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RESEARCH ARTICLE

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Precise determination of the molybdenum isotopic composition of urine by multiple collector inductively coupled plasma mass spectrometry

Jing Zhang[1,2] | Jie Li[1] | Le Zhang[1] | Zhi-bing Wang[1] | Sheng-ling Sun[1] | Zhen-yu Luo[1]

[1]State Key Laboratory of Isotope Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, 510640, China

[2]University of Chinese Academy of Sciences, Beijing, 100049, China

Correspondence
J. Li, State Key Laboratory of Isotope Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences. Guangzhou 510640, China.
Email: jieli@gig.ac.cn

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Rationale: Molybdenum (Mo) is predominantly expelled from the human body in urine. Consequently, urinary variability in the concentration and isotopic composition of Mo may encode valuable clinical information. To access this information, however, it is first necessary to develop and demonstrate a rapid, accurate and precise methodology capable of concentrating Mo from urine for isotope analysis.

Methods: The utility of *N*-benzoyl-*N*-phenylhydroxylamine (BPHA) to effectively separate and purify Mo from urine samples without the need for acid digestion was tested. Following this approach, applying a double-spike mass bias correction, we determined the Mo isotopic compositions of a set of urine samples by multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS).

Results: Based on replicate analyses of an in-house urine standard, this approach demonstrates an external precision on $\delta^{98/95}$Mo values of better than 0.08‰ (2SD, n = 15). Application to a sample set collected from healthy individuals in Guangzhou, China, provides the first suite of $\delta^{98/95}$Mo measurements from urine samples. Samples from the female participants show $\delta^{98/95}$Mo (‰) values (1.31 ± 0.19‰, Ave ± 2SD, n = 14) that are consistently lower than those from the male participants (1.55 ± 0.16‰, Ave ± 2SD, n = 17).

Conclusions: The employed methodology is suitable for rapid, low-blank and high-throughput Mo isotope analysis of urine samples. Although resolvable $\delta^{98/95}$Mo variability is seen in this preliminary dataset, the mechanism driving this variability is unknown. High-precision Mo isotopic analysis might be added to the urinalysis toolkit, with the potential to provide valuable clinical information in the future.

1 | INTRODUCTION

The study of mineral elements (e.g., Mo, Fe, Cu and Zn) is of considerable relevance to human health because of their conflicting roles as essential trace elements as well as toxicants. Molybdenum (Mo) serves as an essential element within enzymatic prosthetic groups and can be found in sulphite oxidase, xanthine oxidase and aldehyde oxidase in human tissues.[1,2] Although Mo is clearly an essential element, at high concentrations it has toxic effects, causing severe gastrointestinal irritation, cardiac failure and bone deformation.[3] In contrast, Mo deficiency has been related to the development of some cancers, such as oesophageal cancer.[4,5]

The isotopic composition of certain elements can be affected by disease. Thus, the isotopic analysis of essential elements has the potential to provide valuable clinical information. For example, the copper isotopic composition of serum can fingerprint hepatocellular carcinoma,[6] Wilson's disease,[7] liver cirrhosis[8] and certain cancers (e.g., breast and colorectal).[9] In addition, calcium isotope ratios can

identify cancer-related bone loss[10] and multiple myeloma[11], while zinc and iron isotope systematics can detect breast cancer[12] and hereditary hemochromatosis,[13] respectively. While the medical application of novel isotope systematics has recently been reviewed,[14,15] it remains a novel and promising field that requires significantly more research to be completely developed as a complementary analytical approach for medical diagnosis and prognosis.

Molybdenum has seven naturally occurring stable isotopes and its redox sensitivity is known to result in substantial mass-dependent isotope variations in Earth sciences, mainly during weathering, sediment deposition, and seafloor alteration.[16,17] In biological systems, previous studies have focused on Mo isotope fractionation by cyanobacteria and *Azotobacter vinelandii* during nitrate utilisation and N_2 fixation,[18] demonstrating that bacteria preferentially assimilate the lighter isotopes of Mo, resulting in fractionations of −0.2 to −1.0‰ for $\delta^{98/95}$Mo values ($\delta^{98/95}Mo = [^{98/95}Mo_{sample}/^{98/95}Mo_{standard} - 1]$).[19] To our knowledge, no studies have explored the utility of Mo isotopes as tracers of either metabolic pathways, or as an isotopic fingerprint of disease. Urine is the major excretory pathway of Mo from the body.[20] Consequently, variations in the concentration and isotopic composition of Mo may provide valuable clinical information about environmental and occupational exposure.[21] Nevertheless, a first step to the clinical application of Mo isotope systematics requires an efficient pre-concentration and measurement protocol that is readily applicable to a large number of urine samples.

Multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS) has been used for almost two decades to make high-precision Mo isotope measurements from numerous geologically and environmentally relevant metrics.[19,22-27] Generally, three strategies are applied to correct for instrumental mass bias during MC-ICP-MS Mo isotope determination, including sample-standard bracketing (SSB), elemental spiking (ES) and double spiking.[28] In SSB, it is assumed that the instrumental mass bias is identical during standard and sample analysis. Unfortunately, however, owing to unstable source conditions, this method requires frequent measurements of bracketing standards, and typically replicate analyses of individual samples to deliver a satisfactory level of precision. In the ES method, the mass fractionations of Sr, Zr, and Pd isotopes are used to correct for instrumental mass bias.[19,24,27] Here, the precision is reliant on identifying the correlation that characterises the mass fractionations of Mo, Sr, or Zr, or Pd isotopes through analysing doped standards that bracket the samples being analysed. Consequently, in practice, both above approaches require much time due to multiple measurements.

Given the time-consuming nature of the SSB and ES approaches to Mo isotope ratio analysis, not to mention the apparent limitations on precision, we adopted a double-spike methodology to correct for instrumental mass fractionation. In detail, the double-spike approach has several advantages over the SSB and ES methods. For example, mass-dependent isotopic fractionation can be determined in a single analysis, avoiding the time-consuming analysis of more standards than samples. The double-spike method is also less susceptible to potential analytical artefacts induced by trace amounts of residual sample matrix and incomplete yields of the analyte following chemical purification.[22] In addition, precise and accurate Mo concentrations can be determined via isotope dilution without separate concentration determination.

Although a variety of pre-concentration and separation procedures have been developed for Mo isotope determination in geological materials,[23-29] no Mo isotope study has been conducted on urine samples. The Mo concentration of urine is low and urine represents a relatively complex organic- (e.g., urea, uric acid, urobilin, bilirubin, and creatinine, and smaller amounts of carbohydrates, proteins, fatty acids, hormones, pigments, and mucins) and inorganic-rich matrix (e.g., sodium, potassium, and calcium). These two factors may provide difficulties in the isolation and detection of Mo. In addition, few geologists have realised that Mo isotope determination could be applied to health issues.

Here we describe a rapid and highly efficient chromatographic procedure capable of separating and pre-concentrating Mo from urine for accurate and precise [Mo] and $\delta^{98/95}$Mo measurement by MC-ICP-MS. To our knowledge, we present the first $\delta^{98/95}$Mo dataset derived from human urine. While we identify isotopic variability, its causes within human body remain unclear. This is a preliminary foray into Mo isotope urinalysis, which may provide valuable clinical information in the future.

2 | EXPERIMENTAL

2.1 | Reagents and materials

2.1.1 | Reagents

Mineral acids (HCl, HNO_3, HF) from the Beijing Institute of Chemical Reagents (Beijing, China) were purified using a sub-boiling distillation system (DST-100, Savillex, Minnetonka, MN, USA). Optima-grade 30% H_2O_2 (Fisher Chemicals, Pittsburgh, PA, USA) was used without further purification. All dilutions and manipulations were performed using Milli-Q water (Elix, Millipore, St Louis, MO, USA; 18.2 MΩ.cm at 25°C).

2.1.2 | ^{97}Mo-^{100}Mo double-spike preparation

A ^{97}Mo–^{100}Mo double spike (DS) was prepared by mixing individual spike solutions enriched in ^{97}Mo and ^{100}Mo (Oak Ridge National Laboratory, Oak Ridge, TN, USA). The compositions of the ^{97}Mo–^{100}Mo double spike and NIST SRM 3134 Mo reference solution (National Institute of Standards and Technology, Gaithersburg, MD, USA) were calibrated by doping with Pd.[22,26]

