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Impact of Sample Preparation History on Isotopic Fractionation: Isotope Ratios of Lead SRM 981

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Electronic supplementary information (ESI) available.

ABSTRACT

Several batches of NIST SRM 981 dissolved in 0.15 mol/kg HNO_3 were used for the determination of *K* factors of isotope ratios ^{204}Pb/^{208}Pb, ^{206}Pb/^{208}Pb, and ^{207}Pb/^{208}Pb applying MC-ICP-MS. Pure solutions of the primary standard (reference) material NIST SRM 981 (w(Pb) ≈ 70 ng/g to 100 ng/g) were compared with those treated with a chromatographic separation using a crown ether resin, and additionally with those treated with microwave digestion followed by chromatographic separation. The procedural blank influence was studied and potential interferences of ^{204}Hg on the ^{204}Pb signal were corrected. An improved chromatographic separation protocol demonstrates the suitability of Pb separation. Mass bias correction factors (*K* Factors) were determined for each ratio and compared for the three procedures. The respective uncertainty budgets for the first time gave direct quantitative indications of differences and uncertainty contributions of the respective digestion and column separation processes compared to untreated solutions. To derive the impact of the preparation steps alone (digestion, column separation), pure SRM® solutions with Pb (no Pb containing matrix samples) were investigated. For K(^{204}Pb/^{208}Pb) an impact of a sample pretreatment cannot be observed and isotopic fractionation can be mainly deduced from the mass spectrometric measurement. In case of the ratios ^{206}Pb/^{208}Pb and ^{207}Pb/^{208}Pb, the *K*

factors of the digested and column separated as well as only column separated samples can be distinguished from the non-pretreated samples for $k = 1$, which suggests a small, but observable impact of the sample history on the K factor although the initial samples were matrix-free.

1. Introduction

The experimental determination of isotope ratios using mass spectrometry is always accompanied by instrumental isotopic fractionation (IIF), often called mass bias. Due to physical and other processes the measured isotope ratios deviate from the “correct” or “true” values up to several percent, when e.g., applying multicollector-inductively coupled plasma mass spectrometry (MC-ICP-MS) which was used in this work. [1-5] To correct for isotopic fractionation in a system with at least two isotopes, one possibility is to use certified reference materials CRMs (preferably traceable to the international system of units SI).[1-7] However, the availability of CRMs is often limited and cost-intensive. Moreover, in the last decade, other sophisticated methods for the absolute determination of K factors have been invented: The most accurate and precise is the “full gravimetric isotope mixture” correction model.[8-11] However, if CRMs are available, it is more convenient to use them although the measurement uncertainty associated with results is limited by the CRMs itself. It is additionally important to use the CRM in the same matrix as the analyte or to remove the matrix material by an adequate process (e.g. digestion and/or analyte-selective extraction chromatographic method). Lead is one of the most intensively studied elements in isotope ratio mass spectrometry.[10,12-17] It consists of a mixture of radiogenic (^{206}Pb, ^{207}Pb, ^{208}Pb) and non-radiogenic (^{204}Pb) isotopes. Due to this fact, no conventional ratio can be fixed which makes Pb attractive for several areas. Among them are geological sciences, forensics, health studies, food chemistry, environmental analysis, and not least metrology in chemistry.[18] The most often used and accepted CRM of lead is the Standard Reference Material® 981 from the National Institute of

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Standards and Technology (NIST), in the following called: NIST SRM 981. Its absolute isotopic composition has been determined in the 1960s, and numerous publications dealing with this very material have been published.[19,20] Most laboratories measuring Pb isotope ratios are applying NIST SRM 981 in solution, dissolved in a few percent of aqueous HNO_3. For this reason, we decided to use this material to study whether sample pre-treatment (digestion, column separation) has a measurable impact on derived *K* factors of the ratios $^{204}Pb/^{208}Pb$, $^{206}Pb/^{208}Pb$, and $^{207}Pb/^{208}Pb$. We wanted to test whether and how common sample preparation procedures (digestion, column chromatography) introduce additional isotopic fractionation. Although in practice microwave digestion is applied mainly on “real” samples in a matrix, we tried to investigate potential influences of the digestion process itself which can be accompanied by e.g. absorption in or adsorption on the surfaces of the digestion vessels which might generate isotopic fractionation. The aim was to quantify the contribution of, e.g. additional digestion of a sample to the *K* factor of a respective isotope ratio compared to the *K* factor of the solution only, and/or the amount of the contribution of column separation compared to the *K* factor of the solution. Thus, we ask whether it is possible to distinguish the contributions of individual pre-treatments to the final *K* factor. To correct for a potential ^{204}Hg interference, the ^{202}Hg signal was monitored throughout all measurements and the ^{204}Pb signal was corrected accordingly. Moreover, it was examined whether a blank correction influences the ratios as well as the derived *K* factors. Using a state-of-the-art commercial column resin material for the separation of lead and a respective digestion and elution protocol,[18,21-23] this study can be taken as a versatile but validated building block for extended isotope ratio measurements of Pb samples in more difficult matrices. For a quantitative analysis of the *K* factor dependence on the sample pretreatment history, the measurements and data evaluation were accompanied by uncertainty budgets according to the rules of the “Guide to the expression of uncertainty in measurement” GUM.[24]

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2. Experimental Section

2.1 Materials and Reagents

High purity water (resistivity ≥ 18 MΩ cm) for cleaning and dilution was generated using a combined Elix™ Essential 5 UV and Milli-Q™ IQ 7000 purification system (Merck Chemicals, Germany). All solutions were based on aqueous nitric acid ($w(HNO_3) = 0.0095$ g/g ≈ 1 %). Initial HNO_3 (65%, EMSURE® p. a., Merck Millipore, Germany) was sub-boiled using an OmniPure™ (Teledyne CETAC Technologies, USA) distillation system and was further diluted. For the digestion, H_2O_2 (30 %, Suprapur™, Merck Millipore) was applied. For column separation, HCl (25 %, EMSURE® p. a., Merck Millipore, Germany) was sub-boiled using a DST-1000 sub-boiling distillation system (Savillex Corporation, Eden Prairie, MN, USA).

Bottles, vials, and surfaces in contact with the solutions were made of PFA (perfluoroalkoxy). The PFA equipment was cleaned according to the following protocol: lab dishwasher; rinsing with: 1. high purity water; 2. 10 % Extran™ (lab detergent, Merck Millipore): 3 days lab shaker; 3. high purity water; 4. 1 % HNO_3 (sub-boiled): 3 days lab shaker; 5. high purity water; drying. Quartz sample vials ($V = 24$ mL) as well as respective PTFE (polytetrafluorethylene) caps for microwave assisted digestion and collection of the Pb fraction after column separation were cleaned as follows: 1. lab dishwasher; 2. rinsing with high purity water; 3. drying; 4. microwave assisted digestion (for purification only) using 5 mL HNO_3 (65 %) and 3 mL H_2O_2 (30 %); 5. rinsing with high purity water; 6. drying. Argon gas used for subsequent mass spectrometric measurements had a purity of 99.999 % (5.0).

