Spectrometric determination of silicon in food and biological samples: an interlaboratory trial

JAAS

Interlaboratory Note

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Accuracy and precision of silicon determination in biological matrices (serum, urine, water, beer and spinach) by spectrometric techniques (when necessary after acid destruction) were assessed by means of a collaborative interlaboratory trial. The trial was set up in accordance with ISO 5725-2 (1994). The relative overall repeatability standard deviation was acceptable. It varied between 4% for spinach powder (mean content: 176 mg kg^{-1}) and 11% for serum (mean content: 5.33 mg L^{-1}). On the other hand, the relative overall between-laboratory standard deviation was found to vary from a satisfactorily 15% for spinach after destruction (mean content: 3.32 mg L^{-1}) to an unacceptable 107% for spinach powder (mean content: 176 mg kg^{-1}). The overall conclusion of the trial was that silicon determination in biological matrices can properly be performed by spectrometric techniques. However, when sample pretreatment (*i.e.*, acid destruction) is needed prior to silicon determination problems still remain.

Introduction

Silicon is, next to oxygen, the second most abundant element in the Earth's crust. In 1972, the research groups of Carlisle and Schwarz proved, in chicks and rats, that silicon is an essential element for these animals.[1,2] The essential character of silicon in man remains largely unexplored. This research is severely hampered by analytical difficulties encountered when determining silicon in biological samples in which it is present in only trace amounts. Moreover, as silicon is a refractory metal, its determination is rather difficult using atomic spectrometric techniques. During the last 20 years, the lack of appropriate certified reference materials has delayed the progress in silicon analysis of biological samples, since accuracy testing was hard to perform. Some research groups[3–6] tried to cope with this by determining the silicon content of commercial reference materials certified for other elements, but varying data were reported. Comparing the newly developed analytical techniques with the AOAC approved molybdenum blue colorimetric technique showed that this technique is useful for the analysis of aqueous solutions, but much less useful for analysis of organic matrices.[7]

The aim of this interlaboratory trial was to check the validity of spectrometric techniques for silicon determination in food and biological matrices.

Experimental

The design, performance and analysis of results of the trial were all done in accordance with the International Standard ISO 5725-2 (1994).[8]

Materials

Various materials were selected for the trial, based on their relevance for silicon research in food and biomedical sciences:

mineral water (Volvic, F), beer (St. Bernardus Abt, B. Verbiest, Belgium), spinach (frozen, sliced spinach, Iglo, Belgium), serum (Seronorm, Nycomed Pharma AS, Norway) and urine (Seronorm^{TM} Trace Elements, Nycomed Pharma AS, Norway). Beer and spinach were supplied as original samples as well as after acid destruction. The original beer was liquid, while the spinach was a freeze-dried powder. These samples needed to be pretreated (destructed) by the participating laboratories. Serum and urine were freeze-dried powders packed in a glass bottle purchased as such from Nycomed.

One litre of mineral water, 990 mL of beer and 900 g of frozen spinach were considered to be sufficient to cover the experiment (allowing for accidental spillage or errors which may necessitate using extra material) and allow an adequate stock. Sample pretreatment, matrix destruction and sample storage were done under the optimal conditions determined earlier.[9–11] These included no use of glassware, sample homogenisation after freeze-drying using a polypropylene mortar and pestle, as well as sample storage in a temperature controlled environment. After thoroughly shaking the entire sample volume, aliquots of about 40 mL of water and fresh beer were dispensed in polystyrene containers. The frozen spinach sample was defrosted, homogenised and divided in 100 g portions prior to freeze-drying. The dry aliquots were pooled, pulverised and homogenised using mortar and pestle. Thirty portions of the homogenised fresh beer ($\pm$2 mL) and spinach powder sample ($\pm$0.2 g) were submitted to an acid, closed vessel for microwave assisted destruction.[12] All resulting 10 mL liquids of each matrix were collected, and after homogenisation divided into 20 mL aliquots for distribution. Every aliquot was weighed in a polystyrene container, which was provided with a label mentioning sample name and code number. The container was further sealed under vacuum in a polyethylene bag, provided with the same label as the one on the bottle. The serum and urine samples remained in their original glass bottles and were further packed in a polystyrene container and polyethylene bag under vacuum. Their label mentioned the reconstitution guidelines, as stated in the product's leaflet.

