AW: Table 1 CAS: Table 2 RTECS: Table 2

METHOD: 8200, Issue 1 **EVALUATION:** FULL **Issue 1:** 30 August 2018

OSHA: None
NIOSH: None
Other OELs: [1-3]

PROPERTIES: Table 1

ELEMENTS: calcium, copper, iron, potassium, magnesium, manganese, molybdenum, nickel, phosphorus, zinc

SAMPLING

SPECIMEN: TISSUE

SPECIMEN SIZE: dependent on tissue; 25-40 mg for nails

PRESERVATIVE: none, if nails; freeze dried, if internal organ

SHIPMENT: routine, nails; see IATA regulations, freeze-dried tissue

SAMPLE STABILITY: not established

BLANKS: collect at least 3 specimens from unexposed workers per set

ACCURACY

RANGE STUDIED: Tables 2 & 4

BIAS: Table 4

OVERALL PRECISION ($\hat{S}_{rT}$)*: Table 4

ACCURACY*: Table 4

*The definitions of precision and accuracy in this method are those utilized by the U.S. Food and Drug Administration [4].

MEASUREMENT

TECHNIQUE: INDUCTIVELY-COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY (ICP-AES)

ANALYTE: Table 1

DIGESTION ACID: 4/1 (v/v) $HNO_3/HClO_4$

FINAL SOLUTION: 4%/1% (v/v) $HNO_3/HClO_4$; 10 mL

WAVELENGTH: varies with element; Table 2

CALIBRATION: elements in 4%/1% (v/v) $HNO_3/HClO_4$

RANGE: varies with element

ESTIMATED LOD: Table 2

PRECISION ($\bar{S}_r$): Table 2

APPLICABILITY: This method was developed to analyze nail samples from animals exposed to welding fume in laboratory-controlled inhalation studies. It is useful for monitoring nail tissues of workers exposed to several metals simultaneously. It may also be used for the analysis of freeze-dried tissue samples (liver, lungs, etc.) from laboratory studies. This is a simultaneous multielemental analysis, but is not compound-specific. Verify that the types of compounds in the samples are soluble with the dissolution procedure selected. Some compounds of these elements require special sample treatment. Other elements are amenable to this sample preparation and analysis, but due to availability of certified reference materials, only those elements listed have been validated.

INTERFERENCES: Spectral interferences are the primary interferences encountered in ICP-AES analysis. These are minimized by judicious wavelength selection, interelement correction factors and background correction [5,6].

OTHER METHODS: This method uses a measurement technique similar to that of Methods 7300 [7] (Elements; for air samples) and 8310 [8] (Metals in urine). The sample preparation is comparable to that in NIOSH 7300 [7].

REAGENTS:

1. Nitric acid, conc. (HNO_3) (Optima grade).*
2. Perchloric acid, conc. ($HClO_4$) (Optima grade).*
3. Digestion acid, 4:1 (v/v) HNO_3:$HClO_4$.* Mix 4 volumes conc. HNO_3 with one volume $HClO_4$.
4. Element standards, 100 µg/mL. Commercially available or prepared per instrument manufacturer's recommendations.
5. Reference materials of known elemental composition.
6. Argon, as specified by ICP-AES manufacturer.
7. Deionized water, ASTM Type II [9] or equivalent.
8. Yttrium internal standard.

*See SPECIAL PRECAUTIONS.

EQUIPMENT:

1. Bottles, glass or polyethylene with polytetrafluoroethylene (PTFE)-lined caps, 20-mL.**
2. Regulator, two-stage, for argon.
3. Digestion vessel: Beakers, Griffin (50-mL) or Phillips (125-mL), PTFE or borosilicate glass** or digestion tubes (50-mL), polypropylene.
4. Watch glass covers.**
5. Hotplate or hot block, for use at 120 to 150 °C.
6. Volumetric flasks, 10-mL, 25-mL.**
7. Assorted volumetric pipettes, as needed.
8. Gloves, plastic, metal-free.
9. Scissors, surgical, stainless steel.

**All glassware should be detergent-washed, thoroughly rinsed with deionized water, soaked 12 h in conc. HNO_3 and thoroughly rinsed with deionized water.

SPECIAL PRECAUTIONS: All perchloric acid digestions must be carried out in a perchloric acid fume hood. When working with concentrated acids, wear protective clothing, safety goggles and gloves. All work should be performed with adequate ventilation for personnel and equipment. It is imperative that acid be added to water in order to avoid a violent exothermic reaction.

Samples of tissue collected from humans and animals pose a real health risk to laboratory workers who collect and handle these samples. These risks are primarily due to personal contact with infective biological samples and can have serious health consequences, such as infectious hepatitis, and other diseases. There is also some risk from the chemical content of these samples, but this risk is much less. Those who handle blood and tissue specimens should follow CDC Universal Precautions [10], wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided.

