Cooperation Centre for Scientific Research Relative to Tobacco

Tobacco and Tobacco Products Analytes Sub-Group

CORESTA Recommended Method No. 93

DETERMINATION OF SELECTED METALS IN TOBACCO PRODUCTS BY ICP-MS

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CORESTA RECOMMENDED METHOD Nº 93

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DETERMINATION OF SELECTED METALS IN TOBACCO PRODUCTS BY ICP–MS

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0. INTRODUCTION

In 2018, the CORESTA Tobacco and Tobacco Products Analytes Sub-Group (TTPA), conducted a collaborative study for the determination of selected metals in unburned tobacco products using inductively coupled plasma with mass spectrometric detection (ICP-MS). This study included four smokeless tobacco products, three cigarette fillers, and three cigar fillers. The metals included in this study were arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), nickel (Ni), lead (Pb), and selenium (Se). Eleven laboratories participated in the study. This study was the basis for this CORESTA Recommended Method (CRM). The repeatability and reproducibility values of this method have been assessed according to ISO 5725-2:1994.

1. FIELD OF APPLICATION

This CRM is applicable to the determination of arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), nickel (Ni), lead (Pb) in tobacco, cigarette filler, smokeless tobacco (e.g. moist snuff, snus, chewing tobacco, and dry snuff), and ground cigars. The calibration range specified is from 0,0 µg/l to 25 µg/l for all analytes. There is an optional high standard of 50 µg/l that may be added. Selenium is included for informational purposes due to the difficulty in effectively removing isobaric interferences.

2. NORMATIVE REFERENCES

CORESTA Smokeless Tobacco Sub-Group. *Smokeless Tobacco Glossary*

CORESTA Guide N° 11 - *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products*

CORESTA Recommended Method N° 76: *Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products*

ISO 3696, *Water for analytical laboratory use – Specification and test methods*

3. TERMS AND DEFINITIONS

No terms and definitions are listed in this document.

4. PRINCIPLE

The metals content of unburned tobacco products is determined by digesting the tobacco in nitric acid by closed vessel microwave digestion followed by the addition of internal standards and dilution in Grade 1 water. Analysis is accomplished by inductively coupled plasma mass spectrometry equipped with collision cell and/or reaction cell technology. The collision cell and/or reaction cell technology is required to ensure removal of isobaric interferences. Isobaric elemental interferences are caused by isotopes of different elements forming atomic ions with the same mass/charge ratio as the analyte of interest. Isobaric molecular interferences are caused by ions consisting of more than one atom. Follow manufacturer's recommendations for the optimization of the collision/reaction cells. The results are reported as nanograms of analyte per gram of tobacco (ng/g) on an as-is or wet-weight basis.

5. APPARATUS

Normal laboratory apparatus is required, in particular, the following items:

5.1 **Analytical balance:** 0,0001 g accuracy

5.2 **Volumetric flasks** of capacities 100 ml, 250 ml and 1000 ml (PTFE or polypropylene)

5.3 **Mechanical pipettes** with disposable plastic tips 10 µl -1000 µl

5.4 **Bottle top dispenser** suitable for dispensing 10 ml of concentrated nitric

5.5 **ICP-MS with collision and/or reaction cell technology**

5.6 **Closed vessel microwave digestion system**

5.7 **Sample tubes** (polypropylene is recommended)

5.8 **General laboratory equipment** necessary for the preparation of samples, standards, and reagents

5.9 **Grinder** suitable for grinding tobacco and tobacco products for trace metal analyses

6. REAGENTS

Use only reagents of recognized analytical grade.

6.1 Nitric Acid 65 % to 70 % (ultrapure grade or higher)

6.2 Water, Grade 1 (refer to ISO 3696)

6.3 Reaction/collision gases as per ICP-MS vendor recommendation and Table 2

6.4 Certified standard solutions (arsenic, beryllium, cadmium, chromium, cobalt, lead, nickel, selenium) in appropriate concentration to make working standards (e.g. 1000 µg/ml stock solutions).

