CONFIDENTIAL

UK SMOKE CONSTITUENTS STUDY

Part 11: Determination of Metals Yields in Cigarette Smoke

Annex A - ICP-MS method

Commissioned by:

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DEFINITIONS

The acronyms used in this procedure are listed and defined below.

(v/v)	Volume/Volume
1R4F	Industry Reference Cigarette produced by the University of Kentucky
Cal	Calibration
Cigt.	Cigarette
Clean Lab	Metal free sample preparation lab
Conc	Concentration
EP	Electrostatic Precipitation
HNO_3	Nitric Acid
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ISO	International Standards Organisation
LOD	Limits of Detection
LOQ	Limits of Quantitation
MeOH	Methanol
MS	Mainstream Smoke
MSDS	Material Safety Data Sheet
N	Number of Replicates
P.E.	Perkin Elmer
QC	Quality Control
RE	Relative Error
RSD	Relative Standard Deviation
SD	Standard Deviation

DETERMINATION OF SELECTED METALS IN MAINSTREAM SMOKE

I. PURPOSE/SCOPE

Metals are a known component of mainstream cigarette smoke. This method describes the procedure for the determination of metals in mainstream cigarette smoke. Note that the term metals in this SOP, refers to the following: Arsenic, Cadmium, Chromium, Lead, Nickel, Palladium and Selenium. The method for mercury is described in another document.

II. PRINCIPLE OF METHOD

The metals are quantitated from mainstream smoke collected from twenty cigarettes smoked under International standards Organisation (ISO) conditions. Smoking is performed on a Borgwaldt RM 20 CSR analytical smoking machine equipped with an electrostatic precipitation (EP) unit. A quartz EP tube is used in the EP unit for smoke collection.

This method describes the multi-element determination of selected trace metals by ICP-MS. The ICP-MS used in the method is a Perkin Elmer ICP-MS equipped with a dynamic reaction cell that allows for the determination of elements, including Chromium and Selenium, which normally suffer from severe polyatomic interferences. Ammonia and methane are used as the reaction cell gas for chromium and selenium, respectively. Samples are introduced by nebulization into a radio frequency plasma where energy transfer processes cause desolvation, atomization, and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and detected on the basis of their mass to charge ratio by a quadrupole mass spectrometer. The ions transmitted through the quadrupole are detected by an electron multiplier and the ELAN software processes the ion information. Instrumental drift is corrected for by the use of indium and gallium as internal standards, which are added online.

The metals are reported in units of mass-to-volume (ng/mL). The measured concentration, the number of cigarettes smoked, and the sample solution volume(s) are also used to calculate the total analyte mass on a per cigarette basis. The measured concentration of the metals and the number of cigarettes smoked are used to calculate the concentration of each element in units of nanograms per cigarette (ng/cigt).

III. APPARATUS, CHEMICALS, AND LABORATORY SUPPLIES

A. Required Chemicals

Chemical	Supplier	Grade or Purity
Aqueous Single Element Standard Stock Solutions 1000μg/mL As, Cd, Cr, Ga, In, Ni, Pb,. Pd, and Se	SPEX Certiprep Inc. (or similar)	Trace ICP/ICP-MS grade
Quality Control Standard 21	SPEX Certiprep Inc. Cat# CL-QC-21 (or similar)	Trace ICP/ICP-MS grade
Methanol	Burdick and Jackson NP230-10 (or similar)	High purity
Nitric Acid	Fisher Scientific A 467-1 (or similar)	High Purity, Optima Grade
Hydrogen Peroxide, 30%	J.T. Baker 7722-84-1 (or similar)	ULTREX II Ultrapure (Ultrex is also acceptable)
Compressed Methane Gas	Air Products CAS NO 74-82-8	99.999% purity
Ammonia, Anhydrous	Scott Specialty Gases, Inc. CAS NO 7664-41-7 (or similar)	99.999% purity
Type 1 Water		