Homogeneity and stability of the samples was thoroughly tested prior to distribution of the samples. Efficacy of homogenisation was tested by analysis of individual sample portions from ten different spots in the homogenised sample pool. From the ten values of all individual portions, the mean and standard deviation (s) were calculated. From the latter the relative standard deviation (RSD) was derived. The maximum tolerable RSD was chosen in accordance with an AOAC publication.[13] It states that, when performing analyses under repeatability conditions, the maximum tolerable relative standard deviation depends on the mean elemental content of the sample. As a consequence, at a concentration level of 1 to 10 mg kg^{-1}, a RSD of 10% is still acceptable. To assess sample stability, eight aliquots were stored at room temperature in polystyrene containers sealed in polyethylene bags under vacuum. Silicon determination was performed at day 0, 1, 2 and week 1, 2, 4, 6 and 8. In this case, the margins were set at double the maximum tolerable RSD for homogeneity testing. For the spinach sample, which might be expected to be the most sensitive to contamination since it grows near to the soil, no homogeneity nor stability problems were encountered when the spinach was analysed after freeze-drying. When testing homogeneity, the following data (expressed per dry weight, $n=10$) were obtained: mean, 6.61 mg kg^{-1}; s, 0.34 mg kg^{-1}; and RSD, 5%. Upon stability testing, the following results (expressed per dry weight, $n=8$) were found: mean, 6.45 mg kg^{-1}; s, 0.25 mg kg^{-1}; and RSD, 4%. Since the RSD remainded below the maximum tolerable value (10% for homogeneity and 20% for stability testing), the freeze-dried spinach powder sample can be considered homogeneous and stable, as far as its silicon content is concerned.

Distribution of the requested samples was primarily done by mail. The samples were accompanied by detailed instructions about sample analysis and data reporting. A blank form for data reporting was enclosed. Sample analysis was asked to be carried out threefold. Frames for identification of the laboratory, for reporting of the analytical technique applied (including sample pretreatment, determination method and validation), and for the three analysis data and eventual remarks were inserted on the blank forms.

Methods

All participating laboratories were randomly numbered from 1 to 14 and will be mentioned by their number.

Laboratories having experience in silicon analysis—preferably by spectrometric techniques—were invited to take part in the trial. The closed vessel, microwave-assisted destruction method with HNO_3–H_2O_2 was strongly recommended for sample pretreatment. Table 1 reviews the techniques applied in each laboratory. Laboratory No. 6 did also analyse the samples by slurry-ETAAS after simple dilution in 5% v/v HNO_3. However, the data obtained by this method revealed so poor a repeatability that they were not retained for statistical analysis. Table 2 summarises the validation parameters (limit of detection, limit of quantification and relative standard deviation under repeatability conditions) of the techniques applied.

Statistical data analysis

Original test results. This collaborative interlaboratory study involved p laboratories called i ($i=1, 2, ..., p$), each testing q materials called j ($j=1, 2, ..., q$) with n replicates (each ij combination). All replicates of each ij combination are assigned to one cell. The cell means and cell spread were calculated as follows:

$$\bar{y}_{ij}=\frac{1}{n_{ij}}\sum_{k=1}^{n_{ij}} y_{ijk} \qquad S_{ij}=\sqrt{\frac{1}{n_{ij}-1}\sum_{k=1}^{n_{ij}}(y_{ijk}-\bar{y}_{ij})^2}$$

where n_{ij} is the number of test results in the cell for laboratory i and material j, and y_{ijk} is any one of these test results ($k=1, 2, ..., n_{ij}$).

Scrutinity of results for consistency and outliers. During the first stage of the evaluation, the data were critically examined in order to identify outlying values and other inconsistencies. Both a graphical consistency technique and numerical outlier tests were applied.