NIST SRM 981 was already dissolved in 1% HNO_3 in our laboratory to produce stock solutions of different Pb mass fractions; these were further diluted and used as the "sample" material. The certified values are used here as a consistent anchor point for comparative purposes, not as an attempt at a new absolute ratio calibration. All dilutions and blends were prepared in a temperature- and humidity-controlled weighing laboratory equipped with anti-electrostatic surfaces. Sample preparation was performed gravimetrically applying air buoyancy correction.

This in turn enabled the mass fraction to be corrected for the inevitable evaporation of acid and water. This was done by weighing any bottle before and after withdrawing an aliquot of solution. Analytical balances used were a five-digit balance Cubis™ MCE225S (resolution: 0.01 mg, Sartorius, Germany) for the blend preparation for digestion samples and a mechanical balance H315 (resolution: 0.1 mg, Mettler-Toledo, Switzerland) for stock solutions and dilutions.

For the study of "matrix-free" sample material based on NIST SRM 981, the following sample solutions were prepared by dilution from respective stock solutions:

z_ref with $w(\text{z\_ref}) = 100$ ng/g, z_14 with $w(\text{z\_14}) = 70$ ng/g, and $w(\text{z\_135}) = 70$ ng/g. z_ref, z_14 and z_135 were derived from three different branches of prepared stock solutions from NIST SRM 981. The *K* factors related to these sample solutions will be denoted as e.g. $K_{\text{z14,sol}}$ in the following.

2.2 Sample Digestion

For the study of the impact of the digestion step on possible isotopic fractionation, sample aliquots of approximately 2.5 g z_14 ($w(\text{z\_14}) = 70$ ng/g) with 2.5 g HNO_3 ($w = 1\%$) (and 2.5 g z_135 ($w(\text{z\_135}) = 70$ ng/g) with 2.5 g HNO_3 ($w = 1\%$), respectively) were weighed into 24 mL quartz vessels (microwave tubes). To each aliquot, 5 mL HNO_3 (65 %) and 3 mL H_2O_2 (30 %) were added. The digestion was carried out with an ultraCLAVE III™ microwave system (MLS, Germany) applying the following parameters: total duration 2 h ($p_0 = 50$ bar Ar, $T = 250$ °C); linear ramp from room temperature to 250 °C (1 h); plateau at constant temperature (1 h, 250 °C, $p_{\text{max}} = 150$ bar); cooling down. The applied microwave power was variable and controlled by the temperature program. However, the applied power was restricted to 1000 W. The microwave system was operated with a base load of 400 mL H_2O, 10 mL H_2O_2 (30 %) and 2 mL H_2SO_4 (96 %). Subsequently, the clear solutions were evaporated to dryness in an aluminum (Al) block on a hot plate (duration: 8 h, $T_{\text{max}} = 125$ °C). After cooling down, 8 mL of HNO_3 ($c = 1$ mol/L) were added to each sample residue prior to column separation. The

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resulting sample solutions (after additional column separation) were denoted as z_14dc and z_135dc (d for digestion and c for column separation).

2.3 Column separation

For the study of the impact of digested and column separated samples, the sample solutions (8 mL in $c(HNO_3)$ = 1 mol/L) were loaded onto commercially available preconditioned Pb resin columns (PB-C20-A™, Triskem, France) with a 2 mL bed-volume.[21-23] The improved separation protocol (Table 1) was based on a former related protocol applied in the CCQM-K158 key comparison.[18] To provide a maximum of contact time of the eluents with the column, the separation is performed under gravity control (not under vacuum, which often reduces the contact time). The sample aliquots were collected in additional 24 mL quartz vessels (microwave tubes) which have been carefully cleaned. The sample solutions were evaporated to dryness in an Al block on a hot plate to remove the 6 mol/L HCl (8 h at 120 °C). The residues were redissolved in 3 mL HNO_3 (w = 65 %) and again evaporated to dryness in an Al block on a hotplate (9 h, 140 °C) to remove potential abraded organic matter or remaining column particles (bed-particle size: 100 – 150 µm).

Table 1. Elution protocol using Triskem PB-C20-A™ resin columns (2 mL bed-volume) for Pb separation. The approximate flow rate is 0.3 mL /min (standard atmospheric conditions); total duration ≈ 270 min. The flow is controlled by gravity to ensure a proper contact time between the sample solution and the resin bed.

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fraction	**eluent**	***c* / (mol/L)**	***V* / mL**	**aliquots**	**action**
1	HCl	6	4	1 × 4	Pb impurities removal
2	HCl	6	4	1 × 4	
2 a	HCl	6	4	1 × 4	
2 b	HCl	6	4	1 × 4	
3	HNO_3	1	4	1 × 4	conditioning
4	HNO_3	1	4	1 × 4	
5	sample in HNO_3	1	8	2 × 4	sample load
6	HNO_3	1	16	4 × 4	matrix removal
7	HCl	6	3	1 × 3	waste
8	HCl	6	12	3 × 4	Pb fraction
9	HCl	6	8	2 × 4	regeneration
10	HCl	6	8	2 × 4	

The dried residues were redissolved in 4 mL HNO_3 (1 %) to yield measurement solutions with w(Pb) ≈ 45 ng/g. The pre-packed columns consist of a crown-ether (4,4'(5')-di-t-butylcyclohexano-18-crown-6) diluted in isodecanol on an inert support capable to effectively capture Pb^{2+} ions in the ether cage.[21-23]

A third set of sample solutions was treated with the column separation only (without digestion) and the respective samples are denoted as z_14c and z_135c.

The recovery of the column separation process has been tested by three individual campaigns applying one point calibration with a reference solution (NIST SRM 981) estimating the measured mass fraction w(Pb) from fraction 8 (Table 1). These data were compared to the gravimetrical determination of w(Pb) yielding the theoretical apparent mass fraction (100 %) of Pb after separation. The determined average recovery is 92 % (different recoveries didn't result in different isotopic fractionations). Thus, an almost complete recovery of the presented separation protocol can be postulated.

2.4 Mass spectrometry

All isotope (intensity) ratio measurements were carried out using a Neptune XT™ multicollector-inductively coupled plasma mass spectrometer (MC-ICP-MS, Thermo Fisher Scientific GmbH, Bremen, Germany) equipped with a high-throughput jet-interface OnToolBooster™ pump (Pfeiffer Vacuum, Germany). The high vacuum region was backed with an oil-free scroll pump (nXDS6i™, Edwards, Germany).