B. Laboratory Apparatus and Supplies

- Perkin Elmer ICP-MS, ELAN 6100 DRC
- Fisherbrand peristaltic pump tubing, black, size 0.030 inch ID, Cat # 14-190-106
- Fisherbrand pump tubing, orange/green, size 0.015 inch ID, Cat # 14-190-103
- Borgwaldt RM 20/CSR smoking machine
- Electrostatic precipitation unit
- Pure Tungsten electrode, 1/16” diameter
- 10-100μL micropipette
- 100 – 1000μL micropipette
- 1-10mL macropipette
- HPLC grade water or water system capable of delivering 18 MΩ-cm water
- Keck Clips No. 29, Fisher Scientific, Cat. No. 05-880F
- Parafilm
- Nitrile gloves
- Quartz EP tubes and end caps
- 100mL polypropylene volumetric flasks
- 1000mL polypropylene volumetric flasks
- 50mL polypropylene centrifuge tubes
- 15mL polypropylene centrifuge tubes
- Temperature programmable digestion block
- 44mm Ribbed polypropylene watch glass, Environmental Express, Cat#SC505, or similar
- 68ml polypropylene graduated digestion vessels, Environmental Express, Cat#SC475, or similar

NOTE: All labware and EP tubes should be pre-cleaned before use by soaking a minimum of 24 hours in 5% nitric acid (aqueous). After soaking, rinse thoroughly with Type I water. Dry in the clean bench and store in plastic bags until needed.

IV. PREPARATION OF ANALYTICAL SOLUTIONS

A. Calibration Dilute Stock Standard I

See Appendix A

B. Calibration Dilute Stock Standard II

See Appendix A

C. Calibration Standards

See Appendix A

D. Internal Standard

See Appendix A

V. PREPARATION OF QUALITY CONTROL SOLUTIONS

A. Quality Control Dilute Stock

See Appendix B

B. Quality Control Dilute Stock II

See Appendix B

VI. SAMPLE COLLECTION AND WORKUP

Authors Comment – conditions for smoke generation and collection are described elsewhere – a summary is reproduced below.

- Cigarettes are conditioned[1] at a temperature of 22 ± 1°C and 60 ± 3 % relative humidity for a minimum of 48 hours but not exceeding 10 days.
- Butt marking will be ISO butt length specifications[2]. Filtered cigarettes will be smoked to a measured butt length equal to either the tipping paper + 3 mm or filter length + 8 mm whichever is longer. The minimum butt length will be 23 mm and this will also be used for non filter brands. All smoking shall be conducted in an environment of temperature 22 ± 2°C and 60 ± 5 % relative humidity[1].
- ISO conditions[3] for smoking cigarettes will apply. The smoking machine puffing parameters will be 35 ± 0.2 cm^3 puff volume with 2.0 ± 0.05 second puff duration once every 60.0 ± 0.5 seconds.
- A minimum of five determinations will be performed for each brand. The smoking of the cigarette brands is randomised so that samples from the same brand are smoked on different days.
- With each batch of samples a 2R4F cigarette is smoked.

A flow diagram of the sample collection and workup procedure is given in Appendix D.

[1] ISO 3402: 2000 - Tobacco and tobacco products – atmosphere for conditioning and testing
[2] ISO :4387: 2000 - Methods for chemical analysis of tobacco and tobacco products – Determination of total and nicotine- free dry particulate matter using a routine analytical smoking machine
[3] ISO 3308:2000 – Routine analytical cigarette smoking machine – 1: Definitions and standard conditions

For mainstream smoke collection, cigarette smoke is collected in a quartz EP tube with a 1/16 inch diameter needle type tungsten electrode and the sample is subsequently analyzed by ICP-MS. Each mainstream sample will consist of the smoke collected from twenty cigarettes smoked under FTC conditions, unless otherwise specified. The sample quantity for product cigarettes is defined in the study-specific Cigarette Product Testing Protocol.

Before and during the smoking process, care should be taken to not touch the ground glass ends or the inside of the EP tubes. Care should also be taken not to contaminate the EP caps by setting them on laboratory surfaces. When the caps are removed from EP tubes, they should be placed in a clean plastic container that has been prepped in the clean room. Also note that before smoking the ends of the EP tubes should be carefully wrapped in Parafilm to avoid contamination from the smoking machine. In the clean room, and wearing clean (new) gloves, carefully wrap the ends of all EP tubes using Parafilm. The Parafilm should cover the ends or tips of the EP tube.