In the graphical consistency technique, two measures called Mandel's h and k statistics are used. Mandel's h statistic is a

Table 1 Analytical techniques applied by the participants of the collaborative interlaboratory trial

	Analytical method	
Laboratory	Sample pretreatment[a]	Determination technique
1, 5, 9	A	ETAAS
2	B	HR-ICP-MS
3, 6	A	ICP-AES
4	B	GEXRF
7	C	ETAAS
8, 11	B	ICP-MS
10, 14	B	ETAAS
12	B	ICP-OES
13	D	ICP-OES

[a]A, Without sample destruction; B, closed vessel, microwave assisted destruction with HNO_3–H_2O_2; C, closed vessel, hot plate destruction with HNO_3–H_2O_2; and D, closed vessel, microwave assisted destruction with HNO_3–H_2O_2–HF.

Table 2 Validation parameters (LOD, LOQ, RSD) reported by the participating laboratories; values in parenthesis are number of replicates

					Beer		Spinach	
Laboratory No.	Parameter	Serum/ μg L^{-1}	Urine/ μg L^{-1}	Water/ μg L^{-1}	Fresh/ mg L^{-1}	Destructed/ mg L^{-1}	Powder/ mg kg^{-1}	Destructed/ mg L^{-1}
1	LOD	4.2	2.2	2.2	2.2			
	LOQ	8.4	4.4	4.4	4.4			
	RSD (%)	4	2	1 (10)	2 (10)			
2	LOD	0.386	0.386	0.386				
	LOQ							
	RSD (%)							
3	LOD			10		0.01		0.01
	LOQ			25000		25.0		25.0
	RSD (%)							
4	LOD	13.7	14	7.6	0.028	0.004	0.009	0.01
	LOQ	46	48	25	0.096	0.014	0.031	0.032
	RSD (%)	8 (3)	7 (3)	4 (3)	8 (3)	11 (3)	6 (3)	3 (3)
5	LOD	15.7	15.7					
	LOQ	52.3	52.3					
	RSD (%)	19 (40)	14 (5)					
7	LOD			17.7			17.7	17.7
	LOQ			35.4			35.4	35.4
	RSD (%)			24 (10)			18 (10)	251 (10)
8	LOD	1.0		1			0.1	0.001
	LOQ	5.0		5			0.5	0.005
	RSD (%)	2 (3)		5 (12)			6 (8)	7 (4)
9	LOD							
	LOQ							
	RSD (%)	7 (5)	6 (5)	4 (5)	2 (5)	3 (5)		5 (5)
10	LOD			3000	6.0		37.5	
	LOQ			6000	12.0		75.0	
	RSD (%)			6 (14)	7 (14)		8 (12)	
12	LOD	3	3	3	3			
	LOQ							
	RSD (%)							
13	LOD			120	120.0	0.12	0.2	0.12
	LOQ			360	360.0	0.36	0.6	0.36
	RSD (%)			1	2	1	3	5
14	LOD	8.9	1.7	1.7	16.0	16.0	0.8	16.0
	LOQ	17.9	3.5	3.5	32.0	32.0	1.6	32.0
	RSD (%)	5 (10)	5 (10)	1 (10)	3 (10)	1 (10)	4 (10)	1 (10)

measure of the between-laboratory consistency, while Mandel's k statistic is a measure of within-laboratory consistency. They are calculated as follows:

$$h_{ij} = \frac{\bar{y}_{ij} - \bar{\bar{y}}_j}{\sqrt{\frac{1}{p_j - 1}\sum_{i=1}^{p_i}(\bar{y}_{ij} - \bar{\bar{y}}_j)^2}} \qquad k_{ij} = \frac{S_{ij}\sqrt{p_j}}{\sqrt{\sum s_{ij}^2}}$$

where p_j is the number of laboratories reporting at least one test result for material j.

Consequently, all laboratories' data were numerically tested to identify stragglers or outliers. Cochran's test is a test of within-laboratory variability. The Grubbs' test is primarily a test of between-laboratory variability.

Cochran's test statistic, C, is

$$C = \frac{s_{\max}^2}{\sum_{i=1}^{p} s_i^2}$$

where $s_{\max}$ is the highest standard deviation in the set.

Using the Grubbs' test, data can be analysed for one outlying value (single Grubbs' test) or two outlying values (double Grubbs' test).