Main parameters of the MC-ICP-MS are listed in Table 2. The measurement sequence was built-up as follows: forward direction: blank, 2 × (z_ref, z_14, z_14c, z_14dc, z_135, z_135c, z_135dc), reverse direction: 2 × (z_135dc, z_135c, z_135, z_14dc, z_14c, z_ref), blank). Due to the limited amount of the digested and column separated samples as well as to avoid potential drift issues of the MC-ICP-MS, the sequences were created to have a duration of approximately 3 h. For the correction of potential Hg interferences, the ^{202}Hg signal was monitored throughout the measurements (L3 detector). Although the ^{202}Hg signal was almost negligible, the ^{202}Hg/^{204}Hg mass bias was estimated via the exponential law. A natural ratio of $n(^{204}\text{Hg})/n(^{202}\text{Hg})$ = 0.23007 mol/mol was used.[25] The resulting mass bias-corrected ^{204}Hg signal was subtracted from the m/z = 204 signal yielding the ^{204}Pb signal used for all calculations.[26] The applied principle of the ^{204}Hg interference correction as well as the used reference data can be tracked in detail in the Supplementary Information.

Briefly, the ^{204}Hg interference correction of the ^{204}Pb signal is performed via the expression

$$U\left(^{204}\text{Pb}\right)=U\left(^{204}\text{Pb}_{\text{nc}}\right)-U\left(^{204}\text{Hg}\right)=U\left(^{204}\text{Pb}_{\text{nc}}\right)-\frac{1}{K_4}\times\frac{x\left(^{204}\text{Hg}\right)}{x\left(^{202}\text{Hg}\right)}\times U\left(^{202}\text{Hg}\right) \quad (1)$$

with the measured ion intensities U (in V); the non-corrected ^{204}Pb signal $U(^{204}\text{Pb}_{\text{nc}})$ and the reference amount-of-substance fractions x of Hg. The calibration factor K_4 is obtained via

$$K_4=K_2^{\gamma} \quad (2)$$

with the calibration factor K_2

$$K_2=\frac{R(^{206}\text{Pb}/^{208}\text{Pb})}{r(^{206}\text{Pb}/^{208}\text{Pb})} \quad (3)$$

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with the ratios R from the certificate (“true” ratios) and the measured ratios r. The exponent γ is determined using

$$\gamma = \frac{\ln \dfrac{M\left({}^{204}\mathrm{Hg}\right)}{M\left({}^{202}\mathrm{Hg}\right)}}{\ln \dfrac{M\left({}^{206}\mathrm{Pb}\right)}{M\left({}^{208}\mathrm{Pb}\right)}} \tag{4}$$

with the respective molar masses of the isotopes (given in the supporting information).

Table 2 MC-ICP-MS (Neptune XT™) parameters

cool gas (Ar) / L min^{-1}	16.0
auxiliary gas (Ar) / L min^{-1}	0.8
nebulizer gas (Ar) / L min^{-1}	1.1
nebulizer (sample flow rate / µL min^{-1})	PFA, self-aspirating (50)
sampler, orifice / mm	Nickel, 1.1
skimmer, orifice / mm	nickel (“H-type”), 0.8
mass resolution $M/\Delta M$	400 (LR, pseudo low resolution mode)
sample introduction system	ASX110FR autosampler (CETAC™) in a class-100 laminar flow hood
spray chamber	double pass cyclonic/Scott (quartz)
torch, injector tube, bonnet	quartz
radio frequency power / W	1200
Data acquisition	
operation mode	static
baseline measurement	defocusing at start (30 s)
integration time / s	0.13
number of integrations / cycle	1
number of cycles / block	1
number of blocks	20
detectors	Faraday cups: L3 (^{202}Hg), L1(^{204}Pb), C (^{206}Pb), H1(^{207}Pb), H2 (^{208}Pb) all with $R = 10^{11}$ Ω

3. Results and discussion

3.1 Blank Influence on Measurements

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The possible impact of the blank was tested by evaluating the data with and without subsequent blank subtraction (all raw data as well as data calculation procedures are provided in the Supplementary Information). Figure 1 displays the effect of blank correction in case of intensity ratios ^{204}Pb/^{208}Pb in a representative sequence for a z_14 sample (SRM 981 in 1% HNO_3 with w(Pb) = 70 ng/g). No significant impact on the ratios could be observed. The relative experimental standard deviation of the mean ranges between 0.013 % and 0.015 % and the data points cannot be separated significantly. Therefore, for the data evaluation in this study and the potential impact of experimental conditions on *K* factors, the not-blank corrected data were chosen.

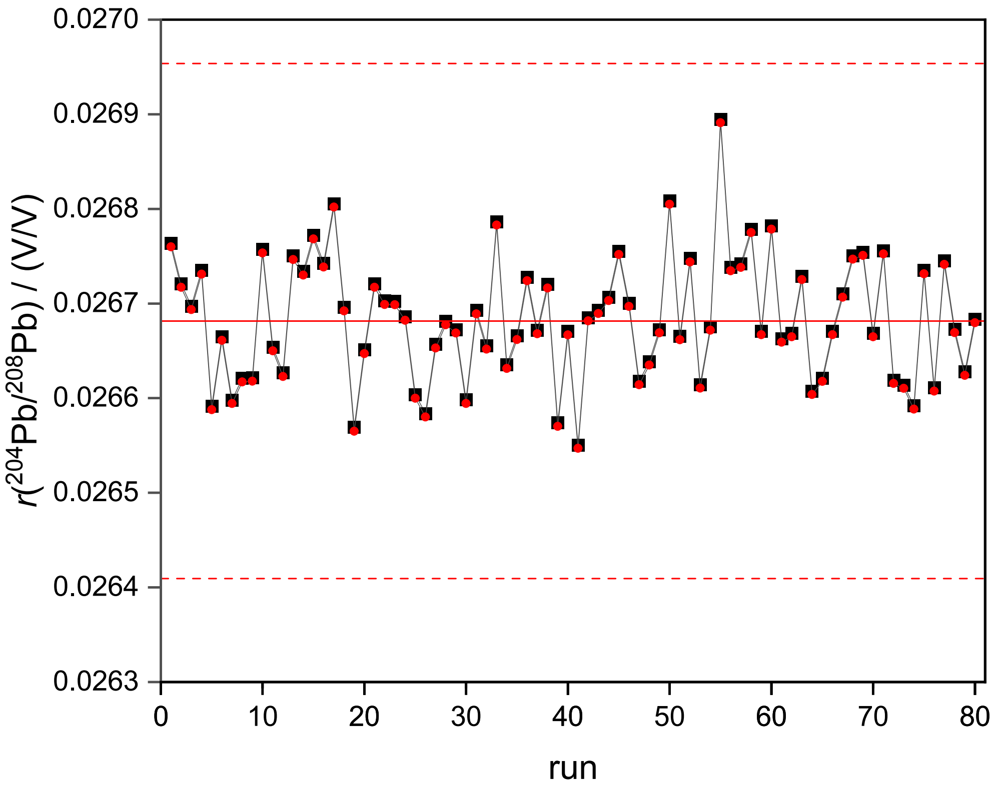

Figure. 1 Measured intensity ratios $r(^{204}Pb/^{208}Pb)$ determined during one sequence using solution z_14 (w(Pb) = 70 ng/g) prepared from NIST SRM 981. Filled black squares: data points without blank correction; red solid circles: data points with blank correction. Solid red line: Average of blank-corrected data; dashed red lines: Upper and lower relative standard

