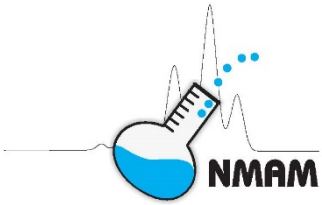

BACKUP DATA REPORT

NIOSH Method No. 8200

Title: Elements in tissue by ICP-AES

Analyte: Elements (Ca, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Zn)

Author/developer: Ronnee N. Andrews, Ph.D., NIOSH

Date: 8/30/2018

NIOSH 8200: Elements in Tissue by ICP-AES Back up Data Report

Method Validation for Rodent Nail Samples

Samples of rodent tissue have been analyzed previously using the acid digestion and sample analysis conditions listed in NIOSH 7300. NIOSH 7300 is an air sampling method and, as such, does not contain sampling details relevant to rodent tissue collection. A new method, NIOSH 8200, was developed to fully address sampling, preparation and analysis of tissue samples. After this method validation, all future rodent nail samples will be prepared and analyzed according to NIOSH 8200.

Sample Preparation:

For the rat nails (NIOSH 8200), the following digestion procedure was followed:

1. Samples transferred to digestion vessels. In this case, the digestion vessels were either 125 mL Phillips beakers or Erlenmeyer flasks.
2. 10 mL 4:1 nitric acid: perchloric acid (concentrated and Optima grade) added to each sample and the samples covered with watchglasses. The amount of acid used initially varied based upon the amount of sample material to be digested. SRM 1577C used 15 mL of 4:1 nitric acid: perchloric acid (concentrated and Optima grade) for digestion.
3. Samples refluxed on hot plate initially at 120 – 130 °C. The temperature was then increased to 150 °C. Samples refluxed until digestion appeared complete (no visible solid).
   NOTE: Control rat nails refluxed for 5-6 days. SRM 1577C samples refluxed for 13 days. These times are highly variable. Recent studies with both nail samples and SRM bulks finished digestion (and sample analysis) in 1 week.
4. Watchglasses removed with a slight deionized water rinse and sample volume reduced to near-dryness by heating at 120 °C.
5. 0.5 mL 4:1 nitric acid: perchloric acid (concentrated and Optima grade) were added to each sample and, upon cooling to room temperature, sample digestates were transferred to dilution vessels and diluted to 10 mL using deionized water. In this case, dilution vessels were 15 mL polystyrene centrifuge tubes.

All samples were analyzed on a Spectro Arcos ICP-AES using external calibration standards.

Determination of method limits of detection (LOD) and quantitation (LOQ)

In order to determine the method limits of detection and quantitation, seven reagent blank samples were digested and analyzed according to the method. In instances where the concentration detected by the ICP is less than the instrument's internally calculated LOD, the result was noted in italics. The limits of detection (LOD) were calculated as 3.143 times the standard deviation of the average concentration and the limits of quantitation (LOQ) were calculated as 10 times the standard deviation of the average concentration.

Please note: All analyzed elements are included below, even though the method is not validated for all of them. Also, the numbers listed next to the element pertain to the monitored wavelength (nm).

Table 1 Reported results from analysis of reagent blank samples used in the calculation of the limits of detection (LOD) and limits of quantitation (LOQ). Results reported in µg/sample.