Given a set of data x_i for $i = 1, 2, ..., p$, arranged in ascending order, the Grubbs' statistic, G_p, should be computed to determine whether the largest observation is an outlier.

$$G_p = \frac{(x_p - \bar{x})}{s}$$

where

$$\bar{x} = \frac{1}{p}\sum_{i=1}^{p} x_i$$

and

$$s = \sqrt{\frac{1}{p-1}\sum_{i=1}^{p}(x_i - \bar{x})^2}$$

To test the significance of the smallest observation, the test statistic G_1 is computed

$$G_1 = \frac{(\bar{x} - x_1)}{s}$$

To test whether the two largest observations may be outliers, the Grubbs' test statistic G_{high} is calculated.

$$G_{\text{high}} = \frac{s_{p-1,p}^2}{s_0^2}$$

where

$$s_0^2 = \sum_{i=1}^{p}(x_i - \bar{x})^2$$

and

$$s_{p-1,p}^2 = \sum_{i=1}^{p-2}(x_i - \bar{x}_{p-1,p})^2$$

and

$$\bar{x}_{p-1,p} = \frac{1}{p-2}\sum_{i=1}^{p-2} x_i$$

Alternatively, to test the two smallest observations, G_{low} is calculated.

$$G_{low} = \frac{s_{1,2}^2}{s_0^2}$$

where

$$s_{1,2}^2 = \sum_{i=3}^{p} (x_i - \bar{x}_{1,2})^2$$

and

$$\bar{x}_{1,2} = \frac{1}{p-2} \sum_{i=3}^{p} x_i$$

Calculation of the general mean and variances. For level j, the general mean is

$$\hat{m}_j = \bar{\bar{y}}_j = \frac{\sum_{i-1}^{p} n_{ij} y_{ij}}{\sum_{i=1}^{p} n_{ij}}$$

Three variances are calculated for each material, *i.e.*, the repeatability variance, the reproducibility variance, and the between-laboratory variance.
The repeatability variance is

$$s_{rj}^2 = \frac{\sum_{i=1}^{p} (n_{ij} - 1) s_{ij}^2}{\sum_{i=1}^{p} (n_{ij} - 1)}$$

The reproducibility variance is

$$s_{Rj}^2 = s_{rj}^2 + s_{Lj}^2$$

The between-laboratory variance is

$$s_{Lj}^2 = \frac{s_{dj}^2 - s_{rj}^2}{\bar{\bar{n}}_j}$$

where

$$s_{dj}^2 = \frac{1}{p-1} \sum_{i=1}^{p} n_{ij} (\bar{y}_{ij} - \bar{\bar{y}}_j)^2$$

and

$$\bar{\bar{n}}_j = \frac{1}{p-1} \left[\sum_{i=1}^{p} n_{ij} - \frac{\sum_{i=1}^{p} n_{ij}^2}{\sum_{i=1}^{p} n_{ij}} \right]$$

Results and discussion

Original test results

All data reported (all means and all spreads) by the 14 laboratories are summarized in Table 3. Few remarks appeared on the blank forms. The majority of remarks concerned the destruction technique. Laboratory 7 reported loss of sample solution during hot-plate digestion (microwave equipment was not available) of beer and spinach. Laboratory 9 had major problems during sample destruction of spinach (no analysis data were sent for this matrix). Laboratory 14 claimed that the high standard deviation for the serum analysis originated from improper dilution of the sample. The difficulties experienced with sample destruction are reflected in the low number (only five) of laboratories (2, 4, 7, 10 and 14) analysing all materials provided.

Scrutinity of results for consistency and outliers

In Figs. 1 and 2 the respective h_{ij} and k_{ij} values are plotted for each cell in order of laboratory, grouped for all materials examined by each laboratory. The horizontal lines on the h and k plots are indicators for Mandel's h and k statistics at the 5% and 1% significance level. These indicator lines serve as guides when examining patterns in the data.

As can be seen in Fig. 1, all laboratories have both positive and negative h values at different levels of the experiment. No laboratory exhibits patterns of results that are markedly different from the other laboratories in the study. However, individual extreme values are reported by laboratory 6 for water and fresh beer, by laboratory 7 for water, by laboratory 12 for urine and by laboratory 13 for spinach powder and spinach after destruction.

