Trace Elements in Cigarette Tobacco by ICP-MS

Scope and Application

An inductively coupled plasma mass spectrometry (ICP-MS) is used for quantitative determination of trace elements in tobacco leaf and tobacco filter material. The current method quantifies the concentration for vanadium, chromium, manganese, cobalt, copper, zinc, molybdenum, cadmium, barium, and lead.

Levels and Limitations

Method Levels and Limitations

	Linear Range	Limit of Detection	Limit of Quantitation
V-51	2 to 100 ppb	0.23 ppb	2 ppb
Cr-52	5 to 200 ppb	3.74 ppb	5 ppb
Cr-53	5 to 200 ppb	3.62 ppb	5 ppb
Mn-55	50 to 2000 ppb	5.23 ppb	50 ppb
Co-59	2 to 100 ppb	0.17 ppb	2 ppb
Cu-63	10 to 1000 ppb	2.87 ppb	10 ppb
Cu-65	10 to 1000 ppb	2.82 ppb	10 ppb
Zn-64	10 to 1000 ppb	2.81 ppb	10 ppb
Zn-66	10 to 1000 ppb	2.36 ppb	10 ppb
Mo-98	5 to 200 ppb	1.06 ppb	5 ppb
Cd-111	2 to 200 ppb	1.67 ppb	2 ppb
Ba-137	5 to 1000 ppb	2.04 ppb	5 ppb
Pb-208	2 to 100 ppb	1.57 ppb	2 ppb

Supplemental Documents

None

Equipment

1. Perkin-Elmer DRC-e Inductively Coupled Plasma Mass Spectrometer or equivalent
2. Perkin-Elmer S10 Autosampler or equivalent (optional)
3. Perkin-Elmer Mixing block for on-line internal standard addition (Part # B050-7962) or equivalent
4. Closed vessel microwave digester with Teflon vessels, stoppers and caps (example: CEM model MARSXpress or equivalent)
5. Analytical balance with at least 4 decimal places
6. Centrifuge 3500 rpm at room temperature
7. Freezer -20°C
8. Functional fume hood
9. Oven capable of reaching a temperature of 100°C
10. Cigarette Grinder (example: Thomas Scientific part# 3383-L10)
11. Desiccator

ELAN ICP-MS Instrument Conditions and Method Parameters

Nebulizer	Meinhard
Spray Chamber	Baffled Cyclonic
Sampler and Skimmer Cones	Nickel
RF Power	1400 W
Plasma gas flow rate	15.0 L/min
Nebulizer gas flow rate	0.85-1.1 mL/min
Auxiliary gas flow rate	1.0 L/min
Sample uptake flow rate	1.0 mL/min
Sweeps/reading	20
Readings/Replicate	1
Replicates	3
Dwell time	50 ms per AMU
Mode of analysis	Standard

RF Power is 1600W for the NexION 300D

Signal Processing

Detector Mode	Dual
Autolens	ON
Spectral Peak Processing	Average
Signal Profile Processing	Average
Measurement Units	Counts per Seconds (CPS)
Blank Subtraction	Subtracted after internal standard
Smoothing	Yes, Factor 5

Interferences

1. Spectral interferences
    a. Isobaric overlap is an interference that comes from different isotopes of other elements in the sample with the same atomic weights or mass numbers as the analyte of interest. See Elements Monitored below and the validation report for further explanation.
    b. Polyatomic interference can occur when two or more atomic ions combine. See Elements Monitored below and the validation report for further explanation.
2. Matrix interference affects ion intensity. This type of interference can be brought on by the level of dissolved solids or acid concentration in the sample. The best way to avoid matrix interference is to make sure that the sample is digested properly (See 8.2. Sample Digestion Procedure).
3. Memory effect occurs when the signal for an analyte is enhanced due to carryover from a previous sample. Memory effect can be detected by monitoring the rinse blanks. If the mean concentration for an analyte in the rinse blank is not less than or equal to a factor of ten of the method detection limit, the analyst has to either program a longer rinse time, dilute the samples causing the carry over or increase the number of rinse blanks in between samples.

Equations / Corrections used for overlaps

Analyte	Mass	Correction
Zn	64	-0.035297* Ni 60
Mo	98	-0.109613* Ru 101
Ba	137	-0.000901 * La 139 – 0.002838 * Ce 140
In (int.std.)	115	-0.014038*Sn118
Pd	208	+Pb206+Pb207

Elements Monitored

Elements	Mass	Mode
V	51	Standard
*Cr	52/53	Standard
Mn	55	Standard
Co	59	Standard
**Cu	63/65	Standard
***Zn	64/66	Standard
Mo	98	Standard
Cd	111	Standard
Ba	137	Standard
Pb	206-208	Standard

* Chromium-52 and 53 will be analyzed because argon and carbon combines to form 40Ar12C for a total mass of 52. The most abundant isotope for chromium is at mass 52. If the values of Chromium-52 and 53 are comparable in the standard reference material, report the value for Chromium-52. If the values are significantly different (ie: the value of Chromium-52 is significantly higher), report Chromium-53.