Sample	R Blk 1	R Blk 2	R Blk 3	R Blk 4	R Blk 5	R Blk 6	R Blk 7
Ag 328.068	0.02006	*-0.024875*	*-0.022434*	*-0.019948*	*-0.017155*	*-0.028587*	*0.00639*
Al 396.152	1.228	1.747	0.617259	0.894621	2.024	0.824497	1.324
As 193.759	0.092774	*-0.050586*	*-0.050557*	*-0.041514*	*-0.034984*	*-0.066909*	0.033453
Ba 455.404	*-0.008365*	*-0.014292*	*-0.016756*	*-0.013449*	*-0.014373*	*-0.016224*	*-0.009579*
Be 313.042	*-0.005285*	*-0.006753*	*-0.00648*	*-0.006015*	*-0.007113*	*-0.007069*	*-0.00617*
Ca 315.887	1.209	1.173	0.652289	0.974644	2.006	0.549532	1.424
Cd 226.502	0.031269	0.016114	0.024518	0.018098	0.019015	0.010278	0.026931
Co 228.616	0.006381	*-0.015048*	*-0.013947*	*-0.012757*	*-0.014204*	*-0.014744*	*-0.00134*
Cr 267.716	0.05222	*-0.00916*	*-0.004945*	*-0.007282*	*-0.002837*	*-0.016587*	0.019082
Cu 324.754	0.040663	*-0.152997*	*-0.152636*	*-0.121204*	*-0.144777*	*-0.171575*	*-0.064351*
Fe 259.941	0.304999	0.254413	0.087568	0.24097	0.556906	0.137938	0.241782
Fe 275.573	0.28761	0.189157	0.01678	0.173546	0.496848	0.065662	0.213135
In 230.606	*-0.057503*	*-0.239714*	*-0.225797*	*-0.200741*	*-0.215013*	*-0.257486*	*0.157600*
K 766.491	0.393265	*-0.266618*	*-0.557722*	*-0.206051*	0.428663	*-0.427756*	*0.181406*
La 408.672	*-0.007089*	*-0.075737*	*-0.070929*	*-0.068543*	*-0.065945*	*-0.08678*	*-0.023689*
Li 670.780	*0.020077*	*0.00387*	*0.003184*	*0.003605*	*0.009343*	*0.002819*	*0.014114*
Mg 279.079	0.137814	*-0.327333*	*-0.294907*	*-0.303189*	*-0.178962*	*-0.351176*	0.083316
Mn 257.611	0.019923	*-0.014685*	*-0.023316*	*-0.020634*	*-0.005878*	*-0.022498*	0.011659
Mo 202.095	0.018616	*-0.01175*	*-0.012744*	*-0.009109*	*-0.002958*	*-0.013848*	*0.000473*
Na 589.592	6.371	6.158	6.324	4.69	9.285	8.149	5.462
Ni 231.604	0.027576	*-0.022321*	*-0.024231*	*-0.021527*	*-0.025661*	*-0.027086*	*0.003997*
P 178.287	0.100522	*0.037285*	*0.027322*	*0.034617*	*0.042917*	*0.033355*	0.084851
Pb 220.353	0.250296	0.157012	0.184983	0.165768	0.182655	0.117439	0.238305
Sb 206.833	0.110342	*-0.036767*	*-0.037742*	*-0.034879*	*0.021114*	*-0.041736*	0.068063
Sc 361.384	*-0.000616*	*-0.008108*	*-0.007714*	*-0.007329*	*-0.00742*	*-0.009515*	*-0.003061*
Se 196.090	0.111126	*-0.075861*	*-0.071874*	*-0.05863*	*-0.052823*	*-0.081248*	0.056318
Sn 189.991	0.037058	*-0.01963*	0.007105	*0.001925*	*0.000415*	0.012423	0.011605
Sr 407.771	*-0.006344*	*-0.008659*	*-0.010108*	*-0.008849*	*-0.009385*	*-0.009859*	*-0.005291*
Te 214.281	0.104075	*-0.064315*	*-0.074428*	*-0.061106*	*-0.053998*	*-0.07684*	0.043781
Ti 334.941	0.011495	*0.000584*	*-0.003835*	*-0.008018*	0.023077	*-0.009707*	0.004449
Tl 190.864	0.062995	*-0.015058*	*-0.014104*	*-0.00985*	*-0.003006*	*-0.012543*	0.032369
V 311.071	0.020219	*-0.008417*	*-0.007008*	*-0.006047*	*0.001233*	*-0.011068*	0.012717
W 207.911	0.058878	*-0.028473*	*-0.038108*	*-0.031176*	0.175817	*-0.042003*	*0.010435*
Y 371.030	*-0.008769*	*-0.021035*	*-0.019946*	*-0.020341*	*-0.020606*	*-0.02419*	*-0.013325*
Zn 213.856	0.628706	0.534376	0.146918	0.425	0.772308	0.078812	0.279197
Zr 339.198	0.0135	*-0.004539*	*-0.014445*	*-0.01153*	0.033322	0.007769	0.009978

Table 2 Calculated average reagent blank response, standard deviation, method LOD, and method LOQ. Results reported in µg/sample.

Sample	Average R Blk	Std. Dev.	LOD	LOQ
Ag 328.068	ND	0.0183	0.057	0.183
Al 396.152	ND	0.510	1.6	5.10
As 193.759	ND	0.0581	0.18	0.581
Ba 455.404	ND	0.00318	0.010	0.0318
Be 313.042	ND	0.000649	0.0020	0.00649
Ca 315.887	ND	0.492	1.5	4.92
Cd 226.502	ND	0.00712	0.022	0.0712
Co 228.616	ND	0.00846	0.027	0.0846
Cr 267.716	ND	0.0238	0.075	0.238
Cu 324.754	ND	0.0748	0.24	0.748
Fe 259.941	ND	0.150	0.47	1.50
Fe 275.573	ND	0.157	0.49	1.57
In 230.606	ND	0.0722	0.23	0.722
K 766.491	ND	0.419	1.3	4.19
La 408.672	ND	0.0281	0.088	0.281
Li 670.780	ND	0.00678	0.021	0.0678
Mg 279.079	ND	0.204	0.64	2.04
Mn 257.611	ND	0.0174	0.055	0.174
Mo 202.095	ND	0.0115	0.036	0.115
Na 589.592	[6.6]	1.57	4.9	15.7
Ni 231.604	ND	0.0207	0.065	0.207
P 178.287	ND	0.0288	0.091	0.288
Pb 220.353	[0.19]	0.0462	0.15	0.462
Sb 206.833	ND	0.0614	0.19	0.614
Sc 361.384	ND	0.00318	0.010	0.0318
Se 196.090	ND	0.0764	0.24	0.764
Sn 189.991	ND	0.0170	0.053	0.170
Sr 407.771	ND	0.00183	0.0058	0.0183
Te 214.281	ND	0.0709	0.22	0.709
Ti 334.941	ND	0.0116	0.037	0.116
Tl 190.864	ND	0.0302	0.095	0.302
V 311.071	ND	0.0119	0.037	0.119
W 207.911	ND	0.0795	0.25	0.795
Y 371.030	ND	0.00532	0.017	0.0532
Zn 213.856	ND	0.255	0.80	2.55
Zr 339.198	ND	0.0166	0.052	0.166

Variability of control rat nail samples

Control rat nail samples were supplied by research collaborators at HELD. Six samples were chosen at random for this experiment and the sample numbers are shown in the table. The average weight of the nail samples was 39.3 mg. The results have been corrected for the average reagent blank. ND indicates the result was not

detected above the method LOD. Results in brackets are between the method LOD and LOQ. Results marked with * are outliers and were not included in the calculations.

Please note: All analyzed elements included below, even though the method is not validated for all of them. Also, the numbers listed next to the element pertain to the monitored wavelength (nm).

Table 3 Blank response for control rat nail samples, labelled A-F. Results reported in µg/g.