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deviations of the average. The relative deviation of the of the not-blank corrected signal from the blank-corrected signal is generally smaller than 0.015 %.

The procedural blank of the column separation process could not be distinguished from the blank found in the solvent (0.15 mol/kg HNO_3). Therefore, the impact of the procedural blank on the isotope ratios was less than one tenth of the uncertainty associated with the isotope ratios.

3.2 Impact of Sample History on *K* Factors

The relation between corrected “true” and measured “biased” isotope ratios is

$$R_{\text{true}} = K \times R_{\text{meas}} \Leftrightarrow R = K \times r \quad \Rightarrow \quad K = \frac{R}{r} \tag{5}$$

with the corrected “true“ ratio (capital *R*) and measured intensity ratio *r*. Thus, measuring *r* in a material with known *R* yields the *K* factor (K_{meas} or K_{total}) of the respective isotope ratio. This *K* factor includes all impact scenarios leading to this actual value which is used to convert the measured into the “true” value. However, several applications e.g. isotope dilution mass spectrometry (IDMS) with the determination of the molar mass of the analyte element, applying the measurement of isotope ratios of a reference material imply the congruence of the *K* factors of the ratios in all samples, disregarding the origin of preparation (dilution only, digestion, column separation etc.). For a certain ratio (e.g $^{204}Pb/^{208}Pb$) the same *K* factor will be applied for all these samples with different preparation history. In this study, we determined *K* factors of ratios $^{204}Pb/^{208}Pb$, $^{206}Pb/^{208}Pb$, and $^{207}Pb/^{208}Pb$ of the same starting material (NIST SRM 981) originating from samples with different origins of preparation. Theoretically, one might expect *K* factors like

- $K_{\text{total,sol}}$, the (measured) calibration factor in the (diluted) solution of the reference material

- $K_{\text{total,sol, col}}$, the (measured) calibration factor in the (diluted) solution of the reference material after column separation
- $K_{\text{total,sol, dig, col}}$, the (measured) calibration factor in the (diluted) solution of the reference material after digestion and column separation

which should theoretically yield the same value within the range of associated uncertainties. It was tried to investigate whether it is possible to separate the contributions of different preparation steps (dilution, digestion, column separation) towards the resulting K factor with the potential aim to improve the preparation step.

The mass fraction of sample z_ref is comparably larger (w(z_ref) = 100 ng/g) than that of the samples z_14 and z_135 to compare a potential - possibly severe - effect of signal intensity or concentration itself on the K factor distribution. Thus, z_ref is used as a kind of a reference marker or anchor in this study. The ^{204}Pb/^{208}Pb ratios used for further evaluations were all corrected regarding ^{204}Pb taking a possible ^{204}Hg interference into account, since it is not possible with the current equipment to obtain a necessary mass resolution $m/\Delta m > 452\,000$ to separate ^{204}Pb from ^{204}Hg. The measured sequences were created in a symmetric order with the sample aliquots measured in a forward manner to the middle of the sequence and then reverse which eliminates drift effects due to carryover or time and/or mass drift of the MC-ICP-MS. Figure 2 shows the K factors of the ratios ^{204}Pb/^{208}Pb, ^{206}Pb/^{208}Pb, ^{207}Pb/^{208}Pb measured in sequences A, B, and C. Due to the physical fact the mass biases of the ratios differ significantly (deviation from unity: ^{204}Pb/^{208}Pb ≥ 2 %; ^{206}Pb/^{208}Pb ≥ 1 %, and ^{207}Pb/^{208}Pb ≥ 0.5 %), the behavior of the sample pretreatment is discussed for each ratio separately.

3.2.1 K Factors for ^{204}Pb/^{208}Pb

The measured ratios $r(^{204}\text{Pb}/^{208}\text{Pb})$ are approximately 2 % lower than the true ratios, so $K \approx 1.02$ (mol/mol)/(V/V). For the diluted samples z_14, and z_135, an average $K_{\text{total,sol}}(^{204}\text{Pb}/^{208}\text{Pb}) = 1.02026(61)$ (mol/mol)/(V/V) was found, whereas z_ref yielded

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$K_{\text{total,sol}}(^{204}\text{Pb}/^{208}\text{Pb})$ = 1.02017(50) (mol/mol)/(V/V), a relative difference of 0.0089 %. Numbers in brackets denote standard uncertainties of the last digits. This clearly proved that the different mass fractions of the pure diluted sample solutions used here (w(z_ref) = 100 ng/g and w(z_14) = w(z_135) = 70 ng/g) have no significant influence on the resulting K factor within the limits of the associated uncertainties. The respective averaged K factors for the diluted and subsequently column-separated sample solutions yielded $K_{\text{total,col}}$ $(^{204}\text{Pb}/^{208}\text{Pb})$ = 1.0209(17) (mol/mol)/(V/V), with an extended uncertainty range (due to column separation and data scattering). The additional digestion and subsequent column separation gave $K_{\text{total,col,dig}}(^{204}\text{Pb}/^{208}\text{Pb})$ = 1.0210(12) (mol/mol)/(V/V), almost the same value. A representative uncertainty budget for K factors of the ratios $^{204}\text{Pb}/^{208}\text{Pb}$ according to the GUM is given in Table 3.[24] The following budgets were calculated using the GUM Workbench Pro™ software (version 2.4.1. 392; Metrodata GmbH, Germany). Obviously, the main uncertainty contribution in all three sample categories (simple dilution; additional column separation; dilution, digestion and column separation) resulted from the measured ratio $r(^{204}\text{Pb}/^{208}\text{Pb})$ (72 % up to 92 %). Especially the digested and column separated samples were dominated by this uncertainty contribution.

