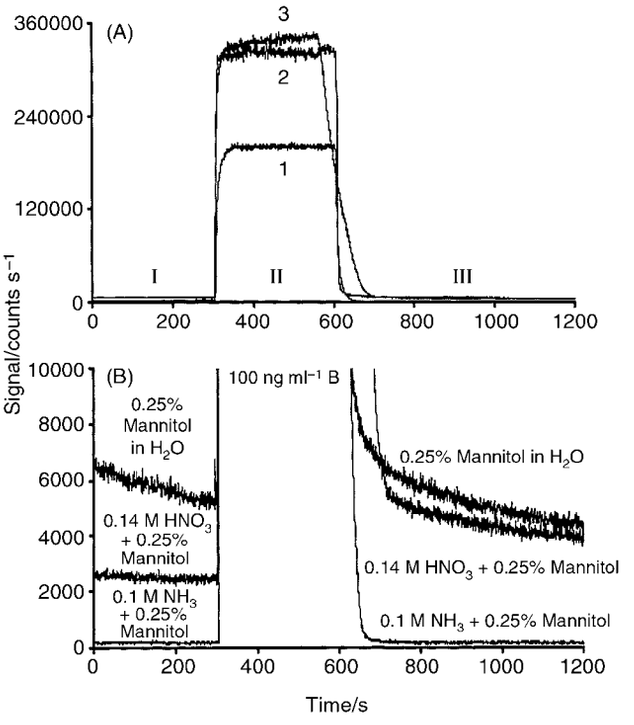

Fig. 3 (A) The time resolved signal (CPS) of $^{10}B^+$: (I) with reagent blanks: (II) 100 ng ml^{-1} of B in different reagents [(1) 0.25% w/v mannitol in H_2O, (2) 0.25% w/v mannitol in 0.14 M HNO_3, and (3) 0.25% w/v mannitol in 0.1 M ammonia]; (III) with reagent blanks as the flush solutions. (B) The enlargement of the lower part of (A).

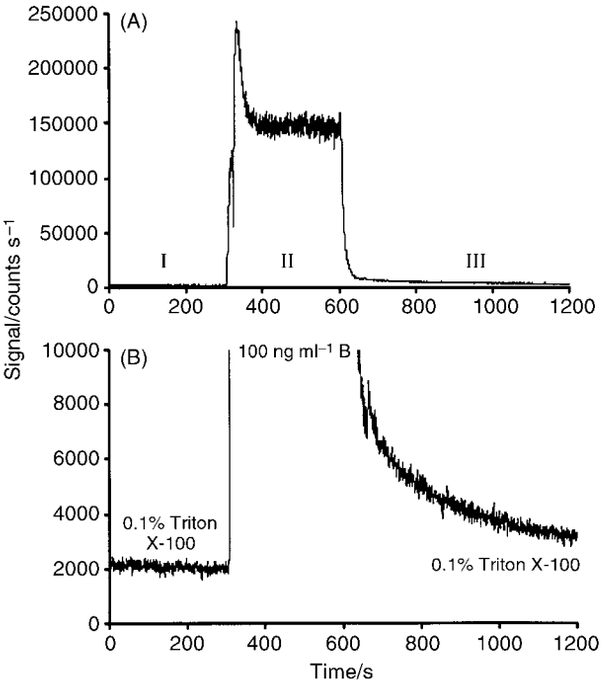

Fig. 4 (A) The time resolved signal (CPS) of $^{10}B^+$: (I) with reagent blank (0.1% w/v Triton X-100): (II) 100 ng ml^{-1} of B in 0.1% w/v Triton X-100; (III) with 0.1% w/v Triton X-100 as the flush solution. (B) The enlargement of the lower part of (A).

Water is the most frequently used rinse solution in ICP-MS. Fig. 2 shows that the signal for B returned rapidly to a relatively low level when H_2O served as the rinse solution. However, a strong signal appeared when a HNO_3 solution (0.14 M) was introduced after a 5 min flushing with H_2O,

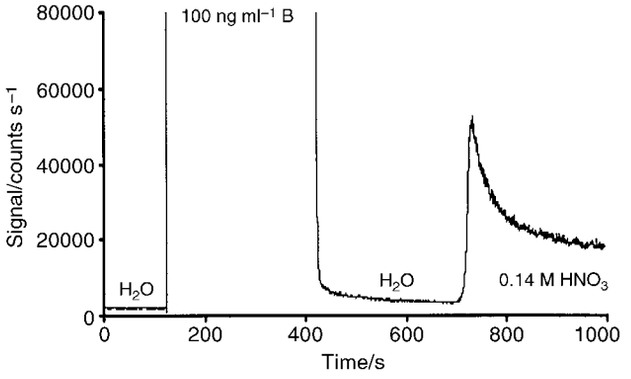

Fig. 5 The memory effects of B with H_2O and 0.14 M HNO_3 as the flush solutions at different times.

indicating that a significant amount of B was accumulating in the spray chamber (Fig. 5).