See Appendix C for detailed instructions on collecting samples with the electrostatic precipitation unit.

VII. PROCEDURE

A. Sample Preparation

NOTE: All sample prep work must be performed in the clean lab.

1. Clip one end of the EP tube using the Keck clips.
2. Remove the cap at the other end of the EP tube.
3. Wash down the inside of the EP tube with approximately 25mL of high purity MeOH using the appropriate Nalgene squirt bottle.
4. Replace the cap on the EP tube and clip it using one of the Keck clips.
5. Carefully shake the EP tube to remove all the smoke condensate from the sides.
6. Remove the Keck clips and transfer the contents of the EP tube into a 60mL polypropylene digestion vessel.
7. Carefully rinse down the insides of the EP tube into the digestion vessel to remove any remaining condensate.
8. Using a micropipette, add 1mL of 50% nitric acid to each sample.
9. Place a watch glass on each sample.
10. Place the samples into the digestion block.
11. Reduce to a final volume of approximately 2mL at 82°C.
12. Remove the samples from the digestion block and reduce the temperature of the Digestion block to 70°C.
13. Cautiously add 5mL of 50% nitric acid to each sample, 1mL at a time.
14. Place the samples back into the digestion block.
15. Heat samples at 70°C for one hour.
16. Increase the temperature of the digestion block to 95°C for 30 minutes and reduce the samples to approximately 2mL.
17. Remove the samples from the digestion block.
18. Carefully add 5mL of concentrated nitric acid to each sample.
19. Place the samples back into the digestion block.
20. Heat the samples at 95°C for 30 minutes or until any reaction subsides.
21. Remove the samples from the digestion block.
22. Let the samples cool completely and set the digestion block to 75°C.
23. Add 1mL of 30% hydrogen peroxide to each sample.
24. Cautiously heat the samples at 75°C for 5-10 minutes.
25. Remove the samples from the digestion block.
26. Add 2mL of 30% hydrogen peroxide to each sample.
27. Heat at 75°C until any reaction subsides.
28. Increase the temperature of the digestion block to 95°C.

29. Heat the samples at 95°C for 30 minutes.
30. Remove the samples from the digestion block and allow them to cool completely.
31. Make each sample up to 50mL with Type I Water.
32. Cap and shake each sample.
33. Samples are now ready for analysis by ICP-MS.

VIII. INSTRUMENT ANALYSIS

NOTE: Before analysis can begin, the instrument must be calibrated by utilizing the calibration capabilities of the ELAN software and standard solutions.

Sample Run Order

Sampling analysis order is as follows:

1) QC = Quality Control Standard
2) Samples 1 – 10
3) QC = Quality Control Standard

The analysis order will be designed so that an equal number of batched samples in groups of 10 or less be analyzed and bracketed by calibration check standards.

Table X.I Operating Parameters of the P.E. Elan 6100 DRC

ICP RF Power	1200 W				
Nebulizer Gas Flow	0.91 L/min				
Number of Sweeps	15				
Number of Readings	1				
Number of Replicates	2				
Sample Flush Time	70 sec				
Sample Flush Speed	30 RPM				
Read Delay Time	20 sec				
Read Delay Speed	16 RPM				
Read Speed	16 RPM				
Wash Time	120 sec		**DRC Gas Used for Analysis**	**DRC Gas Flow Rate**	
Wash Speed	40 RPM				**RPQ**
Dwell Time	Ga 69 *	70 (ms)	NA		0.25
	Cr 52	500 (ms)	Ammonia	0.3 L/min	0.45
	Cr 53	500 (ms)	Ammonia	0.3 L/min	0.45
	In115 *	70 (ms)	NA		0.25
	Se 78	500 (ms)	Methane	0.65 L/min	0.45
	As 75	70 (ms)	NA		0.25
	Ni 60	70 (ms)	NA		0.25
	Ni 62	70 (ms)	NA		0.25
	Pd 105	70 (ms)	NA		0.25
	Pb 206	70 (ms)	NA		0.25
	Pb 208	70 (ms)	NA		0.25
	Cd 111	70 (ms)	NA		0.25
	Cd 114	70 (ms)	NA		0.25

*Note: Ga 69 is added online as the internal standard for Chromium.
In 115 is added online as the internal standard for Selenium, Arsenic, Nickel, Palladium, Lead, and Cadmium.

