Pure & Appl. Chem., Vol. 59, No. 7, pp. 927–936, 1987.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

CLINICAL CHEMISTRY DIVISION
COMMISSION ON TOXICOLOGY
SUBCOMMITTEE ON TOXICOLOGY OF SELENIUM*

Interlaboratory trial on the DETERMINATION OF SELENIUM IN LYOPHILIZED HUMAN SERUM, BLOOD AND URINE USING HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY

Prepared for publication by

BERNHARD WELZ[1], MARK S. WOLYNETZ[2] AND
MARLEEN VERLINDEN[3]

[1]Department of Applied Research, Bodenseewerk Perkin-Elmer & Co GmbH, D-7770 Ueberlingen, FRG
[2]Engineering and Statistical Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6 (Contribution number I-893)
[3]Clinical R&D Department, Janssen Pharmaceutica, B-2340 Beerse, Belgium

*Membership of the Subcommittee during the preparation of this report (1983–85) was as follows:

Chairman: Y. Thomassen (Norway); *Members*: J. Aaseth (Norway); G. Alfthan (Finland); D. Behne (FRG); H. A. Das (Netherlands); H. T. Delves (UK); R. M. Griffin (USA); M. Ihnat (Canada); V. Iyengar (FRG); O. A. Levander (USA); Mirna Mikac-Devic (Yugoslavia); J. Pikkarainen (Finland); Marion F. Robinson (New Zealand); M. Stoeppler (FRG); Christine D. Thomson (New Zealand); R. T. Tulley (USA); C. Veillon (USA); Marleen Verlinden (Belgium); J. H. Watkinson (New Zealand); B. Welz (FRG); G. O. Yang (China).

Interlaboratory trial on the determination of selenium in lyophilized human serum, blood and urine using hydride generation atomic absorption spectrometry

Summary - An interlaboratory collaborative study was conducted on the determination of total selenium (Se) with hydride generation atomic absorption spectrometry. Six different materials were investigated, four lyophilized body fluids, an acid-digested body fluid and an acidified aqueous reference solution. The main objective was to find out if accurate values for Se can be obtained with hydride generation atomic absorption spectrometry when an appropriate sample decomposition technique is used. The proposed procedure included a decomposition with nitric, sulphuric and perchloric acids to a final temperature of 310 °C in special flasks with long necks. The results obtained by 9 laboratories using the proposed decomposition procedure and 4 laboratories using slight modifications of it show excellent agreement with the values established in previous interlaboratory trials or by experienced laboratories using a number of independent techniques.

INTRODUCTION

Selenium has gained substantial interest as an essential trace element for humans as well as for some animals. Its accurate determination, however, is still a major challenge for the analyst. Hydride generation atomic absorption spectrometry (HG AAS) offers the advantages of excellent sensitivity and relatively simple instrumentation; therefore, it is well suited for routine analyses. There has been some doubt, however, regarding the accuracy of the results obtained with this technique. In 1983, an IUPAC interlaboratory trial was conducted to determine the selenium concentration in lyophilized human blood serum which had been proposed as a reference material (Ref. 1). Because three out of four laboratories using HG AAS obtained low values relative to results obtained with other methods, and because of previously published reports casting doubt on the adequate performance of this technique (Ref. 2 - 4), all HG AAS data were excluded in estimating the selenium level.

There are several reports in the literature that the sample decomposition technique is important if the selenium concentration in biological materials is to be estimated accurately (Ref. 5 - 7). In the hydride generation technique, the addition of sodium tetrahydroborate solution to the acid sample digest results in the formation of gaseous hydrogen selenide (H_2Se), which is stripped from the solution and atomized, usually in a heated quartz cell. A prerequisite for this reduction reaction is that selenium be present in its tetravalent ionic form as selenite. Organic selenium compounds must therefore be decomposed completely for the successful application of HG AAS.

In a series of preliminary experiments, the influence of the sample decomposition technique on the accuracy of the selenium values obtained by HG AAS was investigated, and it was found that relatively harsh conditions are required to convert all organically bound selenium into selenite (Ref. 8). Many selenium compounds, however, are volatile and can be lost during an inadequately controlled decomposition procedure. A gradual destruction with nitric, sulphuric and perchloric acids to a final temperature of 310 °C in special flasks with long necks was found to be optimum for the subsequent determination of selenium with HG AAS.