No laboratory stands out on the k plot (Fig. 2) as having more large values than another laboratory. However, individual extreme values are reported by laboratory 1 for water, fresh beer and beer after destruction, by laboratory 10 for beer

Table 3 Cell means and cell spread of silicon content in various materials

	Silicon content/mg kg^{-1} powder or mg L^{-1} liquid						
				Beer		Spinach	
Laboratory	Serum	Urine	Water	Fresh	Destructed	Fresh	Destructed
1	4.570±0.400	5.80±0.26	14.4±1.9	42.4±5.9	9.00±0.78		3.50±0.17
2	6.303±0.151	7.42±0.05	14.1±0.2	43.7±0.9	8.50±0.03	98.6±1.6	3.32±0.21
3			14.2±0.2		8.61±0.09		3.22±0.04
4	6.597±0.528	8.65±0.58	17.2±0.7	57.0±4.6	5.10±0.57	143.3±8.1	4.52±0.14
5	2.533±0.101	6.47±0.85					
6			10.4	105.8[a]	8.35		2.63
7	0.168±0.014	4.92±0.04	21.9[b]±1.3	22.5±0.8	5.17±0.38	46.9±6.2	3.10±0.02
8	11.100±0.170		14.0±0.5			145.3±7.1	3.41±0.23
9		4.27±0.33	14.7±0.6	45.9±0.6	8.53±0.16		2.86±0.12
10	3.657±0.490	10.73±0.85	16.4±1.4	47.7±3.6	9.72±0.79	245.6±31.6[a]	3.81±0.20
11	9.530±0.780	8.83±0.91	14.8±1.1				
12	4.130	14.60	16.4	42.1			
13			15.0	51.3±0.8	9.70	563.0[b]±13.9	10.00[a]
14	3.893±1.243[b]	6.27±0.12	15.0±0.8	38.7±1.8	7.87±0.24	236.0±1.0	3.46±0.08

[a]Outlier. [b]Straggler, indicated by Cochran's test for cell spread (see Table 5) and by single Grubbs' test for cell means (see Table 6); outlying values were not included in calculation of the overall mean and variances.

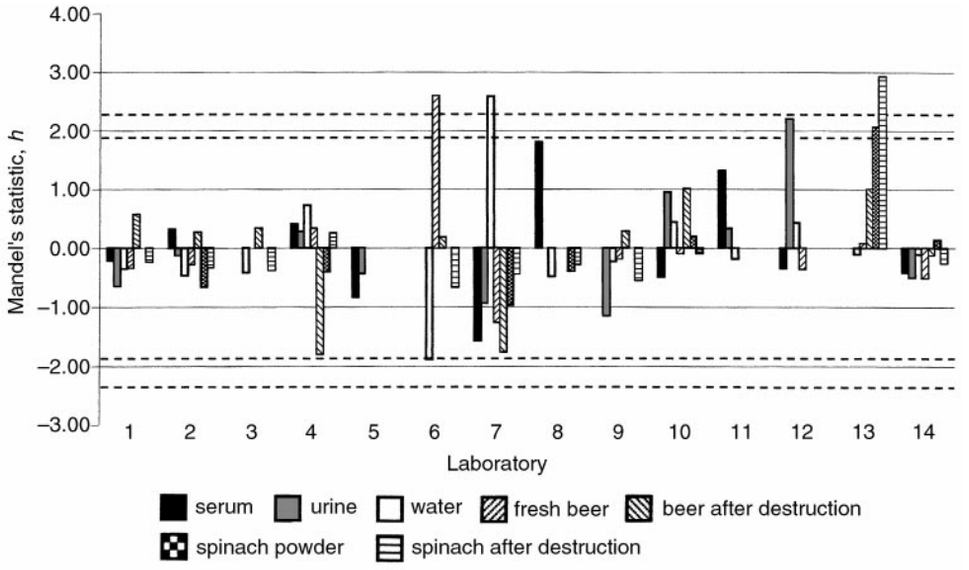

Fig. 1 Mandel's between-laboratory consistency statistic, *h*, grouped by laboratories.