** Copper-63 and Copper-65 will be analyzed. Copper-63 is the more abundant element; however, it has interferences with high sodium-containing solutions due to Argon-Sodium polyatomic species. If the values of Copper-63 and Copper-65 are comparable in the standard reference material, report the value for Copper-63. If the values are significantly different (ie: the value of Copper-63 is significantly higher), report Copper-65.

*** Zinc-64and Zinc-66 will be analyzed. Zinc-64 is the more abundant element; however, it has interferences with Ni-64. If the values of Zinc-64 and Zinc-66 are comparable in the standard reference material, report the value for Zinc-64. If the values are significantly different (ie: the value of Zinc-64 is significantly higher), report Zinc-66.

Internal Standards

Internal Standard	Elements grouped with this Internal Standard
Sc-45	V, Cr(52,53), Mn, Co, Cu(63, 65), Zn(64,66)
Y-89	Mo, Cd, Ba
Tb-159	Pb

NOTE: The internal standard pairings can be changed if necessary. Document any deviations.

Glassware/Plasticware and other Consumables

1. 15 ml Metal Free Centrifuge tube or equivalent
   For example: VWR, purchased from VWR
   Part number: 89049-170
2. 50 ml Metal Free Centrifuge tub or equivalent
   For example: VWR, purchased from VWR
   Part number: 89049-174
3. 125 mL plastic disposable cups
   For example: Fisher Scientific brand, purchased from Fisher Scientific
   Part Number: NC9375658
4. Class A Reusable PMP volumetric flasks of various sizes (50mL, 100mL, 1L)
   For example: Corning 100ml, purchased from Fisher Scientific
   Part numbers: S02288D
5. Disposable Lab Spatulas
   For example: LevGo, Inc., purchased from LevGo
   Part number: 17221
6. Disposable plastic transfer pipettes
   For example: Thermo Scientific purchased from Fisher Scientific
   Part number: S30467-1
7. 125-mm 595 ½ Folded Ashless filters
   For example: GE Healthcare, purchased from Whatman
   Part number: 10311644
8. Plastic graduated cylinders (at least 100 mL)
   For example: 100ml Fisher brand, purchased from Fisher Scientific
   Part Number: 0300741
9. Pipettes and pipette tips of various sizes (10µL to 10 mL)
   For example: Eppendorf Research Pipette 1-10ml, purchased from Fisher Scientific
   Part number: 05-403-121
10. Plastic Funnels
    For example: Fisher Scientific brand, purchased from Fisher Scientific
    Part Number: 10-500

Contamination Control

1. Avoid the use of glassware. Glass vessels can have leachable metals that can contaminate the contents.
2. Avoid cross-contamination from other sampler or standards via test tubes, sampling tubes, etc.
3. Be aware of practices during sample handling that expose samples to risk of contamination and interferences.
4. Use plastic test tubes for samples; do not re-use plastic test tubes.

Acid Washing of Plastic and Glassware

NOTE: The use of glassware for this method is highly discouraged. When possible, use plasticware to prevent contamination.

Clean all glassware and plasticware (with the exception of conical tubes) using the dishwasher or by hand with detergent. Soak plasticware in 10% nitric acid overnight before cleaning if plastic ware is discolored from previous use.

1. Rinse plasticware with 1% nitric acid after washing or before use.
2. Discard all waste according to laboratory's protocol.
3. Plastic ware should be dry before use.
4. Nitric acid should be used under a fume hood.

Acid Washing Digestion Vessels, Plugs and Caps

Teflon vessels, stoppers and caps should be cleaned with a mild detergent by hand or in a laboratory washer. Soak vessels in 10% nitric acid overnight before cleaning if the vessels are discolored from previous use.

1. Under a fume hood, add 10mls of nitric acid to each vessel.
2. Place the vessels in the digestion microwave system with the stoppers installed and caps fully torqued.
3. Based on the number of vessels, select the appropriate method listed in the Optimized Microwave Digestion Program below.
4. Allow vessels to cool.
5. Vent vessels under a fume hood.
6. Store the vessels and caps on their side to dry. Drying vessels on their side dissipates gasses that would otherwise be trapped if the vessels were stored right side up or upside down. Vessels and caps can be dried also in an oven set to at least 75˚C.
7. Discard all waste according to laboratory's protocol.