Table 3. Uncertainty budget of $K(^{204}\text{Pb}/^{208}\text{Pb})$ in NIST SRM 981 for: diluted solution, column separated solution, and column separated solution after digestion. Molar masses M were taken from [27]. Reference (true) ratios of NIST SRM 981were adopted from [19,20].

***K* factors ($x(^{204}\text{Pb})/x(^{208}\text{Pb})$): solution, column, column + digestion**

Quantity	Unit		Best estimate (value)	Standard uncertainty	Sensitivity coefficient	Index
X_i	$[X_i]$	Sample	x_i	$u(x_i)$	c_i	%
		solution				20.9
$R(^{204}\text{Pb}/^{206}\text{Pb})$	mol/mol	column	0.0590420	$18.5\cdot10^{-6}$	17	17.4
		column, digest.				6.3
		solution				7.3
$R(^{208}\text{Pb}/^{206}\text{Pb})$	mol/mol	column	2.168100	$400\cdot10^{-6}$	-0.47	6.0
		column, digest.				2.2

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***K* factors ($x(^{204}Pb)/x(^{208}Pb)$): solution, column, column + digestion**

Quantity	Unit		Best estimate (value)	Standard uncertainty	Sensitivity coefficient	Index
$r(^{204}Pb/^{208}Pb)$	V/V	solution	0.0266886	$15.5\cdot10^{-6}$	-38	71.8
		column	0.0266886	$17.5\cdot10^{-6}$		76.5
		column, digest.	0.0266903	$31.9\cdot10^{-6}$		91.5
Y	$[Y]$		y	$u_c(y)$	$u_{c,rel}(y)$ / %	
$K_{204,208}$	(mol/mol) / (V/V)	solution	1.020364	$699\cdot10^{-6}$	0.069	
		column	1.019982	$765\cdot10^{-6}$	0.075	
		column, digest.	1.02030	$1.28\cdot10^{-3}$	0.13	

Generally, the spread of $u(K(^{204}Pb/^{208}Pb))$ covers all three sample preparation categories, thus there is no need for sample specific distinction of K due to the sample preparation history.

3.2.2 *K* Factors for $^{206}Pb/^{208}Pb$

The K factors correcting the ratio $^{206}Pb/^{208}Pb$ deviate from unity by approximately 1 %. For this ratio, however, a clear dependence on the sample history can be observed: The $K_{total,sol}(^{206}Pb/^{208}Pb) = 1.00968(20)$ (mol/mol)/(V/V) exhibits an elevated K factor compared to the downshifted $K_{total,col}(^{206}Pb/^{208}Pb) = 1.00894(24)$ (mol/mol)/(V/V) and $K_{total,dig,col}(^{206}Pb/^{208}Pb) = 1.00925(21)$ (mol/mol)/(V/V). Although the absolute values as well as the uncertainties of the two latter are similar, both are reduced compared to the respective K factor of the diluted-only sample by 0.0007 and 0.0004 (mol/mol)/(V/V) which is small, but significant with respect to the associated uncertainties as shown in Figure 2. When comparing the uncertainty budgets (Table 4), the main uncertainty contributions result from the certified value $R(^{208}Pb/^{206}Pb)$ (86 %, 81 %, and 72 %). In this case, the measurement of r is by far more precise (due to the intrinsic higher isotopic abundance of ^{206}Pb and ^{208}Pb and a reduced data scattering) compared to the measurement of $r(^{204}Pb/^{208}Pb)$.

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Table 4. Uncertainty budget of $K(^{206}\text{Pb}/^{208}\text{Pb})$ in NIST SRM 981 for: diluted solution, column separated solution, and column separated solution after digestion. Molar masses *M* were taken from [27]. Reference (true) ratios of NIST SRM 981were adopted from [19,20].

***K* factors ($x(^{206}\text{Pb})/x(^{208}\text{Pb})$): solution, column, column + digestion**						
Quantity	Unit		Best estimate (value)	Standard uncertainty	Sensitivity coefficient	Index
X_i	$[X_\text{i}]$	Sample	x_i	$u(x_i)$	c_i	%
$R(^{208}\text{Pb}/^{206}\text{Pb})$	mol/mol	solution	2.168100	$400 \cdot 10^{-6}$	-0.47	85.9
		column				80.9
		column, digest.				72.0
$r(^{206}\text{Pb}/^{208}\text{Pb})$	V/V	solution	0.4568300	$34.1 \cdot 10^{-6}$	-2.2	14.1
		column	0.4570300	$40.9 \cdot 10^{-6}$		19.1
		column, digest.	0.4569500	$52.5 \cdot 10^{-6}$		28.0
Y	$[Y]$		y	$u_\text{c}(y)$	$u_\text{c,rel}(y)$ / %	
$K_{206,208}$	(mol/mol) / (V/V)	solution	1.009639	$201 \cdot 10^{-6}$	0.020	
		column	1.009197	$207 \cdot 10^{-6}$	0.021	
		column, digest.	1.009374	$219 \cdot 10^{-6}$	0.022	

3.2.3 *K* Factors for $^{207}\text{Pb}/^{208}\text{Pb}$

Due to the mass difference of one, the *K* factors correcting the ratio $^{206}\text{Pb}/^{208}\text{Pb}$ deviate from unity by only 0.5 %. When investigating the behavior of this ratio, a clear “stepwise” increase of the *K* factors determined from the column separated and digested and column separated samples compared to the non-pretreated were observed. The average values of $K_\text{total,col}$ $(^{207}\text{Pb}/^{208}\text{Pb}) = 1.00500(30)$ (mol/mol)/(V/V) and $K_\text{total,dig,col}$ $(^{207}\text{Pb}/^{208}\text{Pb}) = 1.00483(29)$ (mol/mol)/(V/V) are elevated by approximately 0.05 % compared to $K_\text{total,sol}(^{207}\text{Pb}/^{208}\text{Pb}) = 1.00449(27)$ (mol/mol)/(V/V). Although the numerical values can be clearly distinguished, the associated uncertainties include all *K* factors despite the history of preparation even with a coverage factor $k = 1$. When analyzing the respective uncertainty budget (Table 5), the measured intensity ratios $r(^{207}\text{Pb}/^{208}\text{Pb})$ contribute only 10 % to 25 % to the overall budget.

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Table 5. Uncertainty budget of $K(^{207}\text{Pb}/^{208}\text{Pb})$ in NIST SRM 981 for: diluted solution, column separated solution, and column separated solution after digestion. Molar masses M were taken from [27]. Reference (true) ratios of NIST SRM 981were adopted from [19,20].