Differences in sensitivity of up to 2.5-fold were obtained for B prepared in the different media, as shown in Fig. 2–4. The highest sensitivities appeared with mannitol in H_2O and in HNO_3 and the lowest ones were observed with H_2O, HNO_3 (0.14 M) and Triton X-100 (0.1%, w/v); medium sensitivities were displayed with ammonia (0.1 M) and with mannitol (0.25%, w/v)–ammonia (0.1 M).

With reference to precision, the results in Fig. 2(A) and Fig. 3(A) show that the signal response did not stabilise after the B solution was introduced for 5 min, the signal kept increasing slowly when H_2O, HNO_3 (0.14 M) or mannitol (0.25%, w/v) in H_2O were selected for solution preparation. Clearly, this situation is not useful for practical analysis. On the other hand, signal fluctuation was observed with ammonia (0.1 M) or mannitol (0.25%, w/v) in 0.14 M HNO_3. The influences of Triton X-100 on the B response were quite different from the other reagents. The signal dropped immediately when maximum counts were reached and took nearly 2 min to reach steady state with significant fluctuation [Fig. 4(A)]. Contrary to all the above situations, a very stable signal was obtained in a medium of mannitol (0.25%, w/v)–ammonia (0.1 M) [Fig. 3(A)].

In summary, the combination of mannitol with ammonia gives the minimum memory effect, the best precision and the lowest background. Furthermore, the best limit of detection was obtained with this combination even though the sensitivity for B was less, compared to the other reagents. The medium of mannitol with ammonia was chosen as diluent in further work.

Fig. 6 shows the count rate (CPS) and the signal-background-ratio (SBR) for 10 ng ml^{-1} of B with different concentrations of ammonia (A) and mannitol (B). The influences of the concentration of ammonia on the count rate is relatively insignificant compared to the effect on the SBR. In consequence a concentration of 0.1 M was chosen for ammonia. The effects of mannitol on signal response are different from those of ammonia. The SBR and the count rate increase with increasing concentration of mannitol from 0 to 0.05%, then the count rate shows very little change while the SBR decreases slowly with further increase of the concentration of mannitol. Considering the wide variety of the matrices and B concentrations in serum, plasma and urine samples, and a relatively stable count rate and SBR with the concentration of mannitol between 0.2% and 0.5%, a concentration of 0.25% w/v mannitol was selected for sample analysis.

Limits of detection, precision and sample dilution

A blank solution (0.25% w/v mannitol and 20 ng ml^{-1} of Be in 0.1 M ammonia) and a solution of B (10 ng ml^{-1}) with 0.25% w/v mannitol–20 ng ml^{-1} Be in 0.1 M ammonia were used to measure the limits of detection and quantitation. The limits of

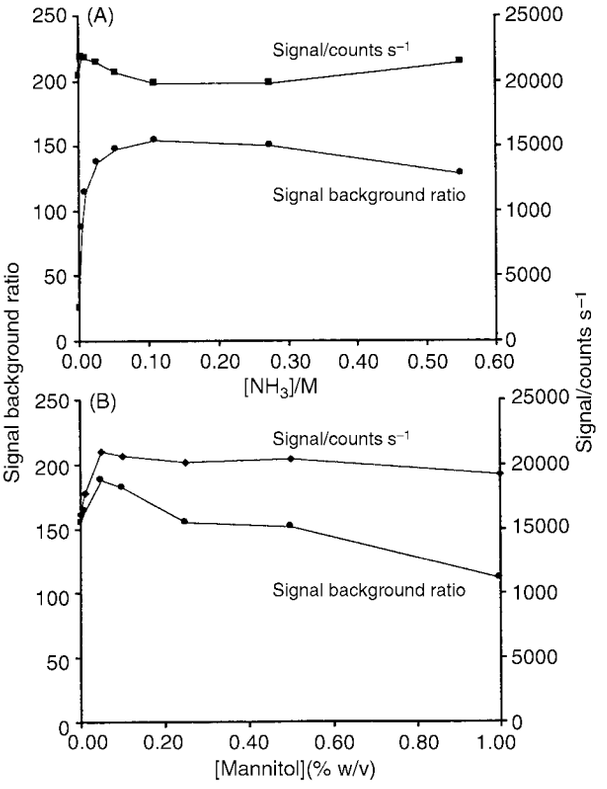

Fig. 6 The signal (CPS) and the signal-background-ratio (SBR) of $^{10}B^+$ with 0.25% w/v mannitol in different concentrations of ammonia (A) and with different concentrations of mannitol (%, w/v) in 0.1 M ammonia (B).