Optimized Microwave Digestion Program

Method	Power (W)	Ramp Time (min)	Temperature(°C)	Hold Time (min)	Cool Down (min)
Tobacco Predigestion -Xpress	400	5	80	15	15
Tobacco Enforce-Xpress	1600	15	200	15	15
1-16 HNO_3 Wash	400	10	150	10	15
17-40 HNO_3 Wash	800	10	150	10	15

Reagents, Standards and Standard Reference Materials

1. Multi-element standard 1 containing 10 ppm (µg/mL) each: Ag, Al, As, B, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ho, K, La, Lu, Mg, Mn, Na, Nd, Ni, P, Pb, Pr, Rb, S, Se, Sm, Sr, Th, Tl, Tm, U, V, Yb, Zn
   For example: Inorganic Ventures, ICPMS Complete Standard, item IV-ICPMS-71A
2. Multi-element standard 2 containing 10 ppm (µg/mL) each: Ge, Hf, Mo, Nb, Sb, Si, Sn, Ta, Te, Ti, W, Zr
   For example: Inorganic Ventures, ICPMS Refractory Elements Standard, item IV-ICPMS-71B
3. Internal standard containing 20 ppm (µg/mL) Sc, Y, In, Tb, Bi
   For example: Inorganic Ventures, Internal Standard, item 2008ISS
4. Second source multi-element standard containing the same concentration and elements as the multi-element standards 1 and 2 but be purchased from a different vendor.
   For example: Spex CertiPrep, Claritas PPT Grade Multi-element Solution, item 2ACLMS-2A
5. Perkin Elmer Smart Tune Solution – Standard Elan and DRC-e, 10 µg/L Ba, Be, Ce, Co, In, Mg, Pb, Rh, and U in 1% HNO3, item N8125040
6. Nitric Acid (HNO_3) 70%, conc., Trace Metals Grade or better
   For example: Fisher Scientific, item NC9092386
7. Hydrogen Peroxide (H_2O_2) 30-32%
   For example: Fisher Scientific, item H323-500
8. Deionized water (18 MΩ or better)
9. Standard Reference Material
   For example: NIST, Tomato Leaves, item 1573a
10. Anhydrous magnesium perchlorate
    For example: Fisher Scientific, item M54-212

NOTE: Single elements can be purchased if needed. For example: Inorganic Ventures, Manganese, item number MSMN-10ppm

Preparation of Reagents and Standards

All plastic and glassware will be acid washed prior to use according to the procedure above. The use of plasticware is highly recommended. Store all solutions in plastic containers.

Reagents

1. **Laboratory Calibration Blank (8% HNO_3)** - Prepare 1L of a solution containing 8% nitric acid by adding 114mL of 70% concentrated trace metal grade nitric acid to about 500mL of deionized water contained in a 1L plastic volumetric flask. Fill flask to volume with deionized water and mix well. Prepare solution under a fume hood. Store solution at room temperature up to one year.
2. **Instrument Rinse / Sample Wash Solution (8% HNO_3)** - Prepare 1L of a solution containing 8% nitric acid by adding 114mL of 70% concentrated trace metal grade nitric acid to about 500mL of deionized water contained in a 1L plastic volumetric flask. Fill flask to volume with deionized water and mix well. Prepare solution under a fume hood. Store solution at room temperature up to one year.
3. **Cleaning Solution (**1% HNO_3**)** - Prepare 1 L of a solution containing 1% nitric acid by adding 14.25mL of 70% concentrated trace metal grade nitric acid to about 500mL of deionized water contained in a 1L plastic volumetric flask. Fill flask to volume with deionized water and mix well. Prepare solution under a fume hood. Store solution at room temperature up to one year.

200 µg/L Internal Standard Solution

Scandium 45, yttrium 89, indium 115 and terbium 159 are used as internal standard elements in this method. Prepare 1 L of a solution by pipetting 10mL of 2008ISS into a 1 L plastic volumetric flask. Fill flask to volume with 8% nitric acid and mix well. Prepare solution under a fume hood. Internal standard can be stored at room temperature for up to three months. The internal standard will be added on-line using a multi-channel peristaltic pump equipped with a mixing block or equivalent.

Calibration Standards

Multi-element standards 1 and 2 are used to prepare the calibration standards. The table below is an example of how to prepare the calibration standards. Pipette the volume of multi-element standards 1 and 2 into 50-mL plastic volumetric flasks. Fill each flask to the final volume of 50 mL with 8% nitric acid solution and mix well. Standards and QCs should be stored at room temperature. Prepare as needed. Store all standards in plastic containers. The shelf life is 3 months.