***K* factors ($x(^{207}\text{Pb})/x(^{208}\text{Pb})$): solution, column, column + digestion**						
Quantity X_i	Unit $[X_\text{i}]$	Sample	Best estimate (value) x_i	Standard uncertainty $u(x_i)$	Sensitivity coefficient c_i	Index %
		solution				44.3
$R(^{207}\text{Pb}/^{206}\text{Pb})$	mol/mol	column	0.914640	$165.5\cdot10^{-6}$	1.1	41.9
		column, digest.				36.5
		solution				46.3
$R(^{208}\text{Pb}/^{206}\text{Pb})$	mol/mol	column	2.168100	$400\cdot10^{-6}$	-0.46	43.9
		column, digest.				38.2
		solution	0.4200000	$34.9\cdot10^{-6}$		9.4
$r(^{207}\text{Pb}/^{208}\text{Pb})$	V/V	column	0.4197600	$44.1\cdot10^{-6}$	-2.4	14.2
		column, digest.	0.4198900	$63.0\cdot10^{-6}$		25.3
Y	$[Y]$		y	$u_\text{c}(y)$	$u_{\text{c,rel}}(y)$ / %	
		solution	1.004434	$272\cdot10^{-6}$	0.027	
$K_{207,208}$	(mol/mol) / (V/V)	column	1.005009	$280\cdot10^{-6}$	0.028	
		column, digest.	1.004698	$300\cdot10^{-6}$	0.030	

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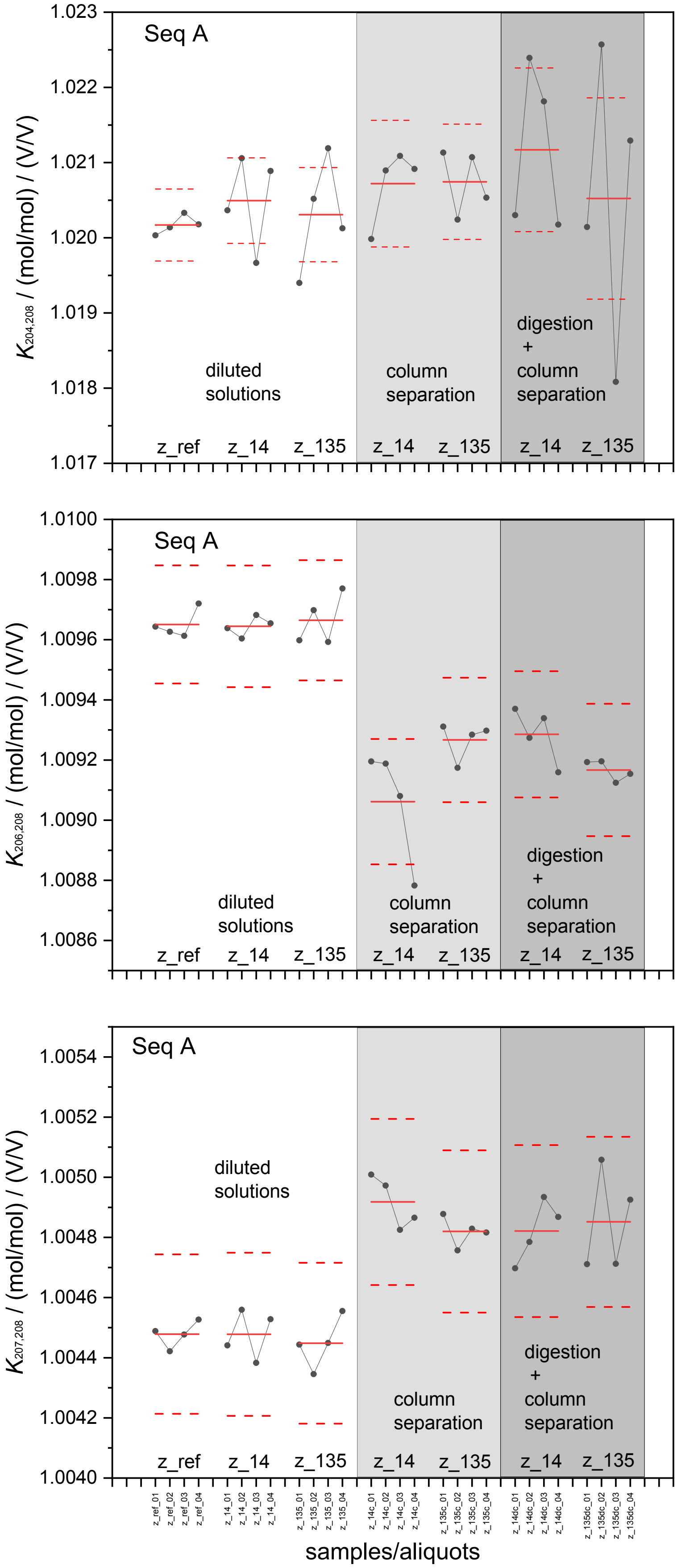

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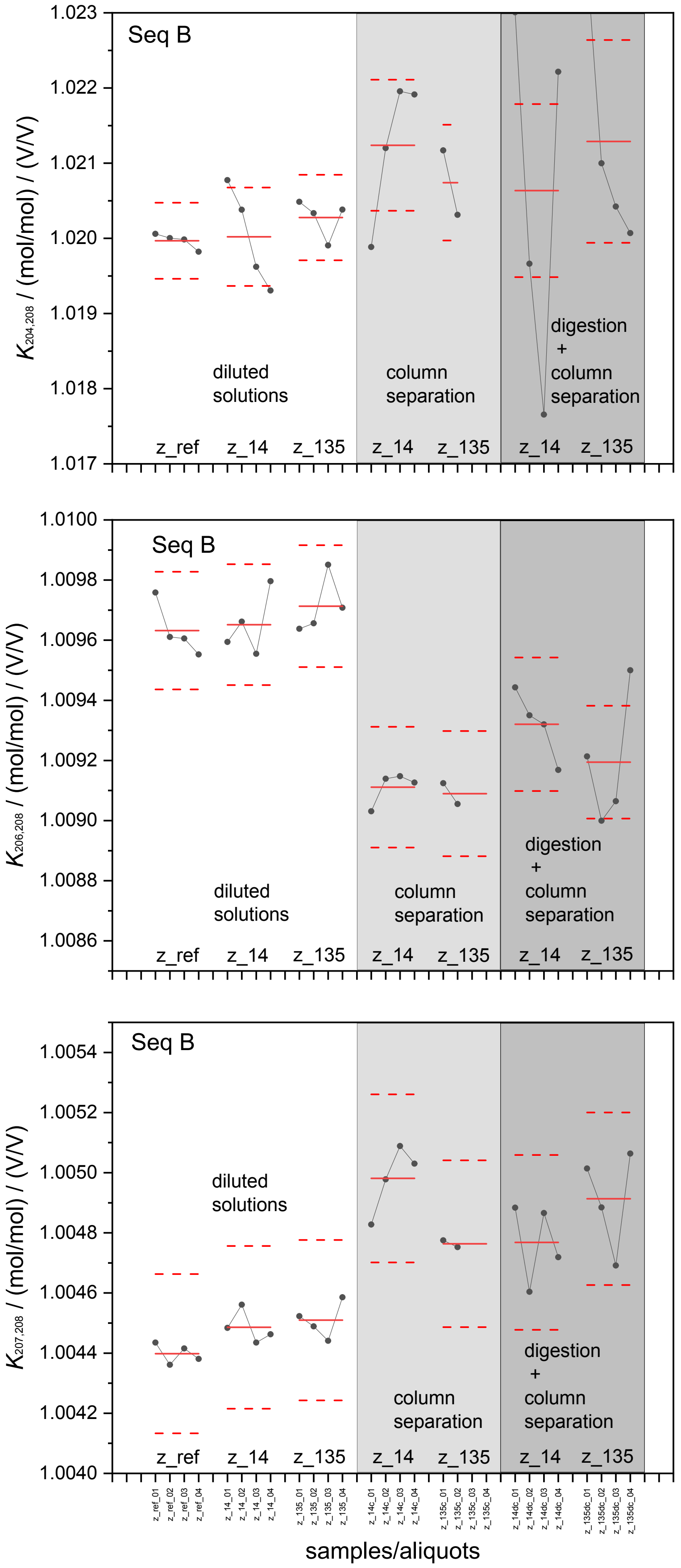