detection (3σ) and quantitation (10σ), which were calculated from the standard deviations of 20 measurements of the blank ratio of $^{10}B^+$ counts/$^9Be^+$ counts, were 0.015 ng ml^{-1} and 0.05 ng ml^{-1}, respectively. The limit of quantitation corresponds to 1.0 ng ml^{-1} of B in the original samples for a 20-fold dilution. The relative standard deviation (RSD) of 10 measurements of 1 ng ml^{-1} B solution with 0.25% w/v mannitol and 0.1 M ammonia performed on different days was between 0.5 and 1.5%. The RSD of 10 measurements of 0.15 ng ml^{-1} (10 times the limit of detection) B solution with the same matrices ranged from 2.5 to 3.5%.

Two levels of dilution (10 and 20 times for serum and plasma; 10 and 100 times for urine) were evaluated according to the degree of signal depression of the internal standard and the analytical precision. A depression of more than 40% on the signal for Be, which was caused from the high contents of dissolved organic and inorganic materials (especially Na), was observed in samples with 10 times dilution compared with that in reagent blank and in standard solutions. When the dilution factor was increased (from 10- to 20-fold for serum and plasma and 10- to 100-fold for urine), the depression was less than 15%. In addition, the precision and spiking recoveries were also significantly improved with additional dilution. Therefore, a 20-fold dilution was made for the determination of B in serum and plasma samples and a 100-fold for urine samples. According to the study of Vanhoe *et al.*[10] the lowest B concentration in serum was 4 ng ml^{-1}, which is higher than the limit of quantitation in the original sample (1.0 ng ml^{-1}).

Sample analysis

Six samples, including two serum (human and horse), two plasma (human and horse) and two human urine samples, were analysed to test the feasibility of the developed method. The accuracy was evaluated by the recoveries of spiked B from the sample matrices, because of the unavailability of body fluid

Table 2 Concentrations of B in 6 different samples and recoveries of spiked B

Sample	Mean concentration of B without spike/ng ml^{-1}	B spiked/ng	B recovered/ng	Recovery (%)
Human serum	38.8	10	10.4	104
		25	25.4	102
Human plasma	32.8	10	9.88	98.8
		25	25.1	100
Horse serum	61.1	10	10.3	103
		25	25.4	102
Horse plasma	45.1	10	9.80	98.0
		25	24.7	98.8
Urine #1	961	250	256	102
		500	507	101
Urine #2	1243	250	245	98.0
		500	504	101

samples with certified values for B. Each sample was accurately transferred to six containers and four of them were spiked with two levels of standard B solutions. Table 2 summarises the analytical values and the recovery data for the spikes. The excellent recoveries of B (98–104%) demonstrate the accuracy of the method. The analytical results show that the concentrations of B in two human urine samples ($\approx$1 μg ml^{-1}) are much higher than those in human serum and plasma samples (32.8–38.8 ng ml^{-1}), suggesting that most of B is rapidly excreted in the urine and does not accumulate in blood. In addition, the concentrations of B in horse serum and plasma (45.1–61.1 ng ml^{-1}) are higher than those in human serum and plasma.

Conclusions

Boron in body fluid samples can be accurately determined by ICP-MS with a simple dilution procedure. Compared to common diluents, such as H_2O, diluted HNO_3, Triton X-100 and ammonia, a solution of mannitol with ammonia not only functions as an effective diluent, but also provides the best measurement precision, the lowest background, and eliminates the memory effect. Good precision and accuracy (recovery of spikes) data can be obtained for serum and plasma samples (20-fold dilution) and urine (100-fold dilution). This method has proved to be simple and reliable for the determination of B in serum, plasma and urine by ICP-MS. A much higher concentration of B in urine was found than that in serum and plasma samples, indicating that most of B is rapidly excreted in the urine and does not accumulate in blood. A further study will be undertaken for multi-element (including B) analysis with the same or a similar procedure.

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