6.5 Certified standard solutions (e.g. germanium, bismuth and indium) to make working internal standard solution (e.g. 1000 µg/ml stock solutions). Other internal standards may also be used if demonstrated to provide equivalent results.

6.6 Certified standard solutions for calibration verification (preferred from a different vendor than the calibration standards or at a minimum a different lot from the same vendor (e.g. multi-element standard)).

WARNING – The use of this method can involve hazardous materials, operations and equipment. This method does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

7. STANDARDS

7.1 Internal Standards Preparation

7.1.1 *Working Internal Standard Solution*: Using a 500 ml polypropylene volumetric flask, fill halfway with Grade 1 water. Add 10 ml nitric acid. Pipet 1,0 ml of each of 1000 mg/l stock solutions of germanium, bismuth and indium (6.4) and dilute to volume with Grade 1 water for a final concentration of approximately 2 mg/l.

7.2 Calibration Standards Preparation

7.2.1 *Calibration Standard Diluent*: Using a 1000 ml polypropylene volumetric flask, fill halfway with Grade 1 water. Add 100 ml nitric acid and bring to volume with Grade 1 water.

7.2.2 *Working Calibration Standards*: Prepare at least five *Working Calibration Standards*. Table 1 provides a recommended calibration range. Using 100 ml polypropylene volumetric flasks, fill halfway with *Calibration Standard Diluent* (7.2.1). Transfer the specified volumes of the *Purchased Certified Standard Solutions* (6.4) and *Working Internal Standard Solution* (7.1.1) according to the table below. Bring to final volume with *Calibration Standard Diluent* (7.2.1) and mix well.

Note: Alternatively, internal standards may be added online during sample introduction instead of adding to standards and samples.

Table 1. Working Calibration Standard Ranges (nominal concentrations)

	Volume of Certified standard solutions (µl)	Volume of Working Internal Standard Solution (ml)	Final Analyte Concentration (µg/l)	Final Internal Standard Concentration (µg/l)
Calibration Blank	0	1,0	0	20
Standard 1	100	1,0	1	20
Standard 2	500	1,0	5	20
Standard 3	1,000	1,0	10	20
Standard 4	2,500	1,0	25	20
Standard 5[1]	5,000	1,0	50	20

[1] May be used if the samples have high levels
[2] Se is included for informational purposes only

7.2.3 The calibration curve is verified using Certified Standard Solutions purchased from a different vendor than those used for the calibration standards. This standard is typically referred to as the Initial Calibration Verification Standard (ICV) and should be prepared near the midpoint of the calibration curve. This solution is prepared at the same acid concentration as the calibration standards and the samples (10 % nitric acid). This standard also contains the internal standards at the same concentration described above (20 µg/l).

7.3 Storage

The standard solutions have been shown to be stable for 2 months when stored at ambient conditions; however, each laboratory should determine stability of all solutions specified in this CRM.

8. SAMPLE PROCEDURE

8.1 Sample Handling

Refer to CORESTA Guide N° 11, *Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products* for sample handling guidelines.

It is recommended that 3 individual sample replicates be digested and analysed in order to generate a standard deviation and a relative standard deviation (%RSD). Data exhibiting a %RSD greater than 25 % may indicate non-homogeneous samples or some other method performance issue and the results should be investigated.

8.1.1 Sample Equilibration:

Samples stored at −20 °C shall be placed unopened in a refrigerator for a minimum of 24 hours to ensure water has fully equilibrated within the product. After equilibration in the refrigerator, samples should be removed from the refrigerator a minimum of 2 hours prior to opening for analysis. The samples should not be opened during the time the samples are equilibrating to ambient temperature. Once opened, the samples shall be stored at approximately 4 °C for up to one week if the analyses will not be conducted immediately.

Note: An insufficient sample equilibration time has been identified as a source of variability.

8.1.2 Sample Preparation:

8.1.2.1 Loose tobacco: Grind a sufficient amount of tobacco to create a homogeneous sample.

8.1.2.2 Portioned smokeless tobacco products: Analyze unit portions (pouches) by cutting the pouch in half and adding the tobacco and pouch material directly into the digestion vessel.