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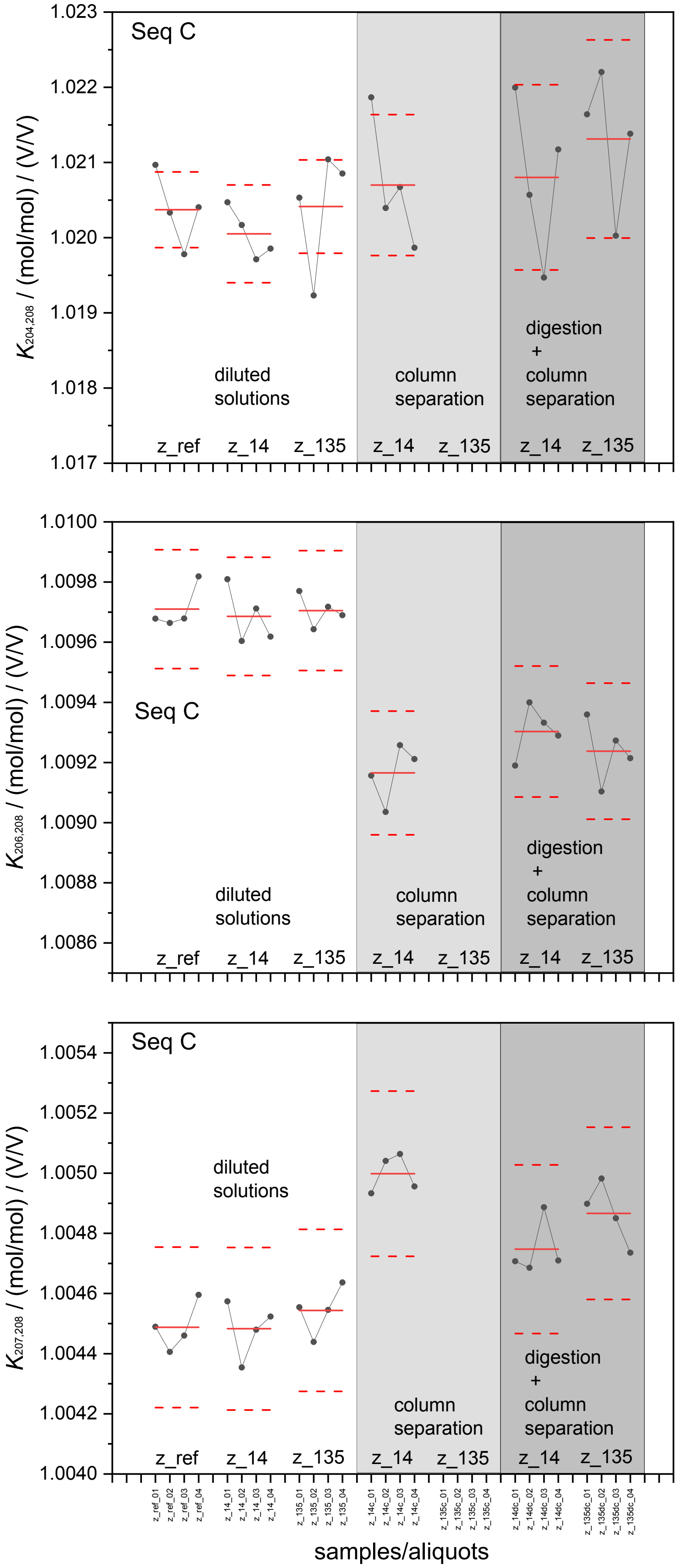

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Figure 2. *K* factors for the correction of the ratios $r(^{204}Pb/^{208}Pb)$, $r(^{206}Pb/^{208}Pb)$, and $r(^{207}Pb/^{208}Pb)$ determined during three sequences A, B, and C with solutions (samples) prepared from NIST SRM 981. Sequences A, B, and C were measured on three separate days with fresh tuning each day. Three samples were taken from diluted stock solutions of NIST SRM 981 in 0.15 mol/kg HNO_3 (z_ref, z_14, and z_135). Two other samples were treated with additional column separation (z_14, z_135, grey shaded area); two additional samples underwent microwave assisted digestion followed by column separation: dark grey shaded area. Single data points represent already average values of aliquots in respective sequences (red lines: averages over all aliquots, dotted lines: upper and lower uncertainties associated with the respective average).

A *K* factor dependence from the sample pre-treatment (history) cannot be generalized. As we observed, first, the isotope ratio must be chosen. In case of the rather small ratios $R(^{204}Pb/^{208}Pb)$, the *K* factors cannot be distinguished due to any pre-treatment, since both the values itself agree as well as their associated uncertainties (which are ten times larger in case of column separated and digested and column separated samples compared to simply diluted samples). However, in case of the ratios $R(^{206}Pb/^{208}Pb)$, a considerable downshift of the *K* factors $K_{206,208}$ of the column separated as well as the digested and column separated samples compared to diluted samples can be observed. This corresponds to a slight increase of the abundance of the ^{206}Pb isotopes relatively to ^{208}Pb in the molecular beam after ionization. For $k = 1$, the associated uncertainties of simple diluted and further prepared (digested and column separated samples) do not overlap, which suggests a clear influence of the sample treatment. Typical instrumental induced fractionation IIF which is induced by non-specified plasma-induced space-charge effects in the interaction region of the skimmer and sampler is usually leading to a decrease of the abundance of the lighter isotope (here: ^{206}Pb) in the beam. This is, because measured ratios are < 1 and all *K* factors are clearly > 1. However, important is the difference of *K* factors between simply diluted and further prepared samples, the latter showing a comparable downward shift in case of $K_{206,208}$. This is a clear indication of additional possible mass-independent fractionation of the digested and separated and only separated samples. This effect in a way superimposes IIF to a significant amount.