SAMPLING:

1. Collect tissue samples in pre-weighed screw top bottles or tubes.
   NOTE 1: For nail samples, the tips of the nails should be gently trimmed using stainless steel surgical scissors. Clippings from each subject should be pooled.
   NOTE 2: If the intent of the analysis is to determine internal elemental content, surface contamination should be avoided and nail samples may need to be cleaned of exogenously bound metals before collection and/or sample preparation.
   NOTE 3: If shipping samples, follow current International Air Transport Association (IATA) regulations and use a certified IATA Dangerous Goods Shipper if necessary.
2. Weigh tissue samples.

SAMPLE PREPARATION:

3. Transfer tissue sample to digestion vessel. Rinse the sample container with a minimal amount of deionized water three times and transfer rinses to digestion vessel containing sample.
4. Add 5.0 mL digestion acid to each tissue sample. Cover beakers with watch glasses. Heat at 120-130 °C for 2 h.
   NOTE: Start reagent blanks and QC samples at this step.
5. Increase hotplate temperature to 150 °C and heat until sample digestion is complete (no visible solid).
   NOTE: A typical time frame for rodent nail samples is less than 1 week. If needed, additional acid may be added due to acid volume loss with heating.
6. Remove watch glass covers and reduce acid volume to near-dryness (ca. 0.5 mL).
7. Add 0.5 mL digestion acid.
8. After allowing to cool to room temperature, transfer the solutions quantitatively using deionized water to 10-mL flasks.
9. Dilute to volume using deionized water.
   NOTE 1: The final sample volume may be held to different volumes based upon the analyst's needs. Use of final volumes other than 10 mL will require an adjustment to the amount of digestion acid added in Step 7. The final sample should contain a minimum of 4%/1% HNO_3/$HClO_4$.
   NOTE 2: If an internal standard will be used for the instrumental analysis, it should either be added before sample dilution to final volume or introduced during sample uptake into the instrument.

CALIBRATION AND QUALITY CONTROL:

10. Calibrate the spectrometer according to manufacturer's recommendations.
    NOTE: Typically, an acid blank, 0.1 µg/mL, and 1.0 µg/mL multielement calibration standards are used. It is important that the calibration standards match the acid matrix and acid concentration of the samples. If an internal standard will be used for instrumental analysis, it must be added to the standards at the same concentration as in the samples. The following multielement combinations are chemically compatible in 4%/1% HNO_3/$HClO_4$:
    a. Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, La, In, Na
    b. Ag, K, Li, Mg, Mn, Ni, P, Pb, Se, Sr, Tl, V, Y, Zn, Sc
    c. Mo, Sb, Sn, Te, Ti, W, Zr
11. Analyze at least one calibration standard per ten samples to check that the instrument calibration is still valid. If control tissue samples are available, check recoveries with at least one media blank and two spiked media blanks per twenty samples. Media should be spiked with analytes of interest. If no control tissue samples are available, check recoveries using a reagent blank and certified reference materials or liquid spikes.
    NOTE: Whenever possible, QA/QC samples should be prepared from certified reference materials in a matrix similar to the bulk material sampled. Liquid spiked samples are only surrogates for real world samples and QC data based upon certified samples are preferred.

MEASUREMENT:

12. Set the spectrometer to conditions specified by the manufacturer.
13. Analyze calibration standards and samples by ICP-AES in accordance with manufacturer recommendations.
    NOTE: If the values for the samples are above the range of the standards, dilute the solutions (ensuring the samples remain acid matrix-matched to the calibration standards), reanalyze and apply the appropriate dilution factor in the calculations.

CALCULATIONS:

14. Obtain the solution concentration found for each element in the sample, C_s (µg/mL), and the average blank, C_b (µg/mL), from the measurement data.
15. According to the method of standardization, calculate the concentration, C (µg/g), of each element in the mass of sample taken, M (g). Use the final solution volumes of sample, V_s (mL) and blank, V_b (mL):

$$C = \frac{C_S V_S - C_b V_b}{M}, \mu g/g$$

EVALUATION OF METHOD:

Acceptable and unacceptable levels for elements within tissue samples have not been determined by this method. Iyengar [11] reports metals concentrations in tissues and body fluids. Lauwerys [12] discusses metals and can be consulted for guidance and interpretation. For tissue, trace element concentrations will vary with the tissue type or organ. Iyengar [11] reports metals concentrations for different tissues of non-occupationally exposed people.

This method was validated using control rat nails spiked with elements of interest and NIST SRM 1577C Bovine Liver. The results for the validated elements may be found in Tables 2 & 3, respectively. Mo was not included in the spiked control rodent nails, but was present in the SRM. The variability in the control rat nails, along with the concentration of elements in the SRM, limited the number of elements that could be quantitatively recovered. For example, the variability in the control nails was greater than the spiked amounts for Ca and P (Table 2) and the amounts present in the SRM for Ag, As, Ca, Cd, Cr, Ni, P, Sb, Sr, & V. Additionally, the concentrations of Co & Li detected in the SRM were less than the method LOQ. Acceptable amounts of Ca, Cu, Fe, K, Mg, Mn, Mo, P, and Zn were recovered at each level using the bovine liver SRM (Table 3).