Sample	A	B	C	D	E	F
Ag 328.068	ND	ND	ND	ND	ND	ND
Al 396.152	351	234	282	158	267	242
As 193.759	35.2	28.4	ND	ND	ND	ND
Ba 455.404	0.926	0.846	1.06	1.13	0.862	1.07
Be 313.042	ND	ND	ND	ND	ND	ND
Ca 315.887	15200	17900	21600	21600	18300	20300
Cd 226.502	[0.64]	[0.96]	[0.34]	[0.97]	[0.75]	[0.31]
Co 228.616	ND	ND	ND	ND	ND	ND
Cr 267.716	ND	[2.6]	ND	ND	ND	ND
Cu 324.754	[16.]	[14.]	[16.]	[16.]	[18.]	[16.]
Fe 259.941	[27.]	[37.]	[20.]	[20.]	[31.]	[22.]
In 230.606	ND	ND	ND	ND	ND	ND
K 766.491	1100	1230	1320	1330	1170	1380
La 408.672	ND	ND	ND	ND	ND	ND
Li 670.780	[1.0]	[0.94]	ND	[0.53]	[1.0]	[0.59]
Mg 279.079	366	422	495	483	423	478
Mn 257.611	ND	ND	ND	ND	ND	ND
Mo 202.095	ND	[1.0]	ND	ND	ND	ND
Na 589.592	2210	2390	3260	2590	2020	2990
Ni 231.604	ND	ND	ND	ND	ND	ND
P 178.287	8040	9220	11100	11000	9390	10200
Pb 220.353	ND	ND	ND	[5.6]	ND	ND
Sb 206.833	203.*	55.3	ND	[6.9]	19.6	16.2
Sc 361.384	ND	ND	ND	ND	ND	ND
Se 196.090	[8.0]	[8.3]	ND	ND	[8.6]	ND
Sn 189.991	7.31	[3.9]	ND	[1.6]	[4.3]	[1.4]
Sr 407.771	3.90	4.51	5.48	5.42	4.38	4.99
Te 214.281	20.2	[15.]	ND	ND	ND	ND
Ti 334.941	9.06	4.84	[1.3]	ND	[1.6]	[0.93]
Tl 190.864	ND	[2.7]	ND	[2.5]	[3.4]	ND
V 311.071	ND	ND	ND	ND	ND	ND
W 207.911	27.4	27.5	ND	ND	[11.]	ND
Y 371.030	ND	ND	ND	ND	ND	ND
Zn 213.856	102	107	97.9	115	109	116
Zr 339.198	ND	ND	6.09*	ND	[1.6]	ND

Table 4 Average control rat nail sample response and method LOD and LOQs (based upon average sample weight of 39.3 mg). Results reported in µg/g.

	Average	Std. Dev.	LOD	LOQ
Ag 328.068	ND	0.361	1.5	4.65
Al 396.152	256	63.4	41	130
As 193.759	[12.]	15.7	4.6	14.8
Ba 455.404	0.983	0.120	0.25	0.810
Be 313.042	ND	0.0308	0.052	0.165
Ca 315.887	19200	2501	39	125
Cd 226.502	[0.66]	0.288	0.57	1.81
Co 228.616	ND	0.205	0.68	2.15
Cr 267.716	ND	0.871	1.9	6.06
Cu 324.754	[16.]	1.38	6.0	19.0
Fe 259.941	[26.]	7.00	12	38.2
In 230.606	ND	3.22	5.8	18.4
K 766.491	1260	107	34	107
La 408.672	ND	0.803	2.2	7.16
Li 670.780	[0.76]	0.246	0.54	1.72
Mg 279.079	444	49.7	16	51.9
Mn 257.611	ND	0.0632	1.4	4.43
Mo 202.095	ND	0.377	0.92	2.92
Na 589.592	2580	472	130	401
Ni 231.604	ND	0.354	1.7	5.28
P 178.287	9800	1153	2.3	7.34
Pb 220.353	ND	2.27	3.7	11.8
Sb 206.833	20.1	20.8	4.9	15.6
Sc 361.384	ND	0.0845	0.25	0.809
Se 196.090	ND	2.68	6.1	19.4
Sn 189.991	[3.3]	2.39	1.4	4.32
Sr 407.771	4.78	0.625	0.15	0.466
Te 214.281	ND	9.01	5.7	18.1
Ti 334.941	3.08	3.30	0.93	2.96
Tl 190.864	[2.5]	0.518	2.4	7.68
V 311.071	ND	0.254	0.95	3.03
W 207.911	[12.]	12.1	6.4	20.2
Y 371.030	ND	0.176	0.43	1.35
Zn 213.856	108	7.28	20	64.9
Zr 339.198	ND	0.513	1.3	4.22

Spiked control rat nail samples

Control rat nail samples were supplied by research collaborators at HELD. Samples were chosen at random for this study and the sample numbers are shown in the following results tables. Samples were spiked with 25.0,

110, or 200 µL of NIOSH-CAL-2A (Inorganic Ventures) and taken through the sample preparation and analysis procedures. Spiked concentrations are listed below.

The individual results have been corrected for the average reagent blank from this sample set and the average control nail response. ND indicates the result was not detected above the method LOD. Results in brackets are between the method LOD and LOQ. In the summation tables for each level, the average recovery shown is the average of the recoveries calculated for each sample in that set and was not calculated based upon the average amount detected. This is due to varying weights of the samples.

The variability in the control nail samples (above section) is greater than the spiked amounts for Ca & P. Using the liquid spike, acceptable amounts of Ag, As, Ba, Be, Cd, Co, Cr, Cu, Fe, K, La, Mn, Ni, Pb, Se, Sr, Tl, V, Y, & Zn were recovered at each level and acceptable amounts of Al & Mg were recovered at the 110 and 200 µL levels.

Please note: The numbers listed next to the element pertain to the monitored wavelength (nm).