Under the auspices of the IUPAC Subcommittee on Selenium, Commission on Toxicology, an interlaboratory trial was conducted in order to demonstrate that the parameters are transferrable and accurate results can be obtained for selenium in human body fluids using HG AAS. Most of the materials that were used in this study had been analysed in previous interlaboratory trials or by experienced laboratories using a number of independent techniques so that the selenium contents can be assumed to be well established. The laboratories participating in this trial, however, had no prior information on the selenium content of the samples that they received. The aim of this trial was to find out if a number of interested laboratories, using the proposed HG AAS procedure, could accurately estimate the selenium concentration in these fluids. The participants were also permitted to modify the proposed procedure slightly in order to investigate the ruggedness of the procedure (i.e. robustness against minor changes in protocol).

EXPERIMENTAL

Samples

Six different materials were sent to each participant. Those participants who had indicated that they wanted to use the recommended procedure plus a different procedure received twice the amount of each material (sample). The six materials were comprised of four lyophilized body fluids, one acid-digested body fluid, and one reference solution.

Seronorm Serum Trace Elements, batch No. 105 (Nyco Med AS, Oslo, Norway) is lyophilized human serum (S 105). This material is normal human serum without added preservatives and is expected to contain endogeneous levels of trace elements. It is available in vacuum-sealed vials to which 3 mL of deionized, distilled water are added for reconstitution. Three vials of this material were sent to each of the participants. Seronorm 105 has previously been analyzed in an interlaboratory trial and a mean selenium concentration of 90.7 $\pm$ 1.1 µg/L has been established for this material (Ref. 9). The measure of dispersion (1.1 µg/L) is the standard error of the mean.

Seronorm Urine Trace Elements, batch No. 108 (Nyco Med AS, Oslo, Norway) is lyophilized normal human urine without added preservatives (U 108). It is available in vacuum-sealed vials to which 10 mL of deionized, distilled water have to be added for reconstitution. One vial was sent to each of the participants. This material was previously analyzed in the same interlaboratory trial; a mean selenium concentration of 49.4 $\pm$ 0.7 µg/L has been established (Ref. 9).

A pilot batch of lyophilized human whole blood (WB) was prepared and made available by Nyco Med AS, Oslo, Norway. The material was shipped in vacuum-sealed vials to which 10 mL of deionized, distilled water should be added for reconstitution. It dissolves easily within one hour; the use of an ultrasonic bath speeds up the dissolution process. One vial was sent to each of the participants. Little information is available for this material; a value of 94 $\pm$ 1 µg/L was obtained in the course of this trial by one of the laboratories (Ref. 10) using fluorimetry, an independent technique, but applying essentially the same acid digestion.

Aliquots of approximately 5 mL of lyophilized bovine serum, NBS Reference Material No. 8419 (US Department of Commerce, National Bureau of Standards, Gaithersburg, MD) were prepared for this study by the Vitamin & Mineral Nutrition Laboratory, US Department of Agriculture, Beltsville MD (RM 8419). Each tube was weighed before freeze-drying, and reconstitution was carried out by adding water until the original wet weight of tube plus serum was reached. One tube was supplied to each of the participants. A selenium content of 16 $\pm$ 2 µg/L has been established for this material by selected laboratories using several independent techniques (Ref. 11).

Also supplied was a bottle of acid-digested serum (DS 105) which was prepared by decomposing Seronorm Serum Trace Elements, batch No. 105, according to the recommended procedure, pooling a sufficient number of decomposition solutions after their dilution to volume, and re-aliquoting them in volumes of approximately 70 mL. The participants were asked to determine selenium directly in aliquots of 5.0 mL or 10.0 mL without any further treatment or dilution.

An acidified, aqueous "reference" solution (RS) containing 50.0 µg/L selenium as the selenite was also supplied. The participating laboratories had no information on the selenium content of RS. Participants were asked to carry this solution through the complete decomposition procedure as for the reconstituted serum, urine and blood samples.

Equipment

An atomic absorption spectrometer equipped with a hydride generation accessory with heated quartz tube atomizer; the AA spectrometers used in this interlaboratory trial included Instrumentation Laboratories Model Video 11 and Perkin-Elmer Models 280, 300, 430, 603, 2380, 3030, 4000 and 5000. The hydride generators used included two different home-made systems, IL Atomic Vapor Accessory and Perkin-Elmer Model MHS-20. All hydride generation accessories were "batch-type" systems.