2.1.3 | *N*-Benzoyl-*N*-phenylhydroxylamine chromatography resin preparation

Analytical grade *N*-benzoyl-*N*-phenylhydroxylamine (BPHA; Aladdin Reagent Inc., Shanghai, China) was purified by recrystallisation from an aqueous solution. BPHA (3 g) was dissolved in ethanol and mixed with Milli-Q water (400 mL) at ~80°C. As the mixture cooled to room temperature, BPHA began to crystallise. Amberchrom CG-71 (Supelco Inc., St Louis, MO, USA; 50–100 μm particle size) was cleaned with a mixed ethanol: HCl:water (2:1:1) solution, and then washed with deionised water until the resin was free from acid. The BPHA resin was obtained by impregnating cleaned Amberchrom CG-71 resin (5 g) with 3% BPHA in ethanol (20 mL).[26]

2.1.4 | Urine samples

We focused on developing a method for Mo isotope determination in urine because, in addition to urine being the major Mo accretionary pathway, this is one of the sample-types that have been targeted by most biomedical studies. To assess the accuracy and precision of the urine-derived Mo isotope measurements, we used an in-house urine standard and IAPSO standard seawater. The urine samples were obtained from 31 healthy individuals, originating from 14 male and 17 female subjects living in Guangzhou, China. The subjects covered the age from 8 to 69 years, and their blood routine, urine routine, liver function, kidney function, ECG, etc., were within the normal range in their medical reports. The urine samples were collected in 50-mL polyethylene bottles and stored at 4°C until chromatography.

TABLE 1 Typical instrumental parameters and the cup configuration employed for MC-ICP-MS analysis herein

Neptune									
RF power	1180 W								
Ar cooling gas flow rate	16.00 L min^{-1}								
Ar auxiliary gas flow rate	0.93 L min^{-1} (optimised daily)								
Ar sample gas flow rate	0.91 L min^{-1} (optimised daily)								
Sample cone	Ni sample cone								
Skimmer cone	Ni X-skimmer cone								
Instrument resolution	~400 (low)								
Nebuliser	MicroFlow PFA-50; ~50uL min^{-1}								
Integration time	4.194 s								
Detection system	Faraday cups								
Amplifier	10^{11}								
Spray chamber	Dual cyclonic-Scott (quartz)								
Idle time	3 s								
Cup configuration	L4	L3	L2	L1	C	H1	H2	H3	H4
	^{91}Zr	^{92}Mo	^{94}Mo	^{95}Mo	^{96}Mo	^{97}Mo	^{98}Mo	^{99}Ru	^{100}Mo

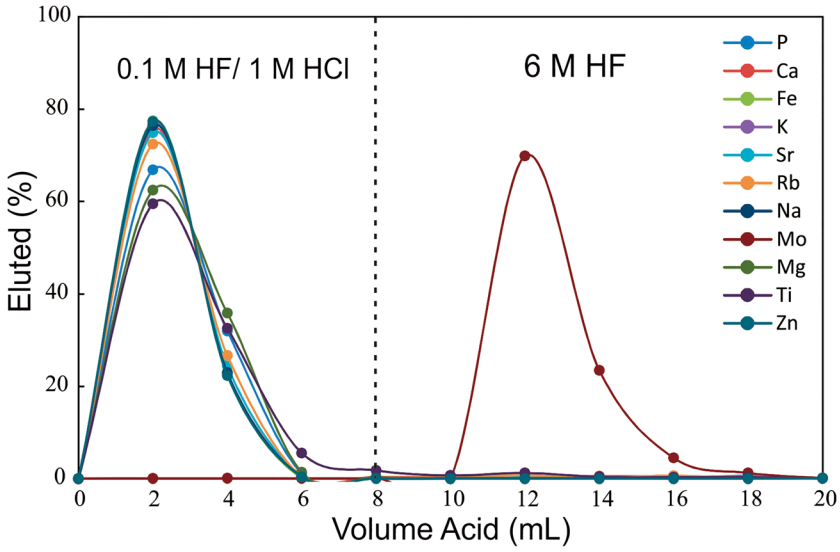

FIGURE 1 Elution curve for the in-house urine standard from the BPHA resin. Elemental concentrations were measured by quadrupole-ICP-MS in aliquots that eluted off the column. Vertical lines represent acid changes. Percent eluted was determined by dividing the total value in all aliquots by the value in a single aliquot. Yields were verified by measuring the elemental composition of the sample before and after chemical purification. All elements had at least 95% yield [Color figure can be viewed at wileyonlinelibrary.com]

2.2 | Column separation

All sample manipulations were carried out in a class 100 cleanroom at the State Key Laboratory of Isotope Geochemistry, Guangzhou Institute of Geochemistry (Guangzhou, China). The PFA beakers (Savillex) used for sample handling were soaked in turn with HNO_3 and HCl (pro-analysis grade) on a hot plate at ~100°C for 8 h and subsequently rinsed repeatedly with Milli-Q water before use.