Table 5 Spiked concentrations (µg/sample) for the 3 levels: 25, 110, and 200 µL.

	25 µL	110 µL	200 µL
Ag	3.13	13.8	25
Al	6.25	27.5	50
As	12.5	55	100
Ba	1.25	5.5	10
Be	1.25	5.5	10
Ca	62.5	275	500
Cd	1.25	5.5	10
Co	1.25	5.5	10
Cr	1.25	5.51	10
Cu	6.25	27.5	50
Fe	6.25	27.5	50
K	93.8	413	750
La	3.13	13.8	25
Li	1.25	5.5	10
Mg	6.25	27.5	50
Mn	1.25	5.5	10
Ni	1.25	5.5	10
P	6.25	27.5	50
Pb	6.25	27.5	50
Se	9.38	41.3	75
Sr	1.25	5.5	10
Tl	6.25	27.5	50
V	3.13	13.8	25
Y	1.25	5.5	10
Zn	18.8	82.6	150

Table 6 Response for control rat nail samples spiked at the 25 µL level, labelled G-L. Results reported in µg/g.

Sample	G	H	I	J	K	L
Ag 328.068	108	87.1	78.0	111	75.9	90.9
Al 396.152	[170]	ND	[93.]	[130]	[120]	ND
As 193.759	433	341	410	522	296	352
Ba 455.404	45.9	36.5	32.5	46.7	32.5	38.7
Be 313.042	46.4	36.9	33.2	47.2	32.4	39.2
Ca 315.887	ND	1070	ND	ND	ND	ND
Cd 226.502	44.1	34.4	30.7	45.0	30.2	36.3
Co 228.616	43.3	34.5	31.2	44.5	30.3	36.7
Cr 267.716	44.1	35.7	32.3	45.7	31.2	37.8
Cu 324.754	237	189	172	244	164	201
Fe 259.941	228	173	143	220	152	181
K 766.491	3900	3510	2793	4074	3010	3027
La 408.672	115	91.9	81.4	117	80.4	96.8
Li 670.780	60.0	47.4	43.8	58.7	43.6	50.4
Mg 279.079	205	186	60.4	158	132	[32.]
Mn 257.611	41.9	33.5	30.4	43.2	29.7	36.0
Ni 231.604	43.5	34.7	31.2	44.9	30.2	36.5
P 178.287	ND	19.0	ND	ND	ND	ND
Pb 220.353	224	180	162	232	156	192
Se 196.090	352	278	269	372	244	290
Sr 407.771	46.0	36.4	32.3	47.5	33.0	38.2
Tl 190.864	216	172	154	222	151	182
V 311.071	115	93.1	84.2	120	81.4	97.7
Y 371.030	44.7	35.7	31.8	45.7	31.4	37.9
Zn 213.856	691	573	503	717	483	583

Table 7 Summation table for control rat nail samples spiked at the 25 µL level. Average and standard deviation reported in µg/g. NOTE: % Recovery based upon average of recoveries for each sample and are not based upon the average amount detected (shown here).ND = not detected; N/A = value could not be calculated

	Average	Std. Dev.	% Recovery
Ag 328.068	91.9	14.9	90.4
Al 396.152	[91.]	62.6	44.3
As 193.759	392	80.5	96.8
Ba 455.404	38.8	6.28	95.5
Be 313.042	39.2	6.38	96.6
Ca 315.887	ND	-	N/A
Cd 226.502	36.8	6.44	90.4
Co 228.616	36.7	6.02	90.4
Cr 267.716	37.8	5.99	93.2
Cu 324.754	201	32.9	99.1
Fe 259.941	183	34.6	89.8
K 766.491	3385	524	111.5
La 408.672	97.1	15.9	95.4
Li 670.780	50.7	7.20	125.1
Mg 279.079	129	69.4	62.8
Mn 257.611	35.8	5.70	88.2
Ni 231.604	36.9	6.16	90.7
P 178.287	ND	-	N/A
Pb 220.353	191	31.4	94.1
Se 196.090	301	50.3	98.7
Sr 407.771	38.9	6.46	95.8
Tl 190.864	183	30.3	90.1
V 311.071	98.6	16.0	97.0
Y 371.030	37.9	6.18	93.2
Zn 213.856	592	95.7	96.9

Table 8 Response for control rat nail samples spiked at the 110 µL level, labelled M-R. Results reported in µg/g.

Sample	M	N	O	P	Q	R
Ag 328.068	338	308	355	329	386	257
Al 396.152	647	503	780	671	908	490
As 193.759	1380	1260	1470	1340	1610	1070
Ba 455.404	143	128	148	135	162	108
Be 313.042	140	129	152	137	165	109
Ca 315.887	4200	1990	1840	ND	2590	7830
Cd 226.502	140	128	147	136	159	106
Co 228.616	135	124	145	132	158	104
Cr 267.716	134	123	144	131	157	103
Cu 324.754	733	663	781	715	844	564
Fe 259.941	672	767	720	653	786	502
K 766.491	13800	12200	13600	12300	15000	10700
La 408.672	352	317	367	336	400	268
Li 670.780	209	188	223	203	241	166
Mg 279.079	643	536	670	518	761	562
Mn 257.611	130	119	140	126	150	99.1
Ni 231.604	132	121	142	130	154	102
P 178.287	ND	ND	ND	ND	ND	2400
Pb 220.353	673	615	722	659	783	515
Se 196.090	1040	950	1100	1010	1190	811
Sr 407.771	141	128	147	132	161	109
Tl 190.864	648	592	691	631	754	498
V 311.071	354	322	377	342	409	271
Y 371.030	141	127	147	134	160	107
Zn 213.856	2240	2050	2390	2190	2590	1710