For sample digestion, graduated quartz or glass flasks with a long neck and a nominal volume of 40 mL as shown in Figure 1 (available through Hans Kürner, Analysentechnik, Herderstraße 2, D-8200 Rosenheim, FRG); flasks of similar design (e.g. Kjeldahl flasks) could also be used. It is important, however, that the flasks have long necks that act as air coolers so that no selenium is lost during the decomposition (Ref. 12).

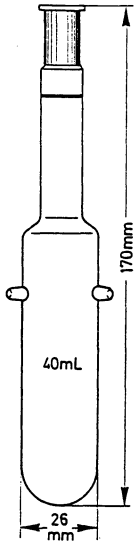

Figure 1.

Recommended acid decomposition flask, 40 mL, with graduation mark and ground glass joint.

Aluminium heating block for six or more digestion flasks which allows accurate temperature programming up to 310 °C; the flasks should be heated to not more than 1/4 to 1/3 of their height in the aluminium heating block.

Reagents

All acids should be of analytical reagent grade purity.

Hydrochloric acid, 5 mol/L
Nitric acid, 65 % (w/v)
Sulfuric acid, 96 % (w/v)
Perchloric acid, 70 % (w/v)

Sample decomposition procedure

- Place 0.5 mL serum, whole blood or urine into digestion flasks. 1.0 mL sample may be used, if necessary.
- Add 1 mL nitric acid.
- Place digestion flask into aluminium heating block.
- Heat slowly (approx. 15 min) to 140 °C and maintain this temperature for 25 min.
- Cool to room temperature.
- Add 0.5 mL sulfuric and 0.2 mL perchloric acids.
- Heat slowly (approx. 15 min) to 140 °C and maintain this temperature for 15 min.
- Increase temperature slowly (approx. 10 min) to 200 °C and maintain for 15 min.
- Increase temperature slowly (approx. 10 min) to 250 °C and maintain for 15 min.
- Increase temperature slowly (approx. 10 min) to 310 °C and maintain for 20 min.
- Let heating block cool to near room temperature.
- Add 20 mL 5 M hydrochloric acid to the residual digestion solution.
- Heat to 90 °C and maintain this temperature for 20 min.
- Cool to room temperature and dilute to volume (40 mL) with deionized water.
- Use aliquots of 5 mL to 10 mL of this solution for selenium determination. Larger aliquots or the total volume (40 mL) may be used for a determination of the lower selenium levels found in urine and bovine serum.

Note: A code of practice for handling perchloric acid has been published by the Society for Analytical Chemistry (Ref. 13), and should be studied by potential users. However, the risk is minimal if the recommended decomposition procedure and acid volumes are used.

Procedure

All participants in the Interlaboratory Trial were requested to analyze the material for total selenium following the recommended procedure and carrying out six independent decompositions and determinations of selenium from each of the four different materials supplied as well as from RS (which was to be treated as a body fluid sample). Six aliquots of DS 105 were to be analyzed for selenium directly without any further acid treatment or dilution. The participating laboratories prepared their own reference solutions in order to calibrate their analytical systems. All participants reported that they used an electrodeless discharge lamp and operated their systems under conditions recommended by the manufacturers. Se was determined at the 196.0 nm resonance line with a spectral bandpass of 2 nm.

Alternative procedures

Participants were allowed to use other procedures (e.g. those that are well established in the participant's laboratory) in addition to or instead of the recommended procedure, but only those procedures which altered the sample treatment or decomposition slightly are included in this study. Data from procedures which included substantially different sample decomposition or techniques for selenium determination other than hydride generation were omitted from the analyses.

Statistical procedures

The data were screened according to several criteria (1) within-laboratory inconsistency and (2) among-laboratory inconsistency. Individual observations identified by criterion 1 were omitted from all analyses. Sets of observations were identified as being aberrant by criterion 2; the formal statistical analyses were performed on the dataset obtained after deleting the aberrant (outlying) values.

Bartlett's test was used to examine the heterogeneity of variance within laboratories (i.e. consistency of within-laboratory precision). Analyses of variance were conducted (1) to compare among- and average within-laboratory variation, and (2) to compute the two variance components, which could then be used to estimate the precision of the determined selenium concentrations in the six materials.