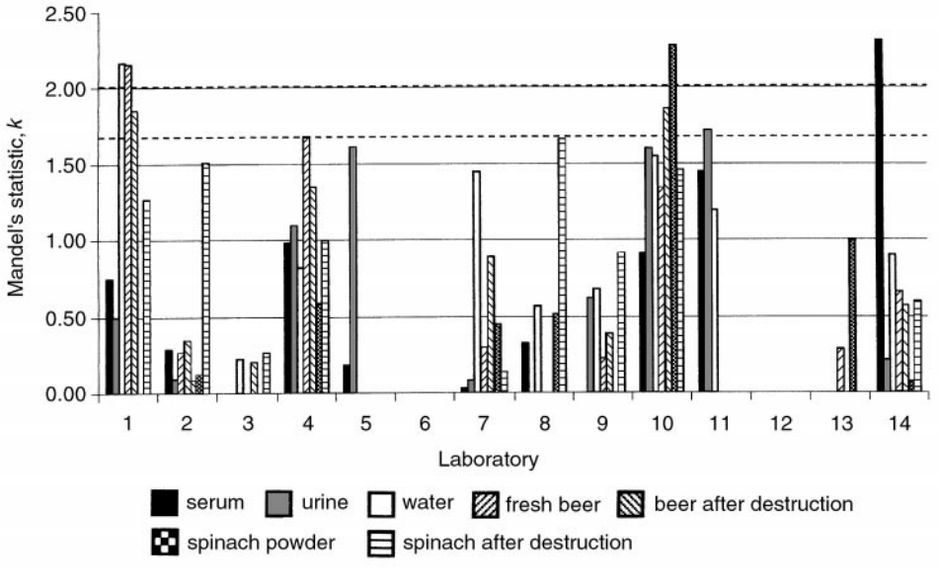

Fig. 2 Mandel's within-laboratory consistency statistic, *k*, grouped by laboratories.

after destruction and spinach powder, by laboratory 11 for urine and by laboratory 14 for serum.

The calculated Cochran's statistics together with the critical values (depending on the number, *p*, of sets of standard deviations) are compiled in Table 4. Upon application of the Cochran's test, only the standard deviation reported by laboratory 10 for spinach powder appeared to be an outlier. For serum the standard deviation of laboratory 14 was classified as a straggler.

Applying the single Grubbs' test, two cell means turned out to be stragglers *i.e.*, water from laboratory 7 and spinach powder from laboratory 13. Two others turned out to be an

Table 4 Application of Cochran's test to cell variances

				Beer		Spinach	
	Serum	Urine	Water	Fresh	Destructed	Fresh	Destructed
Cochran's test statistics							
C	0.534[a]	0.295	0.362	0.463	0.346	0.740[b]	0.250
p	9	9	10	8	8	7	9
Cochran's critical values							
Straggler	0.478	0.478	0.445	0.516	0.516	0.561	0.478
Outlier	0.570	0.570	0.540	0.615	0.615	0.664	0.570

[a]Straggler, indicated by the Cochran's test; outlying values were not included in calculation of the overall mean and variances. [b]Outlier.

Table 5 Application of Grubbs' test to cell means; p, number of laboratories for a given material

Material; p	Single (low)	Single (high)	Double (low)	Double (high)
Grubbs' test statistics				
Serum; 10	1.565	1.803	0.5699	0.3096
Urine; 10	0.647	2.206	0.7638	0.2205
Water; 13	1.891	2.581[a]	0.6407	0.3173
Beer fresh; 10	1.256	2.587[b]	—	—
Beer destr; 10	1.800	1.013	0.1218	0.7181
Spinach powder; 7	0.965	2.064[a]	0.6839	0.2829
Spinach destr; 11	0.659	2.927[b]	—	—

Grubbs' critical values						
Straggler		Single	Double		Single	Double
	$p=7$	2.020	0.0708	$p=11$	2.355	0.1530
	$p=10$	2.290	0.1864	$p=13$	2.462	0.2836
Outlier						
	$p=7$	2.139	0.0308	$p=11$	2.564	0.2359
	$p=10$	2.482	0.1150	$p=13$	2.669	0.2016

[a]Straggler, indicated by the Grubbs' test; outlying values were not included in calculation of the overall mean and variances. [b]Outlier.

outlier *i.e.*, fresh beer from laboratory 6 and spinach after destruction from laboratory 13. After exclusion of the outlying values, the test was repeated at the other extreme cell mean. The double Grubbs' test for two outlying observations was applied only when the single Grubbs' test did not show a cell mean to be an outlier. No stragglers or outliers were detected by the latter test. The Grubbs' statistics as well as the critical values are compiled in Table 5.