8.1.2.3 Cigarettes: remove the filler from a minimum of one pack of 20 and mix well to homogenize.

8.1.2.4 Cigars: Grind a sufficient number of whole cigars (wrapper, binder and filler) to create a homogenous sample. Tips made from materials other than tobacco shall be removed prior to grinding.

Note: Due to the differing physical properties of the wrapper, filler and binder, it may be necessary to cryogrind the cigars by freezing the cigars in liquid nitrogen before adding to the grinder in order to achieve a uniform, homogenous mixture. Grinding should be conducted so as excess heat is not generated.

8.2 Closed Vessel Microwave Digestion

8.2.1 Weigh 0,5 g to 1,0 g of each sample into an appropriate digestion vessel. Record the exact weight to 0,0001 g.

8.2.2 Prepare a blank vessel (Method Blank) to carry through the process to determine background quantities of the analytes of interest. This is done by adding all components to the digestion vessel except for the tobacco sample and treating the Method Blank the same as the samples.

8.2.3 It is recommended that an appropriate Quality Control Sample be prepared with the samples and the Method Blank. This may be a certified reference material, a certified reference tobacco[1], a reference tobacco product, or in-house monitor sample. The results are compared with the certificate of analysis and or control charts to ensure the method is in control.

8.2.4 To each vessel add 10 ml concentrated nitric acid under a chemical fume hood and using the appropriate safety precautions including personal protective equipment.

8.2.5 Close the vessel according to the manufacturer's directions.

8.2.6 Following recommendations from the microwave manufacturer, place the sealed vessel into the microwave and program a ramp time of 15 minutes to a final temperature of 180 °C to 200 °C. Hold this temperature for 15 minutes.

8.2.7 Allow the vessels to cool to room temperature.

8.2.8 Vent the vessels under a chemical fume hood while wearing personal protective equipment. Quantitatively transfer the digested samples, Method Blank(s), and Quality Control Sample(s) to individual 100 ml polypropylene (PP) volumetric flasks. Add 1,0 ml of the *Working Internal Standard Solution* (7.1.1) to each sample and dilute to volume with Grade 1 water.

Note: Alternatively, internal standards may be added online during sample introduction instead of adding to standards and samples.

9. SAMPLE ANALYSIS

9.1 It is required that an ICP-MS with collision cell and/or reaction cell technology be used for this CRM. Follow manufacturer's recommendations for the optimization of the collision/reaction cells and instrument parameters including sweeps/reading, sample flush times, sample speed, peak processing, read delay time, read delay speed, wash time and wash speed among others. This method is not intended to cover the various manufacturers' recommendations for instrument optimization nor is it intended to replace an appropriate method validation.

[1] Polish Virginia Tobacco Leaves and Oriental basma tobacco leaves are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

9.2 Table 2 below lists the recommended masses, corresponding internal standard isotopes, and analysis mode to configure the ICP-MS.

Table 2: Analyte Masses and Associated Internal Standards for Analysis by ICP-MS

Analyte	Mass	Internal Standard	Analysis Mode (ICP-MS)*	Recommended Reaction Gas (refer to instrument supplier recommendations)
Arsenic	75	^{72}Ge	Reaction/Collision Cell Technology	Helium
Beryllium	9	^{72}Ge	Normal Mode	
Cadmium	111	^{115}In	Normal Mode	
Chromium	52	^{72}Ge	Reaction/Collision Cell Technology	Helium or Ammonia
Cobalt	59	^{72}Ge	Reaction/Collision Cell Technology	Helium
Lead	Sum 206,207,208	^{209}Bi	Normal Mode	
Nickel	60	^{72}Ge	Reaction/Collision Cell Technology	Helium
Selenium	78	^{72}Ge	Reaction/Collision Cell Technology	H_2 or Helium

* Reaction/Collision cell technology is required due to interferences. Normal mode may be used for specified analytes. Methane gas is used with some instruments that have reaction cells.