RESULTS AND DISCUSSION

Nineteen laboratories agreed to participate in this IUPAC Interlaboratory Trial. Sixteen laboratories submitted data. Data from one laboratory were received too late to be included in the study. Another laboratory decided to withdraw the submitted values; hence data from fourteen laboratories are included in this report. Nine of the participants used the recommended procedure for sample decomposition and determination of selenium. Three of these submitted additional data using other sample pretreatment procedures. Four laboratories reported only data that were obtained using other sample decomposition techniques, and one laboratory used acid digestion/fluorimetry (ADF). Some of the laboratories did not provide data for all six materials that were distributed. Furthermore, not all laboratories conducted six independent decompositions of each material.

Two sets of data were used in evaluating the performance of the recommended procedure. Firstly, only the values from the nine laboratories that followed the recommended procedure (R) were used. These laboratories are identified as using method R in Tables 1 - 6. Secondly, the data from the four laboratories which used HG AAS and a sample decomposition with nitric and perchloric or nitric, sulfuric and perchloric acids at a sufficiently high temperature ($\geq$ 200 °C) were included (R+ data). The additional data are identified as method MR in Tables 1 - 6. The four remaining sets of data obtained by HG AAS after a decomposition with nitric, sulfuric and perchloric acids at only 100 °C, by HG AAS after nitric acid, perchloric acid and magnesium nitrate dry ashing, or by fluorimetry were omitted from consideration because they provided no direct information on the performance of the recommended HG AAS procedure and because selenium concentrations had been established previously for all materials except whole blood.

S 105 and U 108 have been analyzed previously in another IUPAC trial by 15 laboratories using six inherently distinct analytical methods. The mean values found in that interlaboratory trial were 90.7 $\pm$ 1.1 µg/L and 49.4 $\pm$ 0.7 µg/L, respectively (Ref. 9).

The 9 laboratories using R reported mean values between 84.2 µg/L and 100.7 µg/L (Table 1) with an average of 91.7 $\pm$ 1.7 µg/L (Table 7) for S 105. The mean value is in excellent agreement with that found in the previous trial. For the R+ dataset, the range of means increased slightly to values between 84.2 µg/L and 101.2 µg/L with an average of 93.3 $\pm$ 1.3 µg/L.

It is interesting to note that the agreement among laboratories was worse for DS 105, for which the selenium concentration was to be determined by HG AAS without any pretreatment. After exclusion of the data from laboratory 3 (Table 2), average values of 92.5 $\pm$ 2.5 µg/L for the R data set and 91.0 $\pm$ 2.1 µg/L for the R+ data set (Table 7) were obtained. This indicates that there is no systematic difference in the decomposition procedure carried out in our laboratory and in the participating laboratories.

For U 108 the data from laboratory 6 were excluded. The other 8 laboratories using R reported mean values between 45.8 µg/L and 54.0 µg/L (Table 3) with an average of 49.9 $\pm$ 1.2 µg/L (Table 7). For the R+ dataset, the means ranged from 37.5 µg/L to 54.0 µg/L; the average concentration is 48.4 $\pm$ 1.4 µg/L; which is still in excellent agreement with the previously established selenium content for this material.

Table 1. Selenium determination on serum S 105. All values in µg/L.

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
Method used[1]	R	R	R	R	R	R	R	R	R	MR	MR	MR	MR
Determination values	92	93	101	80	102	96	92	92	94	95	100	95	100
	85	91	89	87	109	83	90	94	93	98	98	97	104
	86	91	92	79	96	86	82	96	95	97	88	98	103
	89	90	104	87	102	63[2]	85	95	94	96	97	93	96
	85	91	88	82	96	90	91	96	93	97	90	97	105
	92	91	99	90	99	85	89	98	94	99	89	95	99
Mean	88.2	91.2	95.3	84.2	100.7	88.0	88.2	95.2	93.8	97.0	93.7	95.8	101.2
Standard deviation	3.3	1.0	6.7	4.4	4.9	5.1	3.9	2.0	0.8	1.4	5.2	1.8	3.4

(1) R = recommended; MR = slight modification of recommended decomposition procedure.
(2) Value deleted because of within-laboratory inconsistency.

Table 2. Selenium determination on acid-digested serum DS 105. All values in µg/L.