Standard Level	Multi-element standard 1 (volume added in µL)	Multi-element standard 2 (volume added in µL)	Final Concentration (µg/L)	Final Volume (mL)
1	5	5	1	50
2	50	50	10	50
3	250	250	50	50
4	500	500	100	50
5	1000	1000	200	50
6	5000	--	1000	50
7	10000	--	2000	50

NOTE: Cu, Zn, and Ba have a linear range up to 1,000 µg/L. Therefor single element standards can be used to make the 1,000 µg/L standard level 6 to prolong the life of the detector. Mn has a linear range up to 2,000 µg/L. Therefor a single element solution containing only Mn can be added to the 1,000 µg/L standard level 6 and used to prepare the 2,000 µg/L standard level 7.

Calibration Curve

Standard Level	Concentration (µg/L)	Element
1	1	V, Co, Pb, Cd
2	10	V, Cr, Co, Cu, Zn, Mo, Cd, Ba, Pb
3	50	V, Cr, Mn, Co, Cu, Zn, Mo, Cd, Ba, Pb
4	100	V, Cr, Mn, Co, Cu, Zn, Mo, Cd, Ba, Pb
5	200	Cr, Mn, Cu, Zn, Mo, Cd, Ba
6	1000	Mn, Cu, Zn, Ba
7	2000	Mn

Calibration Check

An example of how this standard can be prepared is by pipetting the volume of multi-element standards 1 and 2 (from a second source such as Perkin Elmer Internal Standard Mix item number N9303832, and Multi-element Calibration Standard item number N9300233) into a 50mL plastic volumetric flask. Fill each flask to the final volume specified in the table below with 8% nitric acid solution and mix well. Standards and QCs should be stored at room temperature. Prepare as needed. Store all standards in plastic containers. The shelf life is 3 months. ***Bracket every nine to ten samples with the analysis of the quality control standard.***

QC	Final Concentration (µg/L)	Multi-element standard 1 (added in µL)	Multi-element standard 2 (added in µL)	Final Volume (mL)
1	100	500	500	50

Quality Control Samples

1. **Laboratory Reagent Blank (LRB)** - Prepare under a fume hood by adding 4mL of concentrated HNO3 to a microwave digestion vessels followed by the addition of 2mL of 30-32% H2O2 to the vessels. Digest LRB along with the samples to be analyzed. ***At least one LRB must be analyzed with each batch of samples with less than twenty samples or one per twenty samples.***

2. **Laboratory Fortified Blank (LFB)** - Digest LFB along with the samples to be analyzed. As an example the LFB can be prepared by pipetting the volume of ICPMS-71A 10µg/ml multi-element solution and ICPMS-71B 10µg/ml multi-element solution listed in the table below into a microwave digestion vessel. Add 4mL of concentrated HNO3 to the vessels then 2mL of 30-32% H2O2. ***At least one of each LFB must be prepared and analyzed with each batch of samples.***

LFB	ICPMS 71A (added in µL)	ICPMS 71B (added in µL)	Final Concentration (µg/L)	Final Volume (mL)
1	100	100	20	50
2	250	250	50	50

Laboratory Control Standard (LCS)

Certified Tomato Leaf (NIST-SRM 1573a). Refer to instruction provided by NIST for conditioning. The SRM 1573a should be prepared for analysis using the same digestion method that is used for the sample preparation. ***The SRM 1573A must be analyzed at least two times with each batch.***

Preparation of Samples

To perform an ICP-MS measurement, the tobacco sample must be ground to a powder (see Equipment list above) then dissolved prior to analysis. Therefore, samples are digested in nitric acid by closed-vessel microwave digestion using the method below.

1. Samples must be ground to a powder if it is not in powder form.
2. Make sure vessels have been acid washed before use.
3. Prepare a minimum of eight vessels
4. Place vessel into the balance. Tare the weight of the vessel.
5. Add at least 0.25 to 0.5 grams of sample to the vessel then record the weight in a laboratory notebook.
6. Under a fume hood, add 4mL of concentrated HNO_3 to the vessel. Allow acid to settle.
7. Add 2mL of 30-32% H_2O_2 to the vessel. Allow oxidizer to settle.
8. Use 1 to 2mls of deionized water to remove any sample that sticks to the walls of the vessel.
9. Make sure the oven is plugged in, vent is firmly attached and the drainage tube from the microwave is in the waste container.
10. Turn on microwave using the black switch located on the right side of the microwave (see the CEM MARSXpress user manual for additional information).
11. Place the vessels in the microwave system, (with the stoppers installed and the caps fully torqued), and run the Tobacco Predigestion-Xpress method (see Optimize Microwave Digestion Program above).
12. Allow vessels to cool to room temperature.
13. Run the Tobacco Enforce-Xpress method (see Optimize Microwave Digestion Program above).
14. Remove the samples from the microwave.
15. Turn off the microwave using the switch on the right side of the microwave (CEM MARSXpress).
16. Place vessels under a fume hood. Allow vessels to cool to room temperature. Vessels can be placed in a -20°C freezer for about 10 minutes to speed up the process.
17. Vent the vessels under a fume hood by carefully unscrewing the vessel's cap.
18. Allow fumes to be released from the vessels.