9.3 The ICP-MS is calibrated by analysing a blank (Calibration Blank) followed by the *Working Calibration Standards*. It is recommended that the Calibration Blank be analysed following the calibration to ensure that the rinse time is appropriate and that no carryover is observed. A linear calibration model with internal standard should be selected.

9.4 A recommended analysis sequence is as follows:

- Calibration Blank
- Calibration Standards
- Calibration Blank (to monitor carryover)
- ICV
- Method Blank
- Quality Control Sample (reference material)
- Samples 1 through 15
- Calibration Blank (to monitor carryover)
- Samples 16-31 followed by ICV and Calibration Blank
- Further samples can be analysed, continuing to bracket sets of 15 samples with the ICV and the Calibration Blank.

9.5 Ensure that sample concentrations are within the range of the calibration curve. If there are any samples that are above the concentration of the highest calibration standard, dilute using the *Calibration Standard Diluent* (7.2.1) and re-analyze.

9.6 Quality Control: Laboratories should determine quality control procedures that are suitable for the intended purpose of the reported data. The following criteria are recommended:

9.6.1 Method detection limits (MDL) and method quantitation limits (MQL). It is recommended that the MDL and MQL be established for each analyte to ensure results are not reported below the quantitation limit and that background analyte levels are not adversely affecting the analysis. The MDL is the lowest analyte concentration that can be distinguished from the background, while the MQL is the lowest concentration at which the analyte can be quantitated. There are several ways to determine MDL and MQL and labs should use their established method for this. Alternatively, the low standard can be used as the MQL, and the lab should ensure that no results are reported below this level.

Note: One method for experimentally determining the MDL and MQL is by measuring Method Blanks as samples and calculating the standard deviation from these measurements. A minimum of 10 independently prepared method blanks should be used for this measurement. Results of method blanks accumulated over time, and more than the minimum number is optimal for determining the MDL and MQL. The MDL can be defined as 3 × SD, where SD is the standard deviation calculated from measurements of the method blank where the concentration of the analyte of interest is approaching zero. Likewise, the MQL can be defined as 10 × SD. These experiments may require fortification of the Methods Blank if native analyte is not detected.

9.6.2 Additional recommended quality control criteria

- Correlation coefficient is ≥ 0,999
- ICV is ± 10 % of the expected value for all analytes.
- Quality Control Sample (reference material) is within the expected values either from the certificate of analysis or as determined by the use of laboratory control charts.
- %RSD for 3 replicates of samples are ≤ 15 %
- Data for samples is within the range of the calibration curve.
- The prepared method blank results are reviewed to ensure they are below the determined method detection limit (MDL). Method blanks that are above the MDL indicate potential contamination from the sample preparation process or reagents. It is recommended that Method Blanks be below the MDL or the source of contamination identified and the analysis repeated. Subtraction of method blanks is not recommended.

9.7 Determination of the Metals Content of Samples:

The concentration of analytes expressed in nanograms per gram of tobacco is calculated with the formula below:

$$Result\ (ng/g) = \frac{C\ x\ V}{M} x\ 1000$$

Where:

C = is the concentration obtained from the instrument (µg/l)

V = is the final volume (l)

M = is the mass of tobacco digested (g)

10. REPEATABILITY AND REPRODUCIBILITY

In 2018, an international collaborative study involving 11 laboratories was conducted using cigarette filler (1R6F, 1R5F, 3R4F), flavoured cigar filler, unflavoured cigar filler, Swedish style snus pouches (CRP 1.1), American-style loose moist snuff (CRP 2.1), American-style loose dry snuff powder (CRP 3.1), and American-style chopped loose-leaf chewing tobacco (CRP 4.1). Results were analysed according to ISO 5725-2 (1994) and ISO/TR 22971:2005. After removal of outlying data, the final repeatability (r) and reproducibility (R) results were calculated. The r & R results for the study are presented in Table 3. The value of 'N' is the number of laboratories used to determine the statistics after the removal of outliers.