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
Method used[1]	R	R	R	R	R	R	R	R	R	MR	MR	MR	MR
Determination values	83	82	40[2]	84	96	100	95	89	90	82	94	88	81
	87	93	43[2]	84	104	100	107	91	-[3]	93	106	-[3]	88
	86	81	40[2]	86	96	94	100	98	-	81	106	-	98
	86	81	48[2]	87	104	100	92	94	-	80	100	-	92
	87	90	38[2]	-[3]	-[3]	100	100	94	-	89	-[3]	-	-[3]
	86	86	46[2]	-	-	106	103	95	-	87	-	-	-
Mean	85.8	85.5	42.5	85.2	100.0	100.0	99.5	93.5	90.0	85.3	101.5	88.0	89.8
Standard deviation	1.5	5.0	3.9	1.5	4.6	3.8	5.4	3.1	-	5.2	5.8	-	7.1

(1) R = Recommended; MR = slight modification of recommended decomposition procedure.
(2) Values deleted because of inconsistency with other laboratories.
(3) Data not provided

Table 3. Selenium determination on urine U 108. All values in µg/L.

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
Method used[1]	R	R	R	R	R	R	R	R	R	MR	MR	MR	MR
Determination values	49	49	46	53	45	63[3]	53	49	55	53	40	49	50
	51	53	46	53	51	47[2]	50	46	52	53	38	49	42
	51	46	44	59	58	61[3]	43	47	54	53	38	48	38
	52	51	46	52	58	64[3]	41	44	53	53	34	49	39
	49	49	47	53	55	64[3]	41	48	51	55	-[4]	50	40
	49	46	46	-[4]	57	64[3]	49	50	51	54	-	49	41
Mean	50.2	49.0	45.8	54.0	54.0	63.2	46.2	47.3	52.7	53.5	37.5	49.0	41.7
Standard deviation	1.3	2.8	1.0	2.8	5.1	1.3	5.1	2.2	1.6	0.8	2.5	0.6	4.3

(1) R = recommended; MR = slight modification of recommended decomposition procedure.
(2) Value deleted because of within-laboratory inconsistency.
(3) Values deleted because of inconsistency with other laboratories.
(4) Data not provided.

Very little information is available for WB, except for a value of 94 ± 1 µg/L obtained in the course of this trial using acid digestion/fluorimetry. All means obtained by HG AAS using R are between 82.8 and 98.5 µg/L (Table 4). Data from laboratory 7 (mean 91.5 µg/L and standard deviation 11.5 µg/L) were omitted because of the very large within-laboratory standard deviation; the mean of the remaining 8 laboratories is 93.0 ± 1.6 µg/L (Table 7). Including data from the 4 laboratories that used MR increased the range to 82.8 to 103.3 µg/L; the mean of these data is 95.2 ± 1.9 µg/L.

RM 8419 is of particular interest because its low selenium content of 16 ± 2 µg/L is close to those which can be expected in cases of severe selenium deficiency. The results of this study may, therefore, reflect the capability of the proposed procedure to measure low selenium concentrations in human body fluids. The data from laboratory 5 were excluded because they were obviously too high (Table 5). The data from laboratory 7 were excluded because the mean value 9.8 µg/L is much lower and the within-laboratory variance is much higher than those

Table 4. Selenium determination on whole blood WB. All values in µg/L.

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
Method used[1]	R	R	R	R	R	R	R	R	R	MR	MR	MR	MR
Determination values	91	98	89	89	89	84	93[2]	95	99	100	102	100	84
	93	98	87	78	95	100	77[2]	98	99	98	103	105	84
	89	94	100	78	91	83	91[2]	102	99	98	96	105	84
	93	93	92	83	85	94	90[2]	102	90	100	91	102	85
	93	96	102	86	102	92	86[2]	96	94	99	-[3]	103	84
	89	98	101	-[3]	97	94	112[2]	98	93	99	-	105	87
Mean	91.3	96.2	95.2	82.8	93.2	91.2	91.5	98.5	95.7	99.0	98.0	103.3	84.7
Standard deviation	2.0	2.2	6.6	4.9	6.0	6.5	11.5	3.0	3.9	0.9	5.6	2.0	1.2

(1) R = recommended; MR = slight modification of recommended decomposition procedure.
(2) Values deleted because of large within-laboratory variance.
(3) Data not provided.

Table 5. Selenium determination on NBS bovine serum RM 8419. All values in µg/L.

