



**Smokeless Tobacco Sub-Group**

**Technical Report**

**CORESTA Reference Products  
2010 Analysis**

February 2014

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## 1. Introduction

In November 2008, the CORESTA Smokeless Tobacco Sub-Group (CSTS) was established by recommendation of the CORESTA Scientific Commission. At the Amelia Island FL CSTS meeting, October 2009, Working Group Four (WG4) was organized to proceed with the analysis of the four CORESTA Reference Products, CRP-1 through CRP-4, for selected analytes. Ideally, a minimum of three different laboratories would provide data for each analyte. Analytes were selected to represent a wide range of interests from previous data reported in the literature for smokeless tobacco products. Analyte identification and selection criteria are given in APPENDIX I.

Prior to this endeavour, CSTS Working Group Three (WG3) had cooperated to design and manufacture the four reference products. Except for CRP-1, these were intended as fresh replacements for the Smokeless Tobacco Research Products: 2S3 (Moist Snuff), 1S2 (Dry Snuff) and 2S1 (Loose-leaf Chewing Tobacco), which were greater than ten years old. Upon receipt of the new CORESTA smokeless reference products, the early reference products, 2S3, 1S2, and 2S1, were properly destroyed except for a representative sample of each type. CRP-1 through CRP-4 would continue to be stored at -20C and distributed by the North Carolina State University (NCSU) Tobacco Analytical Services Lab under the direction of Dr. David Danehower<sup>1</sup>, Associate Professor in the Department of Crop Science.

In 2012, this responsibility has been assigned to Dr. Ramsey Lewis<sup>2</sup>, with CRP sample requests being directed to Karen Andres<sup>3</sup>.

## 2. Objective

WG4 was organized to provide data for selected analytes in the four CORESTA Reference Products and also to provide reference points of comparison for the respective commercial product categories. It was intended that a minimum of three laboratories would report results for each analyte. Contract laboratory analysis was utilized to supplement the analyses which were beyond the current analytical capabilities of the participating laboratories. After thorough discussion and review, the data could then be submitted for publication, provided it was considered to be of sufficient quality to do so.

## 3. Time schedule

Samples of the four reference products were shipped frozen to each of the participating laboratories of WG4 during the week of December 27<sup>th</sup>, 2009. It was recommended that the analyses be completed by January 22, 2010 to allow for tabulation and submission of the preliminary data to WG4 prior to the Innsbruck CSTS Meeting on January 28<sup>th</sup>, 2010.

## 4. WG4 Participating Laboratories

The nine laboratories which agreed to participate in the analyses of the CORESTA Reference Products are listed here below:

1. Altria (United States)
2. American Snuff Company (United States)

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<sup>1</sup> David\_Danehower@ncsu.edu

<sup>2</sup> Ramsey\_lewis@ncsu.edu

<sup>3</sup> Karen\_Andres@ncsu.edu

3. British American Tobacco (United Kingdom)
4. China National Tobacco Company (Zhengzhou, China)
5. Eurofins Food & Agro (Sweden)
6. India Tobacco Company (India)
7. Japan Tobacco International –Ökolab (Austria)
8. Labstat International (Canada)
9. Swedish Match Northern Europe (Sweden)

Arista Laboratories (United States) provided supplemental analyses for the analytes, as required.

## **5. Samples**

### **5.1 Selection**

The CORESTA Smokeless Reference Products are the following:

- CRP-1: Swedish style snus pouch
- CRP-2: American-style loose moist snuff
- CRP-3: American-style loose dry snuff powder
- CRP-4: American-style loose-leaf chewing tobacco

Composition details of these reference products are included in APPENDIX II.

### **5.2 Supply and Transportation**

Dr. David Danehower and Karen Andres of NC State University were provided with the shipping information and shipped the samples from -20C storage, by FedEx, to the WG4 participating laboratories. They also notified the participating laboratories of the actual shipping date and FedEx tracking information so that the receiving laboratories could prepare for receipt of the samples.

### **5.3 Quantity**

Each participating laboratory received approximately three hundred grams of each reference product.

### **5.4 Receipt**

The samples were to be signed for immediately upon receipt and stored in a freezer until the analyses could be performed. The freezer temperature was not specified.

### **5.5 Within Laboratory Sample Preparation**

The samples were to be thawed at room temperature for at least 2 hours before use. After this initial thawing, the samples were to be stored in a refrigerator in between use. The Snus pouches (CRP-1) were to be cut into two halves directly into the extraction vessel. Both the tobacco and pouch were to be included in the analyses. The Loose Leaf reference product (CRP-4) was to be ground according to the participating laboratory's standard procedure. The Moist Snuff reference product (CRP-2) and the Dry Snuff reference product (CRP-3) did not require sample grinding of this nature prior to sample analysis.