Table 9 Summation table for control rat nail samples spiked at the 110 µL level. Average and standard deviation reported in µg/g. NOTE: % Recovery based upon average of recoveries for each sample and are not based upon the average amount detected (shown here).ND = not detected; N/A = value could not be calculated

	Average	Std. Dev.	% Recovery
Ag 328.068	329	43.9	85.9
Al 396.152	667	161	86.4
As 193.759	1360	184	88.9
Ba 455.404	137	18.3	90.0
Be 313.042	139	19.2	90.8
Ca 315.887	2630	3424	38.7
Cd 226.502	136	18.1	89.2
Co 228.616	133	18.3	87.1
Cr 267.716	132	18.3	86.2
Cu 324.754	717	96.7	93.9
Fe 259.941	683	103	89.7
K 766.491	13000	1516	113.4
La 408.672	340	45.0	88.8
Li 670.780	205	26.3	134.5
Mg 279.079	615	93.2	81.0
Mn 257.611	127	17.7	83.4
Ni 231.604	130	18.0	85.2
P 178.287	ND	-	N/A
Pb 220.353	661	91.9	86.6
Se 196.090	1020	131	88.9
Sr 407.771	136	17.7	89.3
Tl 190.864	635	87.4	83.2
V 311.071	346	47.3	90.3
Y 371.030	136	18.1	89.2
Zn 213.856	2200	300	95.8

Table 10 Response for control rat nail samples spiked at the 200 µL level, labelled S-X. Results reported in µg/g.

Sample	S	T	U	V	W	X
Ag 328.068	854	732	684	828	735	735
Al 396.152	1760	1630	1340	1860	1510	1520
As 193.759	3520	3060	2830	3400	3060	3050
Ba 455.404	363	306	290	350	311	310
Be 313.042	357	312	291	352	311	307
Ca 315.887	3070	10600	10100	7270	6980	13000
Cd 226.502	341	288	272	330	295	292
Co 228.616	341	292	271	326	295	291
Cr 267.716	344	294	271	327	294	293
Cu 324.754	1830	1570	1470	1770	1580	1570
Fe 259.941	1740	1460	1370	1650	1470	1460
K 766.491	36300	29500	29200	34400	30500	31000
La 408.672	908	761	722	873	774	770
Li 670.780	504	449	425	505	447	447
Mg 279.079	1480	1400	1310	1480	1310	1440
Mn 257.611	333	282	264	320	284	283
Ni 231.604	336	287	266	322	289	286
P 178.287	ND	ND	275	ND	ND	1520
Pb 220.353	1690	1450	1340	1620	1460	1450
Se 196.090	2520	2280	2140	2500	2340	2300
Sr 407.771	353	303	287	344	304	300
Tl 190.864	1640	1400	1310	1570	1420	1400
V 311.071	924	781	734	889	786	785
Y 371.030	362	304	287	348	309	307
Zn 213.856	5180	4500	4240	5100	4520	4470

Table 11 Summation table for control rat nail samples spiked at the 200 µL level. Average and standard deviation reported in µg/g. NOTE: % Recovery based upon average of recoveries for each sample and are not based upon the average amount detected (shown here).ND = not detected; N/A = value could not be calculated

	Average	Std. Dev.	% Recovery
Ag 328.068	761	65.3	89.0
Al 396.152	1600	186	93.6
As 193.759	3160	254	92.3
Ba 455.404	322	28.4	94.0
Be 313.042	322	26.6	94.0
Ca 315.887	8490	3465	50.8
Cd 226.502	303	26.7	88.6
Co 228.616	303	25.8	88.5
Cr 267.716	304	26.5	88.8
Cu 324.754	1630	137	95.3
Fe 259.941	1530	140	89.2
K 766.491	31800	2890	124.0
La 408.672	801	72.5	93.7
Li 670.780	463	33.4	135.4
Mg 279.079	1400	75.5	82.2
Mn 257.611	294	26.2	86.0
Ni 231.604	298	26.0	87.0
P 178.287	ND	-	N/A
Pb 220.353	1500	128	87.0
Se 196.090	2350	145	91.7
Sr 407.771	315	26.6	92.1
Tl 190.864	1460	124	85.2
V 311.071	816	73.3	95.4
Y 371.030	320	28.9	93.4
Zn 213.856	4670	382	91.0

SRM 1577C: Bovine Liver samples

SRM 1577C: Bovine Liver is the closest standard or certified reference material for animal tissue samples that we could obtain. It was prepared in 3 sets at 3 different target weights (levels): 100 (A-F), 150 (G-L), and 200 (M-R) mg. SRM 1577C contains many elements: some that we do not typically report. Analyte concentrations in SRM 1577C are found in Table 12. Results for the elements that we typically report are listed below.

Table 12 Concentrations of elements in SRM 1577C

Element	Concentration (µg/g)	Designation
Ag	0.0059	Certified
As	0.0196	Certified
Ca	131	Certified
Cd	0.097	Certified
Co	0.3	Certified
Cr	0.053	Certified
Cu	275.2	Certified
Fe	197.94	Certified
K	10230	Certified
Mg	620	Certified
Mn	10.46	Certified
Mo	3.3	Certified
Na	2033	Certified
Ni	0.0445	Certified
Pb	0.0628	Certified
S	7490	Certified
Se	2.031	Certified
Sr	0.0953	Certified
V	0.00817	Certified
Zn	181.1	Certified
Cl	2870	Reference
Cs	0.0217	Reference
H	73500	Reference
Hg	0.00536	Reference
N	103000	Reference
P	11750	Reference
Rb	35.3	Reference
Sb	0.00313	Reference
Li	0.012	Information
Si	6	Information

The individual results have not been corrected for the average reagent blank response, but the average result for each concentration level has been corrected for it. ND indicates the result was not detected above the method LOD. Results in brackets are between the method LOD and LOQ. Standard deviations and % RSDs calculated before average reagent blank correction. Results marked with * are outliers and were not included in the calculations. N/A indicates the value couldn't be calculated.