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
Method used[1]	R	R	R	R	R	R	R	R	R	MR	MR	MR	MR
Determination values	13	15	16	15	52[2]	22	16[2]	13[2]	14	16	10	13	16
	14	16	16	15	55[2]	21	13[2]	13[2]	14	16	10	12	15
	15	14	17	16	55[2]	17	9[2]	10[2]	15	15	9	12	13
	14	14	17	19	47[2]	15	6[2]	29[2]	15	15	9	13	13
	14	15	16	16	45[2]	18	5[2]	14[2]	13	16	-[3]	-[3]	13
	-[3]	14	-[3]	-[3]	58[2]	21	10[2]	24[2]	15	15	-	-	12
Mean	14.0	14.7	16.4	16.2	52.0	19.0	9.8	17.2	14.3	15.5	9.5	12.5	13.7
Standard deviation	0.7	0.8	0.5	1.6	5.0	2.8	4.2	7.5	0.8	0.5	0.6	0.6	1.5

(1) R = recommended; MR = slight modification of recommended decomposition procedure.
(2) Value deleted because of inconsistency with other laboratories and/or large within-laboratory variance.
(3) Data not provided.

from other laboratories using the recommended method; the data from laboratory 8 were excluded because of the large within-laboratory variation. For the remaining 6 laboratories that used R, the means ranged from 14.0 to 19.0 µg/L; the mean value is 15.8 ± 0.8 µg/L (Table 7). Three of the 4 laboratories using MR report means below 14.0 µg/L (9.4 - 13.7 µg/L) suggesting that the recommended method may have to be followed more closely at low selenium concentrations.

The acidified aqueous RS with a nominal selenium content of 50.0 µg/L was carried through the same decomposition procedure as were the body fluids. Data from laboratory 6 was discarded because of inconsistency with the other laboratories (Table 6). The other 8 laboratories using R reported mean values between 44.7 µg/L and 54.6 µg/L (Table 6) with an average of 49.7 ± 1.0 µg/L (Table 7). When the data from the MR laboratories are included 12 out of the 13 mean values are between 44.7 µg/L and 55.3 µg/L with an average of 50.5 ± 2.1 µg/L. The same laboratory that reported the high value of 62.5 µg/L using R reported a value of 64.2 µg/L using an alternate technique (data not shown in Table 6). This indicates that an improper standard may have been used, an assumption which is supported by the fact that some of the other results reported by this laboratory for the body fluids were high as well.

Table 6. Selenium determination on "reference" solution RS. All values in µg/L.

Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
Method used[1]	R	R	R	R	R	R	R	R	R	MR	MR	MR	MR
Determination values	50	50	58	52	46	61[2]	47	52	50	54	52	52	48
	50	52	50	47	51	63[2]	42	47	48	55	59	50	46
	51	50	57	45	53	63[2]	50	47	50	55	59	49	48
	50	50	50	47	49	63[2]	42	54	49	56	51	51	54
	51	49	57	48	49	60[2]	45	55	48	57	52	49	48
	51	51	57	47	53	66[2]	42	44	50	55	-[3]	50	-[3]
Mean	50.5	50.3	54.8	47.7	50.2	62.7	44.7	49.8	49.2	55.3	54.6	50.2	48.8
Standard deviation	0.5	1.0	3.8	2.3	2.7	2.1	3.3	4.4	1.0	1.0	4.0	1.2	3.0

(1) R = recommended; MR = slight modification of recommended decomposition procedure.
(2) Values deleted because of inconsistency with other laboratories.
(3) Data not provided.

Table 7. Summary of data obtained from laboratories using the recommended method (R laboratories) or slight modifications of it (MR laboratories)[1].

	n	n_L	s^2_L	s^2	mean	sd_1	sd_2
R laboratories							
S 105	53	9	23.36	16.09	91.7	1.7	6.3
U 108	47	8	9.46	9.88	49.9	1.2	4.4
WB	47	8	17.29	22.42	93.0	1.6	6.3
RM 8419	33	6	3.28	2.17	15.8	0.8	2.3
DS 105	39	8	45.60	15.42	92.5	2.5	7.8
RS	41	8	6.94	7.52	49.7	1.0	3.8
R and MR laboratories							
S 105	77	13	23.64	14.54	93.3	1.4	6.2
U 108	69	12	21.46	8.82	48.4	1.4	5.5
WB	69	12	31.05	17.56	94.1	1.7	7.0
RM 8419	53	10	5.50	1.71	14.6	0.8	2.7
DS 105	54	12	42.91	20.55	92.0	2.0	8.0
RS	70	12	8.41	7.16	50.5	1.0	3.9