TABLE 2 Molybdenum concentration and isotope data from replicate analyses of IAPSO seawater (A) and the in-house urine (B) standards. Asterisks denote samples that were acid digested

Standards	Mo (ng g^{-1})	$\delta^{98/95}$Mo (‰)	2SE
(A) IAPSO			
1	10.2	2.05	0.04
2	10.1	2.05	0.04
3	10.6	2.06	0.06
4	10.9	2.06	0.06
5	10.7	2.08	0.05
6	10.7	2.02	0.06
7	10.6	2.11	0.06
8	10.7	2.07	0.04
9	10.6	2.06	0.04
10	10.8	2.04	0.03
11	10.3	2.02	0.03
12	10.6	2.06	0.05
13	10.6	2.06	0.06
14	10.2	2.09	0.05
15	10.2	2.06	0.05
16	10.3	2.07	0.04
AVE ± 2SD	10.5 ± 0.5	2.06 ± 0.05	
Ref.23	10.0 ± 0.9	2.09 ± 0.05	
Ref.34	10.4 ± 0.6	2.03 ± 0.06	
(B) In-house urine standard			
1	54.90	1.69	0.03
2	54.93	1.76	0.04
3	54.81	1.70	0.03
4	54.68	1.66	0.05
5	54.87	1.64	0.03
6	54.90	1.72	0.03
7	54.59	1.65	0.04
8	54.68	1.67	0.05
9	54.87	1.64	0.03
10	54.90	1.70	0.03
11	54.59	1.65	0.04
12*	54.88	1.65	0.04
13*	54.96	1.71	0.03
14*	55.05	1.68	0.06
15*	54.79	1.66	0.02
AVE ± 2SD	54.8 ± 0.3	1.68 ± 0.07	

As the sample/spike ratio is known to affect the accuracy of $\delta^{98/95}$Mo determinations, the Mo concentration of each urine sample was pre-determined by inductively coupled plasma mass spectrometry (ICP-MS).[30] Optimal amounts of $^{97\text{-}100}$Mo double spike (^{98}Mo/^{100}Mo = 0.2–0.5[31]) were then weighed into 15-mL PFA beakers alongside 1–10 mL of urine (~100 ng of sample-derived Mo). After being heated on a hot plate at 100°C for 4–6 h, the equilibrated spiked samples were allowed to cool ready for column separation.

To demonstrate that acid digestion was not needed prior to Mo separation using a BHPA column, four aliquots of the in-house urine standard were dried and digested in a mixture of concentrated HNO_3 (2 mL) and HCl (1 mL) in sealed beakers at 140°C for 12 h. These sample solutions were then dried and redissolved in concentrated HNO_3 (0.2 mL) and 30% H_2O_2 (1 mL) before being once again evaporated to dryness. This final oxidative step was repeated until high aqueous surface tension indicated that the samples were mostly free of dissolved organic material. These organic-free residues were finally dissolved in 0.1 M HF/1 M HCl (2 mL) ready for column separation.

Based on our previous method,[26] separation and purification of Mo were achieved using our in-house BPHA resin. Briefly, ~0.4 mL of BPHA resin was packed in a Poly-Prep column (Bio-Rad Laboratories Inc., Berkeley, CA, USA; 8 × 40 mm). The sample was loaded in 2 mL of 0.1 M HF/1 M HCl. Continued washing with 6 mL of 0.1 M HF/1 M HCl effectively removed the matrix and interfering elements (Fe, Mn, Zr, Ru, Cu, Zn, Ni, etc.) from the sample, leaving the adsorbed Mo that was eluted with 6 M HF (8 mL), which is different from the 6 M HF/0.1 M HCl (8 mL) used in the previous study.[26] The purified Mo was collected in 15-mL PFA beakers and evaporated to dryness on a hot plate at 120°C. Three drops of concentrated HNO_3 and H_2O_2 were added to the dried and purified Mo to decompose any organic residue before it was dissolved in 1 mL of 3% HNO_3 (*v*/*v*) in preparation for mass spectrometry.