19. Transfer the digested sample to a plastic 50 ml volumetric flask.
20. Rinse vessel with deionized water.
21. Transfer rinse to plastic volumetric flask containing sample.
22. Repeat steps 20 and 21.
23. Fill flask to volume with deionized water
24. Cap flask then mix well.
25. Centrifuge samples (3,500 rpm for 15 to 20 minutes) or let the samples sit at room temperature until the samples are clear (samples might be yellow, orange or green in color because of the nitric acid or presence of silicone. This is fine as long as the samples are clear)
26. Filter samples with ashless filters if sample is not clear.

NOTE: Samples, standards, QCs, LRB and LFBs can be prepared by weight (see Appendix)

Procedures

The instrument should be optimized as per vendor instructions and/or internal guidelines.
After analysis, allow the flow of 8% nitric acid rinse / wash solution to continue for at least 15 minutes to clean the system.

Example Sequence Table

On the Perkin-Elmer Autosampler: Positions 1-8: use 50 mL conical tubes; Positions 9 and up: use 15 mL conical tubes

Example Sequence

Sequence Line	*Sample Position*	*Sample Description*	*Sample Type*
1	1	Sample Wash	Sample
1	1	Sample Wash	Sample
2	55	Calibration Blank	Blank
3	**2**	**1 µg/L**	**Standard**
4	**3**	**10 µg/L**	**Standard**
5	**4**	**50 µg/L**	**Standard**
6	**5**	**100 µg/L**	**Standard**
7	**6**	**200 µg/L**	**Standard**
8	**7**	**1000 µg/L**	**Standard**
9	**8**	**2000 µg/L**	**Standard**
10	1	Sample Wash	Sample
11	**51**	**20 µg/L LFB**	Sample
12	1	Sample Wash	Sample
13	**52**	**50 µg/L LFB**	Sample
14	1	Sample Wash	Sample
15	**53**	**1573 SRM**	Sample
16	*9*	*Sample 1*	Sample
17	*10*	*Sample 2*	Sample
18	*11*	*Sample 3*	Sample
19	1	Sample Wash	Sample

20	*12*	*Sample 4*	Sample
21	*13*	*Sample 5*	Sample
22	*14*	*Sample 6*	Sample
23	1	Sample Wash	Sample
24	*15*	*Sample 7*	Sample
25	*16*	*Sample 8*	Sample
26	*17*	*Sample 9*	Sample
27	1	Sample Wash	Sample
28	**54**	**100 µg/L**	Sample
29	**55**	**1573 SRM**	Sample
30	**50**	**LRB**	Sample

1. At least one **LRB** must be analyzed with each batch of samples with less than twenty samples or one per twenty samples.
2. At least one of each **LFB** must be prepared and analyzed with each batch of samples. LFBs can be analyzed after every ten samples for additional quality control confirmation.
3. **SRM 1573A** must be prepared like a sample with each batch and analyzed in duplicate.
4. Bracket every nine to ten samples (not including sample wash, LFB and SRM) with the analysis of the Calibration Check (**100 µg/L quality control)** standard.
5. Run a sample wash at least once after every three samples.

Quality Controls

1. **Calibration Curve** - Each analyte in the calibration curve should have a concentration that is within 90-110% of the theoretical value. If a point is not within range it should be prepared over again.
2. **Internal Standard** - When using online internal standardization, drift of the internal standard may be from the wear of the internal standard tubing. Therefore, internal standard tubing should be changed daily. The internal standard intensity should not drift by more than +/- 40% of the original intensity in the LCB analyzed at the beginning of the run. Samples with drift more than +/- 40% should be analyzed again. See validation report for other alternatives.
3. **LRB** – Data for an analyte in a sample cannot be reported if the concentration in LRB for that analyte(s), bracketing the sample, is 2.2 times the analyte' s limit of quantitation. This is an indication of contamination for that analyte. The results for the remaining analytes can be reported if no signs of contamination can be detected in the LRB. A fresh aliquot of sample must be analyzed if the data for that analyte is needed. The source of the contamination must be determined if the contamination is detected after analyzing the fresh aliquot of samples. If it is decided that the data for that analyte is not needed for that run then the source of the contamination must be determined before analyzing samples thereafter.
4. **LFBs** - Calculate accuracy as percent recovery. Date for an analyte(s) outside the applicable LFB control limit of 85-115% cannot be reported. The run must be repeated if the result for that analyte is needed. An investigation must be carried out to identify the source of the problem if the analyte(s) is out of control after repeating the analysis. If it is decided that the data for that analyte is not needed for that run then the source of the problem must be determined before analyzing samples thereafter. The limit of quantitation for Mn is above 20µg/L. Therefor the 20 µg/L LFB does not apply to Mn.