(1) n = number of observations;
n_L = number of laboratories;
s^2_L = among-laboratory component of variance;
s^2 = within-laboratory component of variance;
sd_1 = standard deviation of the mean
$= \sqrt{s^2_L/n_L + s^2/n}$
sd_2 = standard deviation of a single observation
$= \sqrt{s^2_L + s^2}$

Table 8. Summary of Bartlett's test for homogeneity of variance within laboratories

	R laboratories			R and MR laboratories		
	chi-square	df[1]	prob[2]	chi-square	df	prob
S 105	27.6	8	< 0.001	37.6	12	< 0.001
U 108	21.2	7	0.004	40.6	11	< 0.001
WB	12.8	7	0.08	34.7	11	< 0.001
RM 8419	17.0	5	0.005	26.7	9	0.002
DS 105	10.5	6	0.11	13.5	9	0.14
RS	26.0	7	< 0.001	36.6	11	< 0.001

(1) df = degrees of freedom.
(2) prob = probability of observing a more extreme chi-square value.

Table 9. Analysis of variance of selenium concentrations[1].

		R laboratories		R and MR laboratories	
		df	ms	df	ms
S 105	labs	8	153.62	12	154.53
	error	44	16.09	64	14.54
U 108	labs	7	65.44	11	132.09
	error	39	9.87	57	8.82
WB	labs	7	123.94	11	195.90
	error	39	22.42	57	17.56
RM 4819	labs	5	20.17	9	30.80
	error	27	2.17	43	1.71
DS 105	labs	7	233.91	11	210.84
	error	31	15.42	42	20.55
RS	labs	7	49.16	11	56.22
	error	40	7.52	58	7.16

(1) In all analyses, among laboratory differences are significant at $p < 0.001$.

The foregoing discussion has concerned itself with the accuracy of the HG AAS method for the measurement of selenium concentrations in fluids. However, another aspect of any methodology is its precision. The within-laboratory variance was not homogeneous ($P < 0.01$) for the R data set with the exception of the data for DS 105 and for WB (Table 8). For the R+ data set, the within-laboratory precision was heterogeneous ($P < 0.01$) for all materials except DS 105. These findings indicate that the varying precision among laboratories may be due in large measure to the decomposition step in the recommended procedure. The decomposition procedure had not been used in most of the laboratories prior to this study. Furthermore it is interesting to note that heterogeneity of within-laboratory precision was also observed for the aqueous RS, which was to be decomposed in the same way as the body fluids. On the other hand, the pooled estimate of the within-laboratory variance for DS 105 was not less than that for S 105 in the R+ data set (Table 7).

Ignoring the heterogeneity of within-laboratory variance, an analysis of variance was carried out in order to compare among- and within-laboratory variation. In all analyses, the among-laboratory variation was significantly ($P < 0.001$) larger than the within-laboratory variation (Table 9), even after omitting those data that were obviously inconsistent with the remainder. Except for DS 105, the mean value from the R laboratories was significantly ($P < 0.01$) different from that of the MR laboratories.

CONCLUSION

An interlaboratory trial on the determination of selenium in four lyophylized body fluids, an acid-digested body fluid and an acidified aqueous "reference" solution using HG AAS has been successfully completed. Using the recommended procedure almost all participating laboratories obtained values for selenium which are in good agreement with previously established values. The mean values observed in this study are in excellent agreement with those previously established. This shows that systematic errors which were observed in earlier interlaboratory trials for HG AAS can be avoided if the sample decomposition procedure proposed in this study

is applied. Excellent agreement among the results for one material (S 105) was obtained when it was decomposed in the author's laboratory and in the participants' laboratories, respectively. This shows that the proposed decomposition procedure can be reproduced in other laboratories. The decomposition procedure, however, which had not been used in most of the laboratories prior to this study, contributed substantially to the within-laboratory variance.

Another interesting comparison is that between the urine sample and the aqueous "reference" solution. The selenium contents of both materials are very similar, and both were subjected to the same pretreatment procedure. The results from 8 out of 9 laboratories using the recommended procedure, are very similar. This means that the urine matrix has no influence on the performance of the proposed procedure and that urine, if a proper decomposition procedure is used, can be analyzed as accurately and precisely as an aqueous selenium reference solution with this technique.