2.3 | Mo isotopic analysis

Isotope ratios were measured by MC-ICP-MS (Neptune Plus, Thermo Fisher Scientific, Waltham, MA, USA) using standard skimmer ("H") and sample cones. Sample solutions in 3% HNO_3 were introduced into the plasma through an Aridus II® desolvating sample-introduction system (CETAC Technologies, Omaha, NE, USA). The typical Mo intensity achieved was 180–200 V/μg mL^{-1}. The data acquisition for all samples and reference samples contained six blocks of ten ratios. Subsequently, these 60 ratios were filtered with a one-pass 2 s outlier removal test and the mean value and standard error were calculated. After each analysis, the sample was sequentially washed from the system for 10 min using solutions of 3% HNO_3, 0.1 M HF/3% HNO_3, and 3% HNO_3. The residual Mo signal was typically <1 mv, which is insignificant relative to the ion beams measured during sample analysis. The typical instrumental settings used during this study are shown in Table 1.

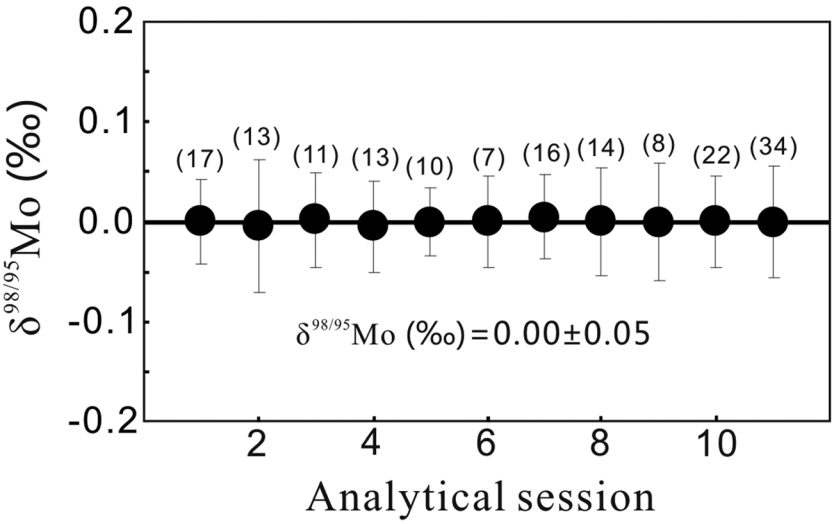

FIGURE 2 Long-term reproducibility of the spiked NIST SRM 3134 Mo standard solution over the course of 10 months. The $\delta^{98/95}$Mo values have been normalised to the daily mean and the number of measurements made during each session is shown in brackets. Error bars represent 2SD of each session, demonstrating that the $\delta^{98/95}$Mo reproducibility within an analytical session was often better than the long-term precision of 0.05‰ (2SD, n = 165)

Mo isotope ratios were reported using standard delta notation.

$$\delta^{98/95}\text{Mo}\,(‰) = \left[\left(^{98}\text{Mo}/^{95}\text{Mo}\right)_{\text{sample}}/\left(^{98}\text{Mo}/^{95}\text{Mo}\right)_{\text{NIST 3134}} - 1\right].$$

The NIST SRM 3134 Mo standard solution is defined as $\delta^{98/95}$Mo (‰) = 0.[32] The accuracy and precision of the Mo double-spike method were calculated via replicate analyses of spiked aliquots of NIST SRM 3134 (n = 165). During an analytical session, the reference solution was measured after each batch of five samples. Over the period of this study, the standard/spike ratios (^{98}Mo/^{100}Mo) were 0.2–0.5 for urine samples and NIST SRM 3134.[31] Correction for mass fractionation during chemical separation and mass spectrometry followed the double-spike deconvolution methods.[22,33]