The above preliminary statistical data were anonymously reported to the participating laboratories, with indication of suspected data for that laboratory. The laboratories were asked to examine whether the suspected values could be explained by some technical error, for example an error in computation or a simple clerical error in transcribing a test result. In these cases, the correct data were asked for. Other possible explanations of a suspect test result were asked to be reported as well.

Laboratory 7 had no obvious explanation for the high mean value found for water. However, data of another run (with a somewhat poorer repeatability) of the sample were provided. Replacing the earlier data with the newly reported ones did not induce more consistency in the overall data, hence the original data were used for further calculations. Laboratory 10 could not provide a theoretical justification for the outlying high standard deviation reported for spinach powder either. Laboratory 11 remarked that a 'within-laboratory consistency' testing of their values was rather inappropriate, since, for every material tested, the two first reported values were obtained after analysis of the original sample on different days, and the third one was obtained after acid destruction of the sample. Since no outlying values had been detected for laboratory 1, the three reported data were further treated as if obtained under repeatability conditions.

As the numerically identified stragglers and statistical outliers remained unexplained, the stragglers were retained as correct items and the statistical outliers were discarded. When the results reported by laboratory 10 for spinach powder were omitted, and the Cochran's test was repeated on the remaining values, no more stragglers or outliers were detected for spinach powder ($C=0.548$, Cochran's critical values ($p=6$): 1% = 0.722, 5% = 0.616). When the Grubbs' test was repeated, after exclusion of the cell means for fresh beer from laboratory 6 and for spinach after destruction from laboratory 13, no more outlying values were obtained (fresh beer: $G_1=1.256$, $G_p=1.409$, $G_{low}=0.2391$, $G_{high}=0.2798$, Grubbs' critical values ($p=9$) single: 1% = 2.387, 5% = 2.215, double: 1% = 0.0851, 5% = 0.1492; spinach after destruction: $G_1=0.659$, $G_p=2.179$, $G_{low}=0.5733$, $G_{high}=0.2723$, Grubbs' critical values ($p=10$) single: 1% = 2.482, 5% = 2.290, double: 1% = 0.1150, 5% = 0.1864).

In Tables 3–5, the discarded values are indicated. The remaining data were used for calculation of the general mean and variances.

Calculation of the general mean and variances

The results of this calculation are compiled in Table 6 for each material (value of j), together with the relative s_{rj} and s_{Lj} values. Reference precision figures for analyses performed under repeatability conditions are tabulated, which have been stated as a function of analyte concentration in the AOAC manual for peer-verified methods.[13]

Comparing the s_{rj} values with the estimated precision data (AOAC), it can be concluded that the overall repeatability was very good for most of the materials tested. It may indicate that the participating laboratories[7,9–12,14–22] did have expertise in

Table 6 Computed values of general mean, s_{rj}, s_{Lj} and s_{Rj} (mg kg^{-1} powder or mg L^{-1} liquid) and relative s_{rj} and s_{Lj} (%) for silicon analysis in food samples and biological matrices

Sample	p_j	Mean	s_{rj}	Rel. s_{rj}	Ref. RSD[a]	s_{Lj}	Rel. s_{Lj}	s_{Rj}
Serum	10	5.33	0.57	11	11	3.33	62	3.38
Urine	10	7.31	0.56	7.6	11	2.82	39	2.88
Water	13	16.4	1.0	6.1	7.3	2.6	16	2.8
Beer								
Fresh	9	41.9	3.0	7.1	7.3	13.1	31	13.4
Destructed	10	8.65	0.47	5.5	11	2.52	29	2.57
Spinach								
Powder	6	176	7	3.9	5.3	189	107	189
Destructed	10	3.32	0.15	4.4	11	0.50	15	0.52

[a]Ref. RSD, Reference precision figures for analyses performed under repeatability conditions, stated as a function of analyte concentration in the AOAC manual for peer-verified methods.[10]

silicon analysis. Only one value equals the reference RSD, *i.e.*, the value for serum. This high variance for serum can be attributed to the high standard deviation of the data reported by laboratory 14, which was found to be a straggler upon application of the Cochran's test.