Acknowledgements

The authors appreciate provision of the materials for this study by Nyegaard & Co AS, Oslo, and by C. Veillon, Vitamin & Mineral Nutrition Laboratory, Beltsville Human Nutrition Research Center, US Department of Agriculture, Beltsville MD 20705, USA. Equally appreciated is the participation and interest in the interlaboratory trial of the analysts listed below:

G. Alfthan, National Public Health Institute, Mannerheimintie 166, SF-00280 Helsinki 28, Finland

F. Alt, J. Messerschmidt, Institut für Spektrochemie und angewandte Spektroskopie, Bunsen-Kirchhoff-Str. 11, D-4600 Dortmund 1, Federal Republic of Germany.

W. Bayer, Laboratorium für spektralanalytische und biologische Untersuchungen, Bopserwaldstr. 26, D-7000 Stuttgart 1, Federal Republic of Germany

S.J. Haswell, School of Chemistry, Thames Polytechnic, Wellington Street, Woolwich SE18 6PF, United Kingdom

U. Kleff, Nattermann, Nattermannallee 1, D-5000 Köln, Federal Republic of Germany

J. Loimaranta, VALIO, Dept. R&C, Kalevankatu 56 B, SF-00180 Helsinki, Finland

G. Norheim, Veterinaerinstituttet, Postboks 8156 Dep, N-0033 Oslo 1, Norway

O. Oster, Abt. Klinische Chemie und Laboratoriumsmedizin, Universitätsklinik Mainz, Langenbeckstrasse, D-6500 Mainz, Federal Republic of Germany

S. Piepponen, Technical Research Centre of Finland, Food Research Laboratory, Biologinkuja 1, SF-02150 Espoo, Finland

L. Pyy, Oulo Regional Institute of Occupational Health, P.O.B. 451, SF 9010 Oulo, Finland

H. Robberecht, Dept. of Pharmacy, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgien

M. Schubert-Jacobs, Bodenseewerk Perkin-Elmer & Co GmbH, P.O.B. 1120, D-7770 Überlingen, Federal Republic of Germany

A.R. Selmer-Olsen, Chemical Analytical Laboratory, Agricultural University of Norway P.O.B. 31, N-1432 Äs-NLH, Norway

N.J. Smith, Health & Safety Executive, 403 Edgware Road, London NW2 6LN, United Kingdom

M. Stoeppler, Institut für Chemie der Kernforschungsanlage, P.O.B. 1913, D-5170 Jülich, Federal Republic of Germany

Y. Thomassen, Institute of Occupational Health, P.O.B. 8149 DEP, N-0033 Oslo 1, Norway

REFERENCES

1. M. Ihnat, M.S. Wolynetz, Y. Thomassen and M. Verlinden, Pure and Appl. Chem. 58 (1986) 1063-1076.
2. M. Ihnat and H.J. Miller, J. Assoc. Off. Anal. Chem. 60 (1977) 1414-1433
3. M. Ihnat and B.K. Thompson, J. Assoc. Off. Anal. Chem. 63 (1980) 814-839.
4. A. Meyer, C. Hofer, G. Tölg, S. Raptis and G. Knapp, Fresenius Z. Anal. Chem. 296 (1979) 337-347.
5. J. Nève, M. Hanocq, M. Molle and G. Lefebvre, Analyst 107 (1982) 934-941.
6. M. Verlinden, Talanta 29 (1982) 875-882.
7. O. Oster and W. Prellwitz, Clin. Chim. Acta 124 (1982) 277-291.
8. B. Welz, M. Melcher and J. Nève, Anal. Chim. Acta 165 (1984) 131-140.
9. M. Ihnat, Y. Thomassen, M.S. Wolynetz and C. Veillon, Acta Pharm. et Toxicologica, 59 Suppl. VII, (1986) 566-572.
10. G. Alfthan, personnal communication (1985)
11. C. Veillon, S.A. Lewis, K.Y. Patterson, W.R. Wolf, J.M. Harnly, J. Versieck, L. Vanballenberghe, R. Cornelis and T.C. O'Haver, Anal. Chem. 57 (1985) 2106-2109.
12. L. Kotz, G. Kaiser, P. Tschöpel and G. Tölg, Fresenius Z. Anal. Chem. 260 (1972) 207-209.
13. Analytical Methods Committee, Analyst 84 (1959) 214.