5. **LCS** - The concentration for the certified analytes should be within the limit of 85-115%. The first SRM sample can be analyzed again if the discovery of the failure is before the analysis of any samples. The run should be repeated if not.
6. **Calibration Check** – Each analyte in the calibration check bracketing samples should have concentrations that are within 85-115% of the theoretical value for the analyte. The instrument should be recalibrated and the samples within the bracket of the calibration check that is out of calibration should be reanalyzed if this is not the case.

Sources of Uncertainty

- Wearing of the internal standard tubing
- Venting of vessels while digesting
- Sample lack of homogeneity
- Environmental temperature variations
- Volumetric pipettes
- Analytical Balance

Calculations

1. LFB % Recovery: $R=[(LFB-LRB)/s]*100$

   R=percent recovery
   LFB = mean conc. generated by the standard curve for an analyte in the LFB
   LRB = background (possible interference) concentration detected for that same analyte in the LRB
   S=concentration equivalent of analyte added to fortify the LRB

2. SRM % Recovery: $R=(SC/CV)*100$

   R=percent recovery
   SC= mean conc. for the analyte generated by the standard curve
   CV=known certified value for the analyte

3. Correlation(r) $r=[N\Sigma XY - (\Sigma X)(\Sigma Y) / Sqrt([N\Sigma X^2 - (\Sigma X)^2][N\Sigma Y^2 - (\Sigma Y)^2])]$

   N = number of values or elements
   X = first Score
   Y = second score
   ΣXY = sum of the product of first and second scores
   ΣX = sum of first scores
   ΣY = sum of second scores
   ΣX^2 = sum of square first scores
   ΣY^2 = sum of square second scores

Reporting Results

1. Values should be reported to the nearest whole number (X ppb or X µg/L).
2. If a data set is reprocessed with edits to the original acquisition method, the method used for reprocessing should be saved. The original acquisition method should not be overwritten.

Safety Notes

Concentrated nitric acid and hydrogen peroxide present various hazards including moderate toxicity and extreme irritation to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield, protective clothing and gloves when working with these reagents. All personnel should be familiar with the laboratory's Chemical Hygiene Plan (CHP). Refer to the Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) that apply for each procedure.

References

1. U.S. Dept. of Health and Human Services: How Tobacco Smoke Causes Disease: The Biological and Behavioral Basis for Smoking - Attributable Diseases. A Report of the Surgeon General, 2010
2. EPA Method 200.8-Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma - Mass Spectrometry, Revision 5.4, EMMC Version.
3. 40 CFR Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11
4. Swami, K.; Judd, C. D.; Orsini, J. Trace Metals Analysis of Legal and Counterfeit Cigarette Tobacco Samples Using Inductively Coupled Plasma Mass Spectrometry and Cold Vapor Atomic Absorption Spectrometry. Spectroscopy Letters, 2009, Volume 42, Pages 479-490.
5. Perkin Elmer Inductively Coupled Plasma Mass Spectrometry with ELAN Software, Rev. C., Appendix A
6. Rechcigl, J.E.; Payne, G.G. Comparison of a Microwave Digestion System to Other Digestion Methods for Plant Tissue Analysis. Poster Session. Annual Meeting of American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, 1989, Las Vegas.
7. Castro, J.; Spraul J.C.; Marcus, R. K. Metal Analysis of Botanical Products in Various Matrices Using a Single Microwave Digestion and Inductively Coupled Plasma Optical Emission Spectrometry Method. The Royal Society of Chemistry, 2009, Pages 188-194
8. Thomas, R. 2008. Practical Guide to ICP-MS: A Tutorial for Beginners. CRC Press, New York
9. Sivakumar, V.; Ernyei L.; Obenauf R. H. Guide for Determining ICP/ICP-MS Method Detection Limits and Instrument Performance. The Application Notebook, Spex CertiPrep, Inc., September 2006, Page 13.
10. Stephens, W. E.; Calder, A.; Newton, J. Source and Health Implications of High Toxic Metal Concentrations in Illicit Tobacco Products. Environ. Sci. Technol. 2005, 39, Pages 479-488.
11. Laboratory Quality Management Services. National Association of Testing Authorities (NATA) Requirements for Accreditation of ICP-MS Techniques. Retrieved March 2011 from website: http://www.lqms.com.au/information/resources/
12. Gaines, P. PhD. Method Validation. Trace Analysis Guide: Part 17. Retrieved August 8, 2011 from website: http://inorganicventures.com/tech/trace-analysis/method-validation
13. CEM. Food, Plant and Animal Tissue, Application Note for Acid Digestion, Retrieved March 2011 from website: http://www.cem.com/downloads366.html
14. Huber, L. Tutorial-Validation of Analytical Methods and Procedures, Located on website: http://www.labcompliance.com/tutorial/methods/default.aspx#08_paramters
15. National Institute of Standards and Technology. Certificate of Analysis, Standard Reference Material 1573A Tomato Leaves. November 22, 1995, Revision October 19, 1993