Generally, the amount of fractionation indicated by the respective *K* factor is similar for both only column separated as well as digested and column separated samples. Thus, it can be deduced that the digestion procedure itself has at least no significant impact on additional fractionation processes. These can be mainly attributed to the column separation. This finding is similar in comparable systems of stable isotopes. To mention only a few, already in 1976 Russell and Papanastassiou have observed isotopic fractionation of calcium due to ion-exchange chromatography.[28] At that time, a physico-chemical explanation was lacking although the observation was explicit. Similar observations and/or explanations have been made for Mo isotopic systems or Nd isotopes and other elements.[29-31] However, fractionation due to column separation usually induces an elevated release of the heavier isotopes compared to lighter ones. This can be interpreted as an enrichment of the heavier isotopes due to the stronger sticking of lighter isotopes on the resin and favoured elution of heavier isotopes from the resin bed which finally is a chemical exchange reaction. Often, the so-called nuclear field shift theory (NFS) can explain this kind of fractionation, which is reported in the literature.[30,32,33] But: In case of the reduction of $K_{206,208}$ – a reverse effect – the increase of the lighter isotope ^{206}Pb (and/or decrease of the heavier ^{208}Pb) was observed. This phenomenon remains unexplained by any current theoretical model and warrants further investigation.

For the ratios $R(^{207}\text{Pb}/^{208}\text{Pb})$, a reverse (and expected) behavior was observed: in case of the column separated and digested as well as column separated samples the correction factors $K_{207,208}$ are shifted toward higher values compared to the diluted samples. This can be translated into a relative lowering of the ^{207}Pb isotopes compared to ^{208}Pb in the beam. A similar observation has been described by Fujii et al..[34] In that study applying ion-exchange

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chemistry using a crown ether for chromatographic Pb separation, the NFS theory has been applied successfully for the explanation of mass-independent fractionation of Pb isotopes leading to a lowering of the abundance of lighter isotopes. This behavior is confirmed also in our study in case of the ^{207}Pb/^{208}Pb ratio leading to a further increase of $K_{207,208}$. Thus, NFS can be assigned as a possible source of the fractionation in case of ^{207}Pb/^{208}Pb by the separation due to chemical exchange.

Additionally, a more detailed hypothetical separation of *K* factor components according to a scheme like

$$K_{\text{total}} = K_{\text{meas}} \tag{6}$$

$$K_{\text{total, sol}} = K_{\text{sol}} \tag{7}$$

$$K_{\text{total, col}} = K_{\text{sol}} \times K_{\text{col}} \tag{8}$$

$$K_{\text{total, dig, col}} = K_{\text{sol}} \times K_{\text{dig}} \times K_{\text{col}} \tag{9}$$

$$K_{\text{sol}} = K_{\text{total, sol}} \tag{10}$$

$$K_{\text{col}} = K_{\text{total,col}} / K_{\text{sol}} \tag{11}$$

$$K_{\text{dig}} = K_{\text{total, dig, col}} / \left(K_{\text{sol}} \times K_{\text{col}}\right) \tag{12}$$

is at least very difficult. In the present study, a *K* factor influence according to eqs. (7), (8), and (9) can be observed and distinguished for the ratios $R(^{206}\text{Pb}/^{208}\text{Pb})$ and $R(^{207}\text{Pb}/^{208}\text{Pb})$. A separation according to eqs. (11) and (12) is not reasonable due to enlarged uncertainties.

4. Conclusion

In the present study the well characterised NIST SRM 981 was used as a pilot material for the study of mass bias correction factor dependencies for the isotope ratios $R(^{204}\text{Pb}/^{208}\text{Pb})$, $R(^{206}\text{Pb}/^{208}\text{Pb})$, and $R(^{207}\text{Pb}/^{208}\text{Pb})$. It is generally known that the correct determination of isotope ratios used for e.g. analysis of concentrations or mass fractions of an analyte element

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in a sample requires a proper pretreatment of the latter: dilution steps, digestion, column separation and other techniques for the separation of matrix and analyte elements in the blank. It is of interest whether the pretreatment processes itself have an impact on isotopic fractionation without considering fractionation due to the mass spectrometric process. For this reason, different pretreated samples of original pure NIST SRM 981 reference material were studied without any matrix which simulates a kind of "undisturbed environment" of the sample: A deduction of the impact of the separation method itself was aimed at. Typical microwave digestion is used to completely dissolve matrix samples. In the current study we investigated potential influences of the digestion itself. Processes like surface absorption or adsorption may induce additional fractionation. This can apply also to samples without matrix undergoing the same digestion procedure as e.g. spiked blends of matrix containing samples when applying blank-matching double IDMS (exact matching). The study suggests that in case of the ratio $R(^{204}\text{Pb}/^{208}\text{Pb})$ the pretreatment has no impact on the K factor mainly due to the already considerably large uncertainties. In case of the ratios $R(^{206}\text{Pb}/^{208}\text{Pb})$ and $R(^{207}\text{Pb}/^{208}\text{Pb})$ however, a clear impact on the K factors of the column separated and digested as well as column separated samples versus non-pre-treated samples can be observed. Fortunately, for $k = 2$, the K factors for all different sample treatments agree within the limits of uncertainties. However, in case of elements in practical samples (e.g. in organic matrices) as shown in the CCQM-K158 study, it is vital to apply a sample pretreatment as digestion and column separation prior to the determination of isotope ratios.[18] This study shows that for isotope ratio measurements it is a safer way to handle all samples and blends in exactly the same way from the beginning if a digestion and column separation is necessary to avoid measurable isotopic fractionation effects. For a given ratio, K factors of both preparation routes (only column separation or digestion with subsequent column separation) are impacted in the same direction and in the same order of magnitude. Therefore, it is assumed that a main

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source of mass-independent fractionation is column separation. NFS can be attributed to additional mass-independent fractionation in case of $^{207}Pb/^{208}Pb$.

Data availability

The data supporting the findings of this study are available within the paper and its supplementary information (ESI) files. Additional data are available upon reasonable request.

Conflict of interest

There are no conflicts to declare.

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Data availability

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