IX. Data Reduction and Example Calculations

This section describes the method used for determining the amount of metals present on a per cigarette basis.

A. Instrument Calibration Calculations

The analysis is carried out using the calibration capabilities of the ELAN software and assumes the operator is already familiar with the procedure for setting up a calibration on the software.

B. Calibration Run Order:

1) Calibration Blank = Reagent blank used for background correction
2) Standard 1
3) Standard 2
4) Standard 3
5) Standard 4
4) Standard 5
5) Standard 6
6) Calibration Blank
7) QC = Quality Control standard
8) Standard 1 treated as a sample

The following table lists the concentrations of the calibration standards.

Table X.2: Calibration Standards

Analyte	Mass	Curve Type	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Units
Ga	69	simple linear							ng/mL
Cr	52	simple linear	0.2	1	5	10	25		ng/mL
Cr	53	simple linear		1	5	10	25		ng/mL
Se	78	simple linear	0.2	1	5	10	25		ng/mL
In	115	simple linear							ng/mL
As	75	simple linear	0.2	1	5	10	25		ng/mL
Ni	60	simple linear	0.2	1	5	10	25		ng/mL
Ni	62	simple linear	0.2	1	5	10	25		ng/mL
Pd	105	simple linear		1	5	10	25		ng/mL
Pb	206	simple linear	0.2	1	5	10	25		ng/mL
Pb	208	simple linear	0.2	1	5	10	25		ng/mL
Cd	111	simple linear	0.2	1	5	10	25	50	ng/mL
Cd	114	simple linear	0.2	1	5	10	25	50	ng/mL

C . Data Reduction of Raw Data from ICP-MS

The analyte concentration is determined by the calibration using the regression equation derived from the calibration curve. Calculation of the analyte is obtained by using the calculation capabilities of the ELAN software.

D. Example Calculation of a mainstream 1R4F sample:

Example calculation:
Mainstream Cigarette Sample Concentration

20 cigarettes were smoked and the final volume after digestion is 50mL. The concentration of the analyte is 0.125ng/mL.

Metal concentration: (ng/cigt.) = (0.125ng/ml*50mL)/20 cigt. = 0.3125ng/cigt.

E. Data Acceptance

In the event of poor data, (failing QC), the lead chemist will be contacted. If the poor data can be attributed to a single event, the standard or sample exhibiting the bad data will be disregarded from any calculations, and a complete explanation will be included with the data.

F. Calibration and Quality Control Standard Acceptance Criteria

Each calibration curve must have a correlation coefficient (r^2) of 0.996 or better. Calibrations that do not meet these requirements should be brought to the attention of the lead chemist immediately. All quality control standards must be within ±15% RE of their calculated values. All quality control standards that do not meet these requirements should be brought to the attention of the lead chemist immediately. If a quality control standard is not ± 15% RE of their calculated values the quality control standard may be replaced at the operators discretion with a different vial of the same standard and re-analyzed. If the new quality control standard is within ±15% RE of its calculated value then sample analysis may continue. If the new quality control standard is not ± 15% RE of the calculated value then the sample analysis must stop, the problem corrected and the instrument recalibrated. Any samples that are bracketed with a failed quality control standard must be re-analyzed.

Appendix A: Preparation of Analytical Solutions and Standards

A. Calibration Dilute Stock Standard I

Transfer approximately 50mL of Type I Water to a clean 100mL polypropylene volumetric flask. Using a macropipette, add 10mL of nitric acid and 1mL each of 1000μg/mL As, Cd, Cr, Ni, Pb, Pd, and Se standards to the same 100mL polypropylene flask. Dilute to volume with Type I Water.