The variability in the control nail samples is greater than the amounts present in the SRM for Ag, As, Ca, Cd, Cr, Ni, Pb, Sb, Sr, & V. The amounts of Co & Li detected in the samples were less than the LOQ for the method. Individual and summation data is displayed for As and Sb, but recovery was not calculated. This is due to artificially high amounts detected due to memory effects [1,2] coupled with amounts in SRM 1577C that are below the method LODs for these elements. Acceptable amounts of Ca, Cu, Fe, K, Mg, Mn, Mo, P, & Zn were recovered at each level using the bovine liver material.

Please note: The numbers listed next to the element pertain to the monitored wavelength (nm).

Table 13 Response for SRM 1577C at the low level, labelled A-F. Results reported in µg/g. Average sample weight is 0.1009 g

Sample	A	B	C	D	E	F
Ag 328.068	ND	ND	ND	ND	ND	ND
As 193.759	ND	ND	ND	ND	1.94	ND
Ca 315.887	130	135	127	133	148	131
Cd 226.502	[0.43]	ND	ND	ND	ND	ND
Co 228.616	ND	ND	ND	ND	ND	ND
Cr 267.716	[0.71]	ND	ND	ND	ND	ND
Cu 324.754	259	261	263	271	269	269
Fe 275.573	190	188	188	195	182	193
K 766.491	10400	10600	10800	11400	11100	10900
Li 670.780	[0.60]	1.39	[0.65]	[0.59]	0.685	[0.58]
Mg 279.079	536	542	546	566	563	557
Mn 257.611	8.64	8.72	8.72	9.01	9.02	9.03
Mo 202.095	3.04	3.16	3.10	3.15	3.05	3.20
Na 589.592	2930	2910	2980	3050	3110	3000
Ni 231.604	ND	ND	ND	ND	ND	ND
P 178.287	10700	10400	10800	11300	11200	11100
Pb 220.353	5.18	[2.0]	[2.6]	[2.7]	[2.8]	[3.1]
Sb 206.833	[5.0]	ND	ND	[2.2]	41.4*	ND
Se 196.090	[4.4]	[3.6]	[4.4]	[4.0]	[4.3]	[4.3]
Sr 407.771	ND	[0.080]	ND	ND	ND	ND
V 311.071	ND	ND	ND	ND	ND	ND
Zn 213.856	165	166	164	179	183	176

Table 14 Summation table for SRM 1577C at the low level. Average and standard deviation reported in µg/g. % Recovery is based upon the average response. NOTE: ND = not detected; N/A = value could not be calculated

	Average	Std. Dev.	% RSD	% Recovery
Ag 328.068	ND	-	-	N/A
As 193.759	ND	-	-	N/A
Ca 315.887	134	7.63	5.7	102.3
Cd 226.502	ND	-	-	N/A
Co 228.616	ND	-	-	N/A
Cr 267.716	ND	-	-	N/A
Cu 324.754	265	4.98	1.9	96.4
Fe 275.573	189	4.48	2.4	95.6
K 766.491	10800	370	3.4	105.9
Li 670.780	ND	-	-	N/A
Mg 279.079	552	11.9	2.2	89.0
Mn 257.611	8.85	0.182	2.1	84.7
Mo 202.095	3.12	0.0632	2.0	94.5
Na 589.592	2940	74.3	2.5	144.5
Ni 231.604	ND	-	-	N/A
P 178.287	10900	343	3.1	92.8
Pb 220.353	ND	-	-	N/A
Sb 206.833	ND	-	-	N/A
Se 196.090	[4.2]	0.314	7.5	205.2
Sr 407.771	ND	-	-	N/A
V 311.071	ND	-	-	N/A
Zn 213.856	172	8.16	4.7	94.9

Table 15 Response for SRM 1577c at the mid-level, labelled G-L. Results reported in µg/g. Average sample weight is 0.1510 g.

Sample	G	H	I	J	K	L
Ag 328.068	ND	ND	ND	ND	ND	ND
As 193.759	[1.7]	[1.6]	[1.3]	[1.6]	[1.5]	[1.5]
Ca 315.887	127	131	127	120	117	130
Cd 226.502	ND	ND	ND	ND	ND	[0.14]
Co 228.616	[0.21]	[0.22]	[0.19]	[0.21]	[0.19]	[0.23]
Cr 267.716	ND	ND	ND	ND	ND	ND
Cu 324.754	262	271	251	257	249	265
Fe 275.573	188	193	176	183	176	186
K 766.491	10507	10897	10023	10245	10062	10709
Li 670.780	[0.43]	[0.43]	[0.39]	[0.41]	0.453	[0.37]
Mg 279.079	535	550	512	520	506	539
Mn 257.611	8.75	9.09	8.31	8.63	8.33	8.81
Mo 202.095	3.14	3.30	2.96	3.12	3.02	3.22
Na 589.592	3180	3260	2920	3110	2990	3120
Ni 231.604	ND	ND	ND	ND	ND	ND
P 178.287	10600	11100	10100	10400	10000	10800
Pb 220.353	[1.7]	[1.8]	[1.7]	[1.5]	[1.6]	[1.5]
Sb 206.833	10.3	[1.7]	ND	8.92	[1.6]	[2.2]
Se 196.090	[4.0]	[4.2]	[3.6]	[4.0]	[3.8]	[3.9]
Sr 407.771	[0.044]	ND	[0.040]	ND	ND	ND
V 311.071	ND	ND	ND	ND	ND	ND
Zn 213.856	164	176	165	163	157	170