16. Method Detection Limit MDL by Hubaux and Vos method http://www.chemiasoft.com/chemd/node/59
17. Matrix effects of the elements on the sensitivity of inductived plasma coupled mass spectrometry measurements, http://iramis.cea.fr/en/Phocea/Vie_des_labos/Ast/ast_visu.php?id_ast=884

Required Training, Certification, and Re-certification

1. In-house training by a certified chemist in the theory and operation of ICPMS, including software, maintenance, and troubleshooting.
2. Analyst has demonstrated competency after successfully obtaining 7 replicates of an unknown within the QC acceptance limits.
3. Chemists will be recertified periodically using proficiency test results and/or re-demonstration of competency.

Revision History

Rev. 1 – Initial revision

Appendix A

Definitions

The following definitions are taken from the Environmental Protection Agency Method 200.8 and Perkin Elmer Inductively Coupled Plasma Mass Spectrometry with ELAN Software, Rev. C.

1. **Calibration Check** - Is used to verify that the instrument is still in calibration. It should come from a secondary source.
2. **Calibration Curve** - The instrument is calibrated using a calibration curve. The correlation coefficient ($R^{2)}$ of calibration curves should be 0.995 or greater.
3. **Cleaning Solution** - A solution that is used to clean the cones.
4. **Instrument Rinse / Sample Wash Solution** - A solution used in between injections as needed to prevent carryover from one sample to the next. It is also used to clean the system.
5. **Internal Standard Solution** (IntStd) - A solution used to compensate for matrix effect[], signal drift and some interferences. The analytes are selected to match the mass range used in the method.
6. **Isobaric Overlaps**- A spectral interference where one or more atom or element having the same atomic weight or mass number as the analyte of interest.
7. **Laboratory Calibration Blank (LCB)** - A volume of reagent water acidified with the same acid matrix as in the calibration standards. It is analyzed at the beginning of each sequence. The calibration blank is a zero standard and is used to calibrate the ICP. Any signals found in the calibration blank analyzed before the standard curve is subtracted out by the software after the analysis of the standard curve.
8. **Laboratory Fortified Blank (LFB)** - An aliquot of Laboratory Reagent Blank to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.
9. **Laboratory Reagent Blank (LRB)** - An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.
10. **Polyatomic Overlaps**- A spectral interference produced by the combination of two or more atomic ions
11. **Standard Reference Material (SRM)** - It is accompanied by documentation issued by an authoritative body using valid certification procedures and providing specified values for specified properties with associated uncertainties and with specified traceability. Can be employed for cross-checking accuracy and recovery of a method when prepared and analyzed exactly as a sample
12. **Tuning Solution** - A solution which is used to determine acceptable instrument performance prior to calibration and sample analyses

Appendix B

An alternative method for preparing standards, QCs and samples is by weight. See examples below.

Calibration Standards

Prepare each level of the calibration standards listed in the table by weighing the amount of ICPMS-71A 10µg/ml multi-element solution and ICPMS-71B 10µg/ml multi-element solution into a tarred 50mL conical tube. Fill each conical tube to 50 grams (± 0.05) with 8% HNO_3 and mix well. Standards and QCs can be stored at room temperature.

Standard Level	Final Concentration (µg/L)	ICPMS-71A (Grams)	ICPMS-71B (Grams)	Final weight (Grams)
1	1	0.0050 ± 0.0001	0.0050 ± 0.0001	50 ± 0.05
2	10	0.0500 ± 0.0005	0.0500 ± 0.0005	50 ± 0.05
3	50	0.2500 ± 0.0020	0.2500 ± 0.0020	50 ± 0.05
4	100	0.5000 ± 0.0050	0.5000 ± 0.0050	50 ± 0.05
5	200	1.0000 ± 0.0050	1.0000 ± 0.0050	50 ± 0.05
6	1000	5.0000 ± 0.0500	5.0000 ± 0.0500	50 ± 0.05
7	2000	10.0000 ± 0.0500	10.0000 ± 0.0500	50 ± 0.05

NOTE: Cu, Zn, and Ba have a linear range up to 1,000 (µg/L). Therefore single element standards can be used to make the 1,000 (µg/L) standard level 6 to prolong the life of the detector. Mn is the solution containing only Mn can be added to the 1,000 (µg/L) standard level 6 and to prepare the 2,000 (µg/L) standard level 7 to prolong the life of the detector.