Metals concentration = 10mg/L As, Cd, Cr, Ni, Pb, Pd and Se

B. Calibration Dilute Stock Standard II

Transfer approximately 50mL of Type I Water to a clean 100mL polypropylene volumetric flask. Using a micropipette, add 10mL of nitric acid and 1mL of Calibration Dilute Stock Standard I to the same polypropylene flask. Dilute to volume with Type I Water.

Metals concentration = 100μg/L As, Cd, Cr, Ni, Pb, Pd, and Se

C. Calibration Standards

- Using a micropipette, pipette 10mL of Nitric Acid into each flask. Fill partially with Type 1 water.
  Using a micropipette, pipette the volumes of the Stock Solutions as shown in Table 1 into 100mL polypropylene volumetric flasks.
- Make up to volume with Type I Water

Table 1

Standard No.	Calibration Dilute Stock I (μL)	Calibration Dilute Stock II (μL)	Final Volume (mL)	Final Concentration (μg/L)
Blank	-	-	100	-
Standard 1	-	200	100	0.2
Standard 2	-	1000	100	1
Standard 3	50	-	100	5
Standard 4	100	-	100	10
Standard 5	250	-	100	25
Standard 6	500	-	100	50

Note: Standard 1 is used in the calibration for all elements except Cr and Pd. Standard 6 is used in the calibration for Cd only.

D. Internal Standard Solution

Transfer approximately 500mL of Type I Water to a clean 1000mL polypropylene volumetric flask. Using a 50 mL polypropylene centrifuge tube or polypropylene graduated cylinder, add 100mL of nitric acid. Using a micropipette, add 30μL of 1000μg/mL Indium, and 60μL of 1000μg/mL Gallium to the flask. Dilute to volume with Type I Water. Cap and mix well.

Final Concentration = 30μg/L In and 60μg/L Ga

NOTE: Table 2 lists the recommended shelf life of the prepared standards.

Table 2

Solution	Shelf Life
Calibration Stock Standard I	12 months
Calibration Stock Standard II	12 months
Calibration Standards 1-5	1 month
Internal Standard	12 months

APPENDIX B

Preparation of Quality Control Solutions

A. QC Dilute Stock

Transfer approximately 50mL of Type I Water to a clean polypropylene volumetric flask. Using the appropriate macropipette, add 10mL of nitric acid and 3mL of 100μg/mL Quality Control Standard 21 to the same 100mL polypropylene flask. Dilute to volume with Type I Water. If Pd is of analytical interest, 300μL the 1000mg/L Pd standard must also be added due to the fact that Pd is not present in QC 21.

Final Concentration = 3000μg/L As, Cd, Cr, Ni, Pb, Pd, Se

B. QC

Transfer approximately 50mL of Type I Water to a clean 100mL polypropylene volumetric flask. Pipette 10mL of nitric acid into the flask. Using a micropipette, pipette 100μL of the QC Dilute Stock solution into a clean 100mL polypropylene volumetric flask. Dilute to volume with Type I Water and mix well.

Final Concentration = 3.0μg/L As, Cd, Cr, Ni, Pb, Pd, Se

NOTE: Table 1 lists the recommended shelf life for the prepared QC solutions.

Table 1

Solution	Shelf Life
QC Dilute Stock	3 months
QC	Prepare daily or as needed

Note: These solutions are stable for the listed times in the table, or until the standard and the QC no longer agree.

APPENDIX C

Electrostatic Precipitation

The following steps should be used when collecting samples with the electrostatic precipitation unit:

1. For the steps described below, care should be taken to not touch the ground glass ends or the inside of the EP tubes. Care should also be taken not to contaminate the EP caps by setting them on laboratory surfaces. When the caps are removed from EP tubes, they should be placed in a clean plastic container that has been prepped in the clean room.

b. Determine the number of runs to be made that day from the run sheet.

c. In the clean room, label the center of the capped EP tubes with the run number using a permanent marker. Also number the EP caps.

d. Record the weight of each capped tube by placing the tube into a clean, empty plastic beaker.

e. In the clean room, and wearing clean (new) gloves, carefully wrap the ends of all EP tubes using Parafilm. The Parafilm should cover the ends or tips of the EP tube. Care should be taken not to contaminate the ground glass joints.

f. Loosely, recap the EP tubes and place in a clean, covered, plastic container for use in the smoke lab.