3 | RESULTS AND DISCUSSION

3.1 | Improvements in matrix and isobaric interference element separation

Chromatographic separation must be optimised for different sample matrices. Molybdenum has been isolated from various siliceous rock and seawater samples using BPHA as the stationary phase.[26] However, in biological samples such as urine, the concentrations of Mo are substantially lower and the matrices are much more complex than in rock samples. Figure 1 illustrates the elution behaviour of elemental constituents of the in-house urine standard from the BPHA resin. The elution behavior of this urine sample was consistent with that observed during the elution of a synthetic solution described previously.[26] *N*-Benzoyl-*N*-phenylhydroxylamine is known to be highly selective towards tetra-, penta-, and hexavalent elements, whereas other matrix elements display little affinity for the resin.[26,34] Fluorides, however, have a strong negative effect on the distribution coefficients of some of these higher-valency elements,[35] hindering the absorption of Ti-Zr-Hf-Nb-W and other matrix elements at low concentrations of HF (0.1 M HF). In contrast, Mo is retained on the resin, being eluted quantitatively in 6 M HF.

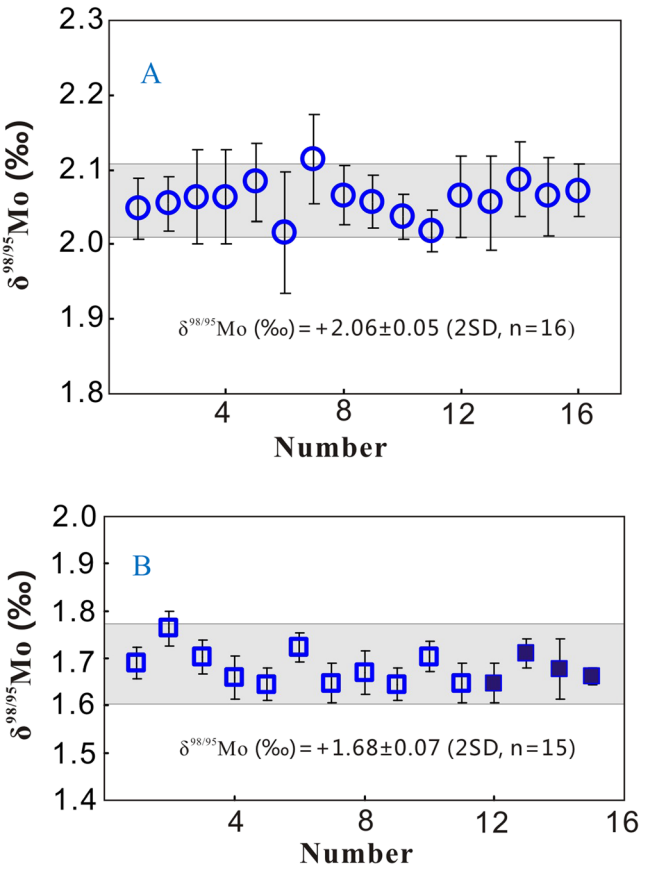

FIGURE 3 $\delta^{98/95}$Mo data for repeat analyses of A, the IAPSO seawater and B, the in-house urine standard. Error bars represent the 2SE of each measurement, whilst the grey band represents the 2SD of all analyses. Samples determined with acid digestion (closed blue triangles) and without acid digestion (open blue triangles) [Color figure can be viewed at wileyonlinelibrary.com]

To evaluate the need for acid digestion prior to BPHA separation, the Mo abundance and isotopic composition of the in-house urine standard were determined with and without acid digestion. The Mo concentrations and $\delta^{98/95}$Mo values for acid digested samples were 54.92 ± 0.22 ng g^{-1} and 1.67 ± 0.05‰ (Ave ± 2SD, n = 4), respectively; and for non-acid digested samples were 54.79 ± 0.26 ng g^{-1} and 1.68 ± 0.08‰ (Ave ± 2SD, n = 11), respectively. These results obtained with and without acid digestion were indistinguishable from each other (Table 2 and Figure 3), thus confirming that the Mo within the urine samples can be directly separated by BPHA column chromatography without acid digestion.