## **6. Analysis**

### **6.1 Analyte Assignments**

The choice of analytes reflects to some extent current regulatory interest, country-specific tobacco reporting requirements, and basic chemical composition; however, the list is not intended to be comprehensive. The analytical capabilities of the participating laboratories were determined in advance by questionnaire. All analytes, except nitrososarcosine and Polonium 210, were then assigned to three respective laboratories with specification for triplicate analysis. Analysis for Polonium 210 was arranged through a contract laboratory. No contract laboratory for nitrososarcosine could be located. Later during the course of the analytical portion of the study, one of the WG4 laboratories volunteered to report results for nitrososarcosine with a newly developed method.

### **6.2 Timing**

The analyses were to be completed and reported, if possible, by January 22, 2010.

## **7. Data Reporting**

The participating laboratories were requested to provide the standardized method used or a description of the analytical instrumentation or procedures used to analyze the samples, including sample sizes, extraction techniques, detection limits, quantification limits and other relevant information. In most cases, the methods used were considered by the reporting laboratory to be Internal/Proprietary methods. A Microsoft Excel spreadsheet was provided for data reporting and other relevant information. It was requested that all test results should be reported “as is” or on a wet weight basis (wwb), with no correction for moisture content, in order to provide consistency in the values reported. Dry basis values, if needed, could be converted afterwards using the moisture content. The results were not to be rounded and were to be reported to at least one more digit than specified in the standard or internal method used by the laboratory for the test.

## **8. WG4 CRP Data Summary**

The data for all selected analytes was summarized according to analyte name, units of measurement, range, number of observations and number of laboratories. These results are included in Tables 1 through 4 in APPENDIX III. The methods used for these analyses are listed in APPENDIX IV. If a standardized method was used then it is listed as such. If non-standardized methods were used, only an instrumental technique is listed. Most of the methods were non-standardized and the between laboratory variability (reproducibility) obtained with the methodology is currently unknown.

## **9. Data Interpretation**

In some cases, more than three laboratories voluntarily reported results for analytes which had not been assigned. These results were included along with the others. In a few cases, some of the laboratories reported duplicate results instead of triplicate. Finally, in some cases, laboratories did not report results for the analytes assigned. It should be noted that it is not known whether the range of levels of analytes represented by data from this small number of laboratories using their own methodologies represents the full range of variability that might be found within a formal collaborative study on a standardized methodology.

All reported data were included in the data ranges for each analyte without determination and exclusion of outliers. For some analytes the summarized range of data from the four reference products should be useful for comparison of results from future analyte determinations of the CRPs utilizing validated methods, or for validating new standard methods. It is beyond the scope of the present report to compare these values to those previously reported in the literature for commercial smokeless tobacco products appropriate for these categories, since a comprehensive review of these data have not been reported in the literature.

## 10. Final Summary, Conclusions, Recommendations or Next Steps

In many cases, data for the analytes demonstrated a wide range of values. This is expected since individual laboratory methods were used for most of the analytes covered in this study and the methods were not harmonized. It is recommended that future studies of this nature should incorporate specified methods of analysis for each analyte.

In order to determine when it is necessary to manufacture new quantities of these CORESTA Reference Products, it is recommended that their stability be monitored by WG4 on an annual basis, for the following selected analytes: nicotine, pH, moisture (oven volatiles) and tobacco specific nitrosamines (TSNAs). It is further recommended that activities begin for the development of a CORESTA Recommended Method for the manufacture of these reference products.

## 11. References

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## APPENDIX I: Analyte Identification and Selection Criteria

Analyte Identification	Selection Criteria	Reference
Aflatoxin B1,B2,G1,G2	Mycotoxin found in tobacco	1
Ochratoxin A	Mycotoxin found in tobacco	1
Nicotine	CDC Analyte, Hoffmann Analyte	2, 3
Nornicotine, anatabine, anabasine	Supplemental product information	6
Total sugars	Supplemental product information	7
Acetaldehyde	Hoffmann Analyte	3
Acrolein	Hoffmann Analyte	3
Crotonaldehyde	Hoffmann Analyte	3
Formaldehyde	Hoffmann Analyte	3
pH	CDC Analyte	2
Ash	Supplemental product information	7
Calcium (Ca)	Supplemental product information	7
Potassium (K)	Supplemental product information	7
Sodium (Na)	Supplemental product information	7
Arsenic (As)	Hoffmann Analyte, GothiaTek® Analyte	3, 4
Cadmium (Cd)	