Table 3: Repeatability (r) and Reproducibility (R) Limits

Analyte	Product[1]	N° of Labs[2]	Mean (ng/g)	Repeatability		Reproducibility	
				r	% r	R	% R
Arsenic	1R5F Ground Filler	8	318	29,6	9,3	118	37,2
Arsenic	1R6F Ground Filler	10	268	32,6	12,2	96	35,9
Arsenic	3R4F Cigarette Filler	10	266	50,9	19,1	138	51,9
Arsenic	Cigar Filler #1	9	1036	68,1	6,6	318	30,7
Arsenic	Cigar Filler #2	9	701	75,9	10,8	256	36,5
Arsenic	CRP 1.1	10	55.7	11,4	20,4	28	49,4
Arsenic	CRP 2.1	10	80.7	22,6	28,1	42,3	52,4
Arsenic	CRP 3.1	11	214	19,5	9,1	110	51,5
Arsenic	CRP 4.1	10	87.4	11,3	12,9	41,0	46,9
Arsenic	RT6 Cigar Filler	9	267	29,2	10,9	112	41,8
Beryllium	1R5F Ground Filler	7	25.93	9,2	35,6	13,32	51,4
Beryllium	1R6F Ground Filler	8	17.03	8,34	49,0	11,26	66,1
Beryllium	3R4F Cigarette Filler	8	16.13	4,0	24,8	12,39	76,8
Beryllium	Cigar Filler #1	7	34.59	8,3	23,9	15,23	44,0
Beryllium	Cigar Filler #2	7	31.31	10,6	34,0	16,30	52,1
Beryllium	CRP 1.1	8	7.93	2,2	28,1	3,40	42,8
Beryllium	CRP 2.1	7	11.75	2,3	19,7	3,77	32,1
Beryllium	CRP 3.1	9	19.46	3,4	17,6	11,25	57,8
Beryllium	CRP 4.1	6	6.65	1,0	14,3	3,25	48,9
Beryllium	RT6 Cigar Filler	8	36.60	4,2	11,6	19,49	53,3
Cadmium	1R5F Ground Filler	8	1457	58,2	4,0	393	27,0
Cadmium	1R6F Ground Filler	10	1060	82,9	7,8	303	28,6
Cadmium	3R4F Cigarette Filler	10	1102	96,1	8,7	325	29,5
Cadmium	Cigar Filler #1	9	1061	60,6	5,7	253	23,9
Cadmium	Cigar Filler #2	9	1154	42,0	3,6	322	27,9
Cadmium	CRP 1.1	11	240	34,7	14,4	91	37,9
Cadmium	CRP 2.1	11	706	145,4	20,6	353	50,0
Cadmium	CRP 3.1	11	1360	69,2	5,1	462	33,9
Cadmium	CRP 4.1	11	339	37,0	10,9	116	34,1
Cadmium	RT6 Cigar Filler	9	1028	71,5	7,0	327	31,8
Chromium	1R5F Ground Filler	9	1805	265	14,7	2741	151,8
Chromium	1R6F Ground Filler	10	1165	398	34,1	1357	116,5
Chromium	3R4F Cigarette Filler	10	972	304	31,3	1018	104,7
Chromium	Cigar Filler #1	9	2126	354	16,6	2028	95,4
Chromium	Cigar Filler #2	9	1854	310	16,7	1757	94,8
Chromium	CRP 1.1	10	350	62,8	17,9	279	79,6
Chromium	CRP 2.1	11	416	165	39,7	465	111,8