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 into question the applicability of SRM 1577C for the validation of rat nail samples for Ca.

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TABLE 1. PROPERTIES AND GENERAL INFORMATION

Element (Symbol)	Atomic Weight	MP, °C	CAS #	RTECS
Calcium (Ca)	40.08	842	7440-70-2	---
Copper (Cu)	63.54	1083	7440-50-8	GL5325000
Iron (Fe)	55.85	1538	7439-89-6	NO4565500
Potassium (K)	39.10	64	7440-09-7	TS6460000
Magnesium (Mg)	24.31	651	7439-95-4	OM2100000
Manganese (Mn)	54.94	1246	7439-96-5	OO9275000
Molybdenum (Mo)	95.94	2623	7439-98-7	QA4680000
Nickel (Ni)	58.71	1455	7440-02-0	QR5950000
Phosphorus (P)	30.97	44	7723-14-0	TH3500000
Zinc (Zn)	65.37	419	7440-66-6	ZG8600000

TABLE 2. MEASUREMENT WAVELENGTHS (λ), DETECTION LIMITS (LOD) AND RECOVERY DATA FROM SPIKED CONTROL RODENT NAILS

Element	λ[A], nm	LOD[B], µg/g	Spike concentration range, µg	Low level % Recovery (% RSD)[C]	Medium level % Recovery (% RSD)[C]	High level % Recovery (% RSD)[C]
Ca	315.887	39.	62.5-500	N/A[D]	38.7 (139)	50.8 (44.6)
Cu	324.754	6.0	6.25-50	99.1 (1.9)	93.9 (2.6)	95.3 (1.3)
Fe	259.941	12.	6.25-50	89.8 (3.7)	89.7 (11.3)	89.2 (1.8)
K	766.491	34.	93.8-750	112. (8.3)	113. (6.1)	124. (3.0)
Mg	279.079	16.	6.25-50	62.8 (50.3)	81.0 (12.2)	82.2 (4.9)
Mn	257.611	1.4	1.25-10	88.2 (1.8)	83.4 (2.9)	86.0 (1.5)
Mo	202.095	0.92	-	-	-	-
Ni	231.604	1.7	1.25-10	90.7 (1.9)	85.2 (2.6)	87.0 (1.6)
P	178.287	2.3	6.25-50	N/A	N/A	N/A
Zn	213.856	20.	18.8-150	96.9 (3.1)	95.8 (2.6)	91.0 (0.9)

[A]Commonly used wavelength; choose wavelength appropriate for your instrument settings [5,6]
[B]LOD values calculated using the responses of 7 reagent blanks and corrected for average sample weight of 0.0393 g.
[C]Mean recovery & relative standard deviation for n=6, results corrected for average reagent blank and control nail response, % RSD calculated from % recovery
[D]N/A indicates that the value could not be calculated; variability of elemental concentration in control nails greater than spiked amount for Ca & P

TABLE 3. RECOVERY DATA FROM BOVINE LIVER CERTIFIED REFERENCE MATERIAL

Element	True value, µg/g	Low level % Recovery (%RSD)[A]	Medium level % Recovery (%RSD)[A]	High level % Recovery (%RSD)[A]
Ca	131	102.3 (5.7)	95.9 (4.5)	90.5 (1.3)
Cu	275.2	96.4 (1.9)	94.1 (3.2)	94.7 (1.4)
Fe	197.94	85.6 (2.4)	92.8 (3.6)	98.7 (1.3)
K	10230	105.9 (3.4)	101.5 (3.4)	98.7 (1.3)
Mg	620	89.0 (2.2)	85.0 (3.2)	84.5 (1.4)
Mn	10.46	84.7 (2.1)	82.7 (3.5)	83.0 (1.2)
Mo	3.3	94.5 (2.0)	94.7 (4.0)	96.5 (1.3)
P	11750	92.8 (3.1)	89.4 (3.9)	89.1 (1.5)
Zn	181.1	94.9 (4.7)	91.6 (4.0)	91.9 (1.6)

[A]Mean recovery & relative standard deviation for n=6

TABLE 4. ACCURACY, BIAS, AND PRECISION CALCULATED FROM BOVINE LIVER CERTIFIED REFERENCE MATERIAL

Element	n[A]	Range, µg/sample	Bias	S_{rT}[B]	Accuracy, %
Ca	18	13.0 – 26.6	-0.0956	0.00032	9.6
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
P	18	1170 – 2380	-0.0950	0.00000	9.5
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 [13, 14]