Table 16 Summation table for SRM 1577C at the middle level. Average and standard deviation reported in µg/g. % Recovery is based upon the average response. NOTE: ND = not detected; N/A = value could not be calculated

	Average	Std. Dev.	% RSD	% Recovery
Ag 328.068	ND	-	-	N/A
As 193.759	[1.5]	0.142	9.3	N/A
Ca 315.887	126	5.69	4.5	95.9
Cd 226.502	ND	-	-	N/A
Co 228.616	[0.21]	0.0148	7.0	70.3
Cr 267.716	ND	-	-	N/A
Cu 324.754	259	8.34	3.2	94.1
Fe 275.573	184	6.69	3.6	92.8
K 766.491	10400	356	3.4	101.5
Li 670.780	ND	-	-	N/A
Mg 279.079	527	16.9	3.2	85.0
Mn 257.611	8.65	0.301	3.5	82.7
Mo 202.095	3.13	0.124	4.0	94.7
Na 589.592	3060	123	4.0	150.3
Ni 231.604	ND	-	-	N/A
P 178.287	10500	408	3.9	89.4
Pb 220.353	ND	-	-	N/A
Sb 206.833	4.34	4.14	95.3	N/A
Se 196.090	3.92	0.181	4.6	193.0
Sr 407.771	ND	-	-	N/A
V 311.071	ND	-	-	N/A
Zn 213.856	166	6.64	4.0	91.6

Table 17 Response for SRM 1577C at the high level, labelled M-R. Results reported in µg/g. Average sample weight is 0.2008 g.

Sample	M	N	O	P	Q	R
Ag 328.068	ND	ND	ND	ND	ND	ND
As 193.759	[1.4]	[1.3]	[1.4]	[1.4]	[1.4]	[1.5]
Ca 315.887	118	120	116	119	118	120
Cd 226.502	[0.13]	[0.13]	[0.12]	[0.12]	[0.12]	[0.13]
Co 228.616	[0.24]	[0.25]	[0.23]	[0.20]	[0.22]	[0.23]
Cr 267.716	ND	ND	ND	ND	ND	ND
Cu 324.754	257	260	256	264	261	265
Fe 275.573	180	184	179	186	181	184
K 766.491	10100	10200	9950	10300	10000	10200
Li 670.780	0.398	[0.33]	[0.30]	0.610	0.498	0.388
Mg 279.079	518	526	514	532	524	530
Mn 257.611	8.57	8.72	8.55	8.79	8.67	8.78
Mo 202.095	3.15	3.21	3.15	3.23	3.15	3.23
Na 589.592	3120	3210	3100	3230	3170	3230
Ni 231.604	ND	ND	ND	ND	ND	ND
P 178.287	10400	10500	10300	10600	10400	10700
Pb 220.353	[1.3]	[1.2]	[1.2]	[1.1]	[1.2]	[1.1]
Sb 206.833	[1.3]	[1.5]	[2.2]	3.49	9.28*	[1.2]
Se 196.090	[3.7	[3.8]	[3.7]	[3.7]	[3.7]	3.94
Sr 407.771	0.0419	0.0409	0.0336	0.0337	0.0325	0.0322
V 311.071	ND	ND	ND	ND	ND	ND
Zn 213.856	163	168	163	169	167	169

Table 18 Summation table for SRM 1577C at the high level. Average and standard deviation reported in µg/g. % Recovery is based upon the average response. NOTE: ND = not detected; N/A = value could not be calculated

	Average	Std. Dev.	% RSD	% Recovery
Ag 328.068	ND	-	-	N/A
As 193.759	[1.4]	0.0564	4.0	N/A
Ca 315.887	119	1.50	1.3	90.5
Cd 226.502	[0.12]	0.00564	4.6	127.4
Co 228.616	[0.23]	0.0155	6.7	76.6
Cr 267.716	ND	-	-	N/A
Cu 324.754	261	3.60	1.4	94.7
Fe 275.573	182	2.67	1.5	92.2
K 766.491	10100	128	1.3	98.7
Li 670.780	[0.17]	0.116	27.6	1383.7
Mg 279.079	524	7.09	1.4	84.5
Mn 257.611	8.68	0.104	1.2	83.0
Mo 202.095	3.19	0.0402	1.3	96.5
Na 589.592	3150	55.2	1.7	154.8
Ni 231.604	ND	-	-	N/A
P 178.287	10500	153	1.5	89.1
Pb 220.353	ND	-	-	N/A
Sb 206.833	[1.9]	0.940	48.4	N/A
Se 196.090	[3.8]	0.102	2.7	185.0
Sr 407.771	0.0358	0.00440	12.3	37.6
V 311.071	ND	-	-	N/A
Zn 213.856	166	2.63	1.6	91.9

Accuracy

Table 19 Validation criteria, including range, bias, and accuracy calculated using SRM 1577C samples.

Element	n[A]	Range, µg/sample	Bias	S_{rT}[B]	Accuracy (%)
Ca	18	13.0 – 26.6	-0.0956	0.00032	9.6
Cd	6	0.00964 – 0.0197	0.289	0.582	127.4
Co	12	0.0298 – 0.0610	-0.272	0.185	57.6
Cu	18	27.4 – 55.9	-0.0493	0.00008	4.9
Fe	18	19.7 – 40.2	-0.0649	0.00013	6.5
K	18	1020 – 2080	0.0622	0.00000	6.2
Mg	18	61.6 – 126	-0.155	0.00003	15.5
Mn	18	1.04 – 2.13	-0.165	0.00193	16.9
Mo	18	0.328 – 0.671	-0.0474	0.00774	6.0
Na	18	202 – 413	0.520	0.00002	52.0
P	18	1170 – 2380	-0.0950	0.00000	9.5
Se	18	0.202 – 0.413	0.943	0.0542	103.2
Sr	12	0.00947 – 0.0194	-0.592	0.460	134.9
Zn	18	18.0 – 36.8	-0.0712	0.00019	7.1

[A]n = total number of results reported for each element, ND results not included

[B]S_{rT} = precision [3,4]

Storage

No storage studies were performed. The elements are expected to be stable in the nail structure.