3.2 | Recovery and blank for the separation of Mo from urine samples

Incomplete yields can lead to isotope fractionation that approximates that observed naturally; thus, it is critical to assess the extent of fractionation that could arise from incomplete yields. To evaluate Mo yields, six 3-mL in-house urine standards were prepared with half of them being spiked. Both the spiked and the unspiked standards were then loaded onto the columns and purified. After Mo elution, the ^{100}Mo–^{97}Mo double-spike was added to the unspiked samples before analysis. The Mo concentrations in the spiked and unspiked urine samples were 54.88 ± 0.12 ng g^{-1} and 53.09 ± 0.35 ng g^{-1} (Ave

TABLE 3 Gender, age, [Mo] and $\delta^{98/95}$Mo data from the urine sample set. Each sample was measured once

Sample identifier	Gender	Age	Mo (ng g^{-1})	$\delta^{98/95}$Mo	2SE
HP001	F	68	14.4	1.33	0.05
HP002	F	42	75.9	1.34	0.02
HP003	F	42	106.7	1.45	0.02
HP004	F	24	75.9	1.28	0.02
HP005	F	10	70.2	1.36	0.02
HP006	F	15	50.0	1.24	0.03
HP007	F	24	70.7	1.47	0.02
HP008	F	36	37.0	1.27	0.02
HP009	F	25	111.0	1.20	0.02
HP010	F	40	106.4	1.20	0.02
HP011	F	8	44.3	1.45	0.03
HP012	F	22	82.9	1.23	0.02
HP013	F	56	68.9	1.22	0.03
HP014	F	48	50.7	1.27	0.03
AVE ± 2SD				1.31 ± 0.19	
HP015	M	69	54.4	1.55	0.03
HP016	M	41	22.6	1.58	0.04
HP017	M	33	99.4	1.52	0.03
HP018	M	32	32.1	1.53	0.02
HP019	M	31	46.5	1.43	0.02
HP020	M	39	8.9	1.49	0.06
HP021	M	53	22.9	1.52	0.02
HP022	M	11	36.4	1.76	0.03
HP023	M	29	81.7	1.57	0.03
HP024	M	36	23.8	1.68	0.06
HP025	M	54	13.0	1.54	0.05
HP026	M	45	16.8	1.53	0.06
HP027	M	37	29.8	1.55	0.04
HP028	M	21	113.7	1.66	0.02
HP029	M	26	49.8	1.52	0.04
HP030	M	28	54.2	1.47	0.03
HP031	M	33	122.8	1.48	0.03
AVE ± 2SD				1.55 ± 0.16	

SE: standard error.

± 2SD, n = 3), respectively. Thus, the recovery of Mo was almost quantitative at >96%. The $\delta^{98/95}$Mo values of the spiked samples (1.67 ± 0.06‰, Ave ± 2SD, n = 3) were also indistinguishable from those of the unspiked samples (1.65 ± 0.03‰, Ave ± 2SD, n = 3). Thus, the BPHA column separation is unlikely to cause isotope fractionation of Mo.

The total procedural blanks varied from 0.12 to 0.25 ng (mean = 0.21 ± 0.10 ng; 2SD; n = 5), < 0.1% of the sample-derived Mo. This was deemed to be negligible and no correction was applied.

3.3 | Accuracy and precision

The precision of the individual measurements, referred to as internal precision, was typically ±0.04‰ (±2SE). The daily $\delta^{98/95}$Mo reproducibility was determined via repeated analysis of spiked ~200 ng g^{-1} solution of NIST SRM 3134 Mo, providing a measure of machine behavior during sample analysis. The external precision was quantified by repeated $\delta^{98/95}$Mo measurements over a 10-month analytical period that encompassed 11 analytical sessions. The resulting $\delta^{98/95}$Mo values were normalised to the daily mean, and the external precision of the spiked NIST SRM 3134 Mo standard solution normalised in this manner was ±0.05‰ (2SD, n = 165; Figure 2).

In the absence of a urine sample with a certified $\delta^{98/95}$Mo value, the accuracy of the approach employed herein was initially verified by replicate analyses of the IAPSO seawater standard. Molybdenum concentrations and isotopic ratios are uniform in open seawater because of the long residence time of Mo relative to ocean mixing.[36] After spiking and Mo separation via the BPHA column procedure, the average $\delta^{98/95}$Mo value and the mean Mo concentration of the IAPSO seawater standard were found to be 2.05 ± 0.06‰ and 10.5 ± 0.5 ng g^{-1} (2SD, n = 16), respectively (Table 2 and Figure 3) – values that are in excellent agreement with those previously reported.[32,37,38] In addition to the seawater standard, replicate analyses of an in-house urine standard were performed. This in-house urine standard sample yielded a mean $\delta^{98/95}$Mo value of 1.68 ± 0.07‰ with a mean concentration of 54.8 ± 0.3 ng g^{-1} (Ave ± 2SD, n = 15, Table 2). The external reproducibility for this in-house urine standard is comparable with that observed from replicate analyses of NIST SRM 3134 performed over a 10-month period (±0.05‰, 2SD). These values demonstrated that we can measure the Mo isotope ratios and concentrations accurately in complex matrix samples such as urine using MC-ICP-MS.