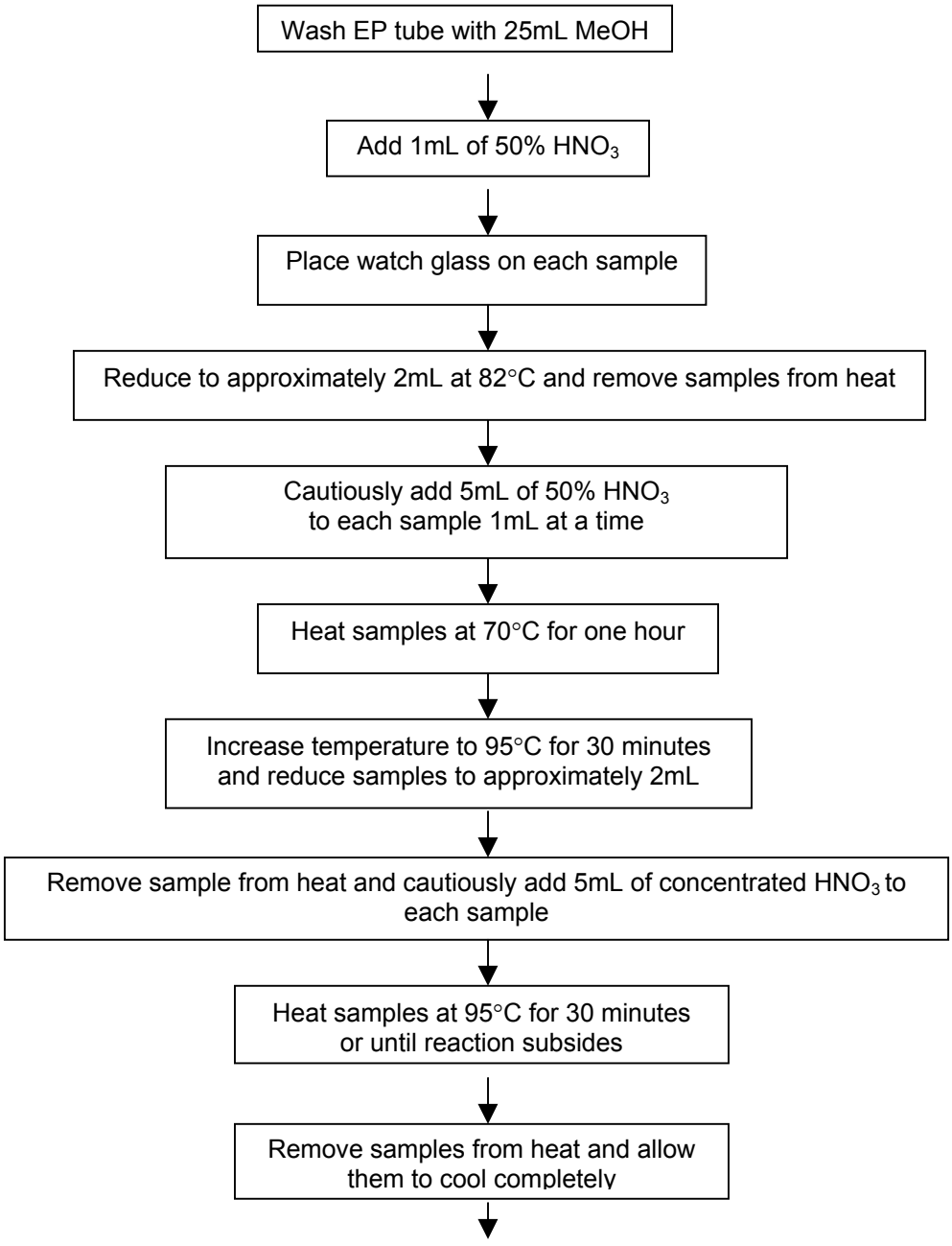
g. Collect the smoke sample. The caps should be stored in a clean plastic container when not on the EP tubes.