The between-laboratory standard deviation (s_{Lj}) and reproducibility (s_{Rj}) values give a very different picture, as they range from a satisfying 15% to over 100% of the mean. This probably is the consequence of a total lack of certified standard reference materials for silicon in bioorganic matrices. The s_{rj} and s_{Lj} values together indeed indicate that the participating laboratories did have expertise in silicon analysis (illustrated by small absolute cell differences, thus small s_{rj},) but none of them could check the accuracy of their work (illustrated by very diverging cell means, thus large s_{Lj} and s_{Rj}). It is not surprising that the smallest relative s_{Lj} values were calculated for materials that did not need any sample pretreatment by the participant prior to analysis *i.e.*, mineral water and the destruction liquids of spinach and of beer. However, a somewhat higher value was obtained for the destruction liquid of beer, compared with the spinach destruction liquid. Only improper calibration, without taking into account the acid matrix of the destruction liquid, could here be pointed out as a reason for low reproducibility. This may account, of course, for the two destruction liquids provided, as well as for the other materials included in the study when the participating laboratory used a destruction procedure prior to analysis (*i.e.*, laboratory 2, 4, 7, 8, 10, 11, 12, 13 and 14). The difficulty of silicon analysis in the complex organic matrix of urine, serum and fresh beer is reflected by the higher relative s_{Lj} obtained in these samples compared with water or the provided destruction liquids. Within this group of samples, the determination of silicon in serum was the most difficult to perform, probably related to the rather low silicon level. A relatively small number of reporting laboratories and a relative between-laboratory standard deviation (s_{Lj}) of more than 100% of the mean content are a clear indication of the lack of appropriate analytical techniques for silicon in silicate-containing materials, such as spinach powder. It appears that the techniques used in this study all have a different ability for silicon determination in silicate-containing materials. Vegetable materials are known to contain silicon as phytolitic silicates, $SiO_2 \cdot nH_2O$ or opal. Therefore, a ranking of the techniques can be established, relying on the amount of silicon detected by the different laboratories in the spinach powder sample. In descending order of power of silicon detection in silicate-containing materials, the techniques can be classified as follows: ICP-OES after destruction with HF > ETAAS after microwave assisted destruction with H_2O_2–HNO_3 > grazing emission XRF (GE-XRF) ≈ HR-ICP-MS after microwave assisted destruction with H_2O_2–HNO_3 > ETAAS after hot plate destruction with H_2O_2–HNO_3.

Though somewhat more optimistic, our findings corroborate the data published by Pavel and Krivan,[23] who claimed in 1997 that determination of silicon traces in biological tissues represents an extremely difficult task which still cannot be solved satisfactorily. In an interlaboratory collaborative study with 13 laboratories they analysed a silicon spiked and unspiked bovine liver sample. A significant difference was observed between the results obtained by different digestion-free methods; results obtained by WDXRF (wavelength dispersive X-ray fluorescence) being higher than those obtained by slurry sampling ETAAS. Results reported for analysis by ETAAS and ICP-AES after sample solubilisation proved to be quite inconsistent, with data obtained by ICP-AES corroborating those of slurry sampling ETAAS, and data obtained by ETAAS confirming those of WDXRF.

The overall conclusion of the present collaborative interlaboratory trial is that silicon determination in a simple matrix can properly be done by spectrometric techniques. Problems arise when more complex, organic matrices are analysed by direct calibration or when silicate-containing materials need sample destruction prior to analysis.

This study stressed the absolute and urgent need for a certified standard reference material for silicon in a bio-organic matrix. The development of such a material certainly is hampered by the 'solubility of silicates' as well. Maybe, 'total' silicon content determination (including the silicates solely soluble in HF, with minor interest in biomedical sciences) is not necessary. For biomedical applications, 'soluble' silicon content determination seems more relevant. However, a scientific discussion on the chemical definition and the most appropriate determination technique of 'available (to biota) silicon' is still to be started.

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