Analyte	Product[1]	N° of Labs[2]	Mean (ng/g)	Repeatability		Reproducibility	
				r	% r	R	% R
Chromium	CRP 3.1	11	2000	146	7,3	1813	90,7
Chromium	CRP 4.1	11	817	449	54,9	994	121,6
Chromium	RT6 Cigar Filler	9	2192	499	22,8	2102	95,9
Cobalt	1R5F Ground Filler	7	718	44,4	6,2	213	29,7
Cobalt	1R6F Ground Filler	9	464	56,1	12,1	113	24,4
Cobalt	3R4F Cigarette Filler	8	429	63,8	14,9	145	33,9
Cobalt	Cigar Filler #1	8	964	43,8	4,5	239	24,8
Cobalt	Cigar Filler #2	8	739	69,2	9,4	204	27,6
Cobalt	CRP 1.1	10	364	33,6	9,2	130	35,7
Cobalt	CRP 2.1	10	502	105,1	20,9	252	50,3
Cobalt	CRP 3.1	10	792	34,0	4,3	269	33,9
Cobalt	CRP 4.1	10	207	23,9	11,6	57,8	28,0
Cobalt	RT6 Cigar Filler	8	802	52,0	6,5	245	30,6
Lead	1R5F Ground Filler	8	1348	208	15,5	459	34,1
Lead	1R6F Ground Filler	9	471	53,8	11,4	121	25,7
Lead	3R4F Cigarette Filler	10	606	218	35,9	314	51,8
Lead	Cigar Filler #1	8	867	195	22,5	305	35,2
Lead	Cigar Filler #2	9	769	138	18,0	289	37,6
Lead	CRP 1.1	10	138	24,7	17,9	67	48,9
Lead	CRP 2.1	10	224	30,4	13,6	158	70,5
Lead	CRP 3.1	10	596	72,1	12,1	162	27,3
Lead	CRP 4.1	11	531	75,9	14,3	194	36,5
Lead	RT6 Cigar Filler	9	1508	442	29,3	610	40,4
Nickel	1R5F Ground Filler	8	2375	121	5,1	669	28,2
Nickel	1R6F Ground Filler	10	1939	403	20,8	593	30,6
Nickel	3R4F Cigarette Filler	10	1751	487	27,8	747	42,7
Nickel	Cigar Filler #1	8	2327	199	8,6	261	11,2
Nickel	Cigar Filler #2	9	2093	224	10,7	548	26,2
Nickel	CRP 1.1	11	885	153	17,3	413	46,7
Nickel	CRP 2.1	11	752	115	15,3	384	51,1
Nickel	CRP 3.1	11	3108	156	5,0	1139	36,6
Nickel	CRP 4.1	11	887	217	24,4	449	50,6
Nickel	RT6 Cigar Filler	9	2283	241	10,6	748	32,8
Selenium[3]	1R5F Ground Filler	7	175	34,6	19,8	271	154,9
Selenium[3]	1R6F Ground Filler	8	115	32	27,5	169	146,8
Selenium[3]	3R4F Cigarette Filler	7	98	29	29,3	133	135,0
Selenium[3]	Cigar Filler #1	7	286	130	45,5	223	77,9
Selenium[3]	Cigar Filler #2	7	265	121	45,6	225	84,8

Analyte	Product[1]	N° of Labs[2]	Mean (ng/g)	Repeatability		Reproducibility	
				r	% r	R	% R
Selenium[3]	CRP 1.1	8	74.8	24,1	32,2	93,6	125,2
Selenium[3]	CRP 2.1	9	91.1	54,6	59,9	140	153,2
Selenium[3]	CRP 3.1	9	161	43,2	26,9	171	106,6
Selenium[3]	CRP 4.1	7	47.5	20,0	42,1	42,6	89,8
Selenium[3]	RT6 Cigar Filler	7	206	39,0	18,9	187	90,9

1. Cigar Filler #1 is a flavoured cigar filler and Cigar Filler #2 is an unflavoured cigar filler
2. The number of laboratory data sets after removal of outliers.
3. Selenium is included for informational purposes due to the difficulty in effectively removing isobaric interferences.

11. TEST REPORT

The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Any statistical analyses should be calculated and expressed before any rounding has taken place. The test report shall state the amount of each analyte of interest in ng/g. The report shall also give all details necessary for the identification of each sample. Moisture content may be determined on separate tobacco aliquots if it is necessary to present the results on a dry-weight basis. This procedure is detailed in CORESTA Recommended Method N˚ 76: Determination of moisture content (oven volatiles) of tobacco and tobacco products.