Independent Laboratory User Check

An independent laboratory user check was performed (under contract #200-2016-M-89668) with Quality Chemical Laboratories (QCL) in Wilmington, NC. After determining that the laboratory could successfully prepare calibration curves and analyst spikes, control nail samples were submitted for preparation and analysis. A blind spiking solution (High-Purity Standards, Charleston, SC) was submitted and used to spike the control nails for the user check. The blind spiking solution contained: Mn and Ni at 5 µg/mL, Cu and Mo at 10 µg/mL, Fe at 15 µg/mL, Zn at 100 µg/mL, Mg at 300 µg/mL, K at 750 µg/mL, P at 5000 µg/mL, and Ca at 7000 µg/mL. Nails were spiked at 3 levels with 6 samples at each level. Spiked levels are shown in Table 19.

Table 20 Spiked concentrations (µg/sample) for the 3 levels of the user check.

Element	Level 1 (µg/sample)	Level 2 (µg/sample)	Level 3 (µg/sample)
Ca	700	1750	2800
Cu	1	2.5	4
Fe	1.5	3.75	6
K	75	187.5	300
Mg	30	75	120
Mn	0.5	1.25	2
Mo	1	2.5	4
Ni	0.5	1.25	2
P	500	1250	2000
Zn	10	25	40

The wavelengths monitored by QCL are listed in Table 20. Additionally, six control nail samples were also prepared and analyzed. The average blank control nail response was used to correct the results for latent analyte content. Average latent analyte content is shown in Table 20.

Table 21 Wavelengths and reported detection limits (DL) and quantitation limits (QL) for the user check.

Element	Wavelength (nm)	DL (ppm)[A]	QL (ppm)[B]
Ca	315.887	0.005	0.015
Cu	324.754	0.001	0.003
Fe	259.941	0.001	0.004
K	766.491	0.003	0.009
Mg	279.079	0.010	0.029
Mn	257.611	0.001	0.002
Mo	204.598	0.004	0.013
Ni	231.604	0.003	0.009
P	213.618	0.009	0.028
Zn	213.856	0.001	0.002

[A] $DL = \frac{3.3*SD}{Slope}$, where SD is standard deviation of seven blank intensities and Slope is taken from the linearity of the curve

[B] $QL = \frac{10*SD}{Slope}$

Table 22 Average latent control nail response.

Element	Average Concentration (µg/sample)
Ca	687.
Cu	0.635
Fe	0.258
K	42.1
Mg	12.9
Mn	ND
Mo	ND
Ni	ND
P	336
Zn	3.30

Average percent recoveries of spiked nails, corrected for the latent analyte content are shown in Table 21.

Table 23 Average percent recoveries of spiked control nails at three levels for the user check samples.

Element	Level 1 (µg/sample)	Level 2 (µg/sample)	Level 3 (µg/sample)
Ca	97.7	105.4	107.2
Cu	107.6	99.3	102.6
Fe	87.4	88.1	87.5
K	95.2	94.3	101.5
Mg	112.3	103.2	106.6
Mn	93.1	93.6	91.4
Mo	95.2	95.5	91.9
Ni	88.2	88.2	86.3
P	94.4	100.0	103.0
Zn	101.1	99.6	106.1

The results show the successful analysis of blind spike control nail samples by an independent laboratory using the conditions described in this method.

Note: the laboratory contractor substituted a hot block digestor for the hot plate initially listed in the method. Before preparing the control nail samples with the hot block digestor, the contractor showed that the sample could adequately be digested using the sample preparation acids and heating temperatures. In fact, the sample preparation time was reduced as compared to that when using the hot plate due to its more controlled heating of the sample. Because of this change, the hot block was added to the method.

Conclusions

The digestion and analysis of rat nail samples using a NIOSH 8200 have been validated for Ca, Cu, Fe, K, Mg, Mn, Mo, P, & Zn. While acceptable amounts were recovered using SRM 1577C for Ca, the large variability (with respect to the amount present in SRM 1577C) in the control nail samples for Ca calls to question the applicability of SRM 1577C for the validation of rat nail samples for Ca.

References

[1] Spandler C, Pettke T, Rubatto D [2011]. Internal and external fluid sources for Eclogite-facies veins in the Monviso Meta-ophiolite, Western Alps: Implications for fluid flow in Subduction Zones, Electronic appendix 2.

Additional note on LA-ICP-MS analytical procedures. J Petrology *52*(6):1207-1236, https://dx.doi.org/10.1093/petrology/egr025.

[2] EPA [1996]. Method 6010B, Inductively coupled plasma-atomic emission spectrometry. Washington, DC: U.S. Department of the Interior, Environmental Protection Agency, https://www.epa.gov/sites/production/files/documents/6010b.pdf.

[3] NIOSH [1995]. Guidelines for Air Sampling and Analytical Method Development and Evaluation. By Kennedy ER, Fischbach TJ, Song R, Eller PM, Shulman SA. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 95–117.

[4] NIOSH [2016]. Measurement Uncertainty and NIOSH Method Accuracy: Chapter UA. In: Ashley K, O'Connor PF, eds. NIOSH manual of analytical methods. 5th ed. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2014-151, https://www.cdc.gov/niosh/nmam/.