Calibration Check

An example of how to prepare the calibration check is to weigh the amount of ICPMS-71A 10µg/ml multi-element solution and ICPMS-71B 10µg/ml multi-element solution listed in the table below into a tarred 50mL conical tube. Fill each conical tube to 50 grams (± 0.05) with 8% HNO_3 and mix well. Standards and QCs can be stored at room temperature.

QC	Final Concentration (µg/L)	ICPMS-71A (Grams)	ICPMS-71B (Grams)	Final Weight (Grams)
1	100	0.5000 ± 0.0050	0.5000 ± 0.0050	50 ± 0.05

Quality Control Samples

1. LRB is prepared, under a fume hood, by adding 4mL of concentrated HNO3 to a microwave digestion vessels followed by the addition of 2mL of 30-32% H2O2. Digest LRB along with the samples to be analyzed.
2. Prepare each LFB listed in the table below by weighing the amount of ICPMS-71A 10µg/ml multi-element solution and ICPMS-71B 10µg/ml multi-element solution into a microwave digestion vessel. Under a fume hood, add 4mL of concentrated HNO_3 to the vessels. Add 2mL of 30-32% H_2O_2 to the vessels. Digest LFB along with the samples to be analyzed.

LFB	Final Concentration (µg/L)	ICPMS-71A (Grams)	ICPMS-71B (Grams)	Final Volume (Grams)
1	20	0.1000 ± 0.0005	0.1000 ± 0.0005	50 ± 0.05
2	50	0.2500 ± 0.0020	0.2500 ± 0.0020	50 ± 0.05

Laboratory Control Standard (LCS)

Certified Tomato Leaf (NIST-SRM 1573a). Refer to instruction provided by NIST for conditioning. The SRM 1573a should be prepared for analysis using the same digestion method that is used for the sample preparation. ***The SRM 1573A must be analyzed at least two times with each batch.***

Sample preparation

To perform an ICP-MS measurement, the tobacco sample must be dissolved prior to analysis. Therefore, samples are digested in nitric acid by closed-vessel microwave digestion using the method below.

1. Samples must be ground to a powder if it is not in powder form.
2. Make sure vessels have been acid washed before use
3. Prepare a minimum of eight vessels
4. Place vessel into the balance. Tare the weight of the vessel.
5. Add at least 0.25 to 0.5 grams of sample to the vessel then record the weight.
6. Under a fume hood, add 4mL of concentrated HNO_3 to the vessels. Allow acid to settle
7. Add 2mL of 30-32% H_2O_2 to the vessels. Allow oxidizer to settle.
8. Use 1 to 2mls of deionized water to remove any sample that sticks to the walls of the vessel.
9. Make sure the oven is plugged in, vent is firmly attached and the drainage tube from the microwave is in the waste container.
10. Turn on microwave using the black switch located on the right side of the microwave.
11. Place the vessels in the microwave system, (with the stoppers installed and the caps fully torque),
12. Run the Tobacco Predigestion-Xpress method (see microwave digestion program table above).
13. Allow vessels to cool to room temperature.
14. Run the Tobacco Enforce-Xpress method (see microwave digestion program table above).
15. Remove the samples from the microwave.
16. Turn off the microwave using the switch on the right side of the microwave.
17. Place vessels under a fume hood. Allow vessels to cool to room temperature. Vessels can be placed in a -20°C freezer for about 10 minutes to speed up the process.
18. Vent the vessels under a fume hood by carefully unscrewing the vessel's cap.
19. Allow fumes to be released from the vessels.
20. Place a 50 ml conical tube into the balance. Tare the weight of the tube.
21. Transfer the digested sample to the tube. Allow the balance to stabilize.
22. Rinse vessel with deionized water two time.
23. Transfer rinse to the tube. Allow the balance to stabilize.
24. Add enough diH_2O to the sample until the final weight is 50 grams ± 0.50 grams.
25. Cap tube then mix well.

26. Centrifuge the samples (3,500 rpm for 15 to 20 minutes) or let samples sit at room temperature until they are clear (the samples might be yellow, orange or green in color because of the nitric acid or presence of silicone. This is fine as long as the samples are clear)
27. Filter samples with ashless filters if sample is not clear.