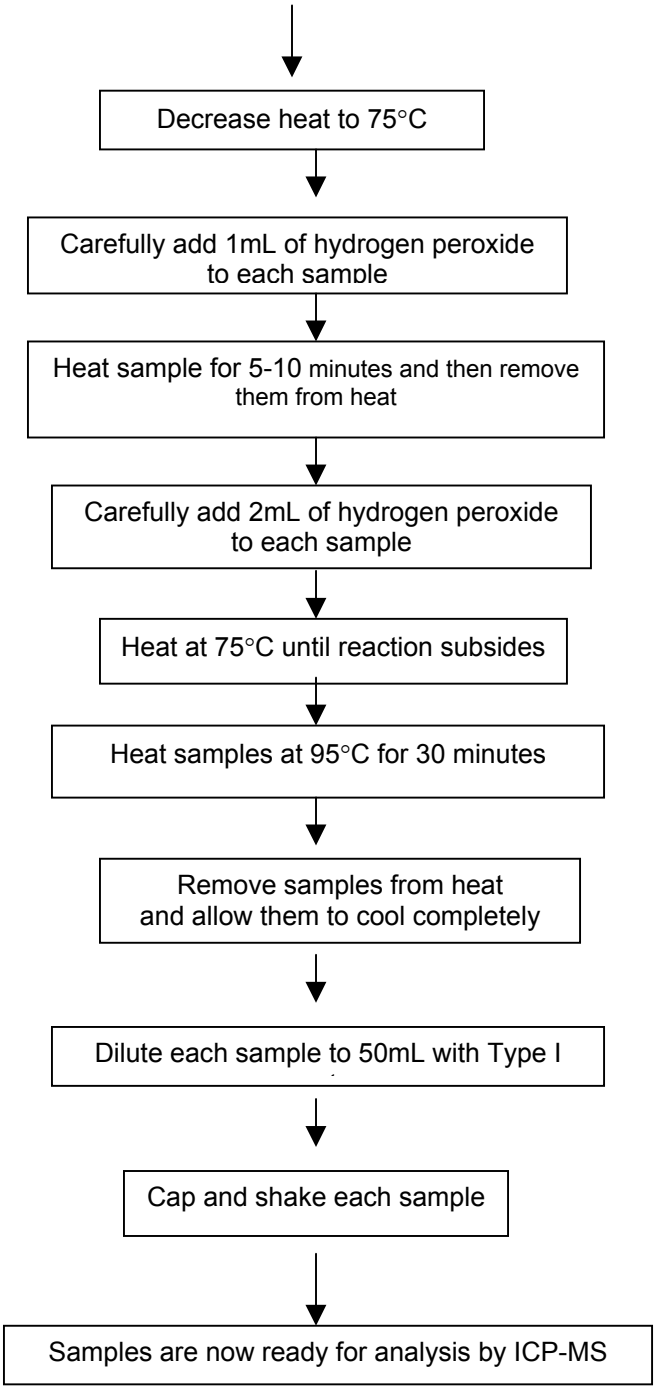
h. Before placing the caps on the EP tube, remove the Parafilm taking care not to not to contaminate the caps or the EP tubes. Next, place the caps on the EP tube and then reweigh.

i. Place the EP tubes back to the covered, plastic container and transport to the clean room.

APPENDIX D

Mainstream Metals Sample Workup




Decrease heat to 75°C
Carefully add 1mL of hydrogen peroxide to each sample
Heat sample for 5-10 minutes and then remove them from heat
Carefully add 2mL of hydrogen peroxide to each sample
Heat at 75°C until reaction subsides
Heat samples at 95°C for 30 minutes
Remove samples from heat and allow them to cool completely
Dilute each sample to 50mL with Type I
Cap and shake each sample
Samples are now ready for analysis by ICP-MS