3.4 | Analysis of urine samples

The concentration and isotopic composition of Mo isolated from urine samples provided by healthy donors were determined, and are summarised in Table 3. The urinary Mo concentrations were 8.9–122.8 ng g^{-1} - within the range of previously published values.[20,30,39-42] Without exception, the urine samples were enriched in ^{98}Mo relative to NIST 3134. These urine samples

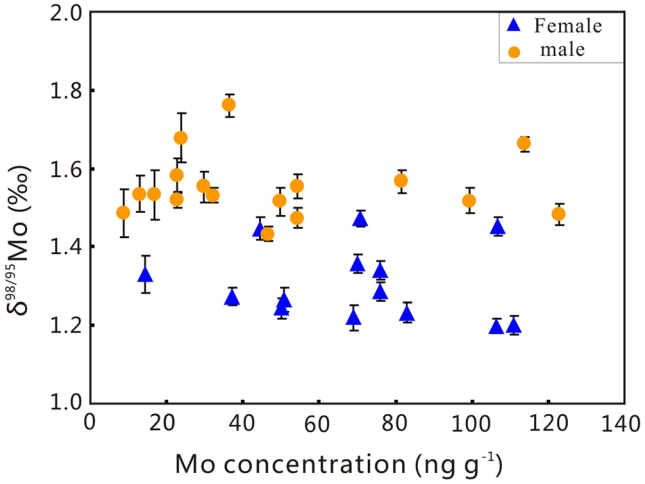

FIGURE 4 Mo concentration versus isotopic information plot for the urine samples of the female and male healthy participants. Error bars represent analytical uncertainty (2SE) [Color figure can be viewed at wileyonlinelibrary.com]

displayed limited isotopic variation ($\delta^{98/95}$Mo = +1.20 to +1.76‰) and yielded a mean $\delta^{98/95}$Mo value of 1.44 ± 0.30‰ (Ave ± 2SD, n = 31). There appears to be an offset in the $\delta^{98/95}$Mo values between the male population (1.55 ± 0.16‰, Ave ± 2SD, n = 17) and the females (1.31 ± 0.19‰, Ave ± 2SD, n = 14) (Figure 4), but that they are not resolved due to the variance. Although a more extensive study needs to be carried out to confirm the natural isotopic variability of Mo in the human body, the results found are promising, pointing in the direction that the combination of Mo concentration and isotopic analysis might serve as a valuable urinalysis tool-kit in clinical assessment.

4 | CONCLUSIONS

We determined natural Mo isotope variations in human urine samples by MC-ICP-MS following isolation and purification of Mo via BPHA column chromatography. Purification by BPHA was quantitative and did not require acid digestion. Chemical and instrumental mass fractionation was efficiently corrected using a double-spike approach. The precision and accuracy of this method were evaluated via replicate analyses of the IAPSO seawater ($\delta^{98/95}$Mo = 2.06 ± 0.05‰, Ave ± 2SD, n = 17), and an in-house urine standard ($\delta^{98/95}$Mo = 1.68 ± 0.07‰, Ave ± 2SD, n = 15). Applying the approach detailed herein to a sample set obtained from healthy participants from Guangzhou, China, we have generated the first urine-derived $\delta^{98/95}$Mo dataset. All samples within this sample set are enriched in ^{98}Mo relative to NIST 3134. Interestingly, within the resolution of our approach, molybdenum isotope variability is observed; however, its cause remains an open question. Although this study deals with human urine, the elemental composition of urine is broadly similar to that of blood and serum, suggesting that this technique could be extended to other biomedical sample types.

This study is encouraging as far as the use of Mo isotopic analysis for understanding of human Mo metabolism and diagnose disorders is concerned, even if further studies with a larger number of samples are needed.

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ORCID

Jing Zhang https://orcid.org/0000-0002-2010-9690
Le Zhang https://orcid.org/0000-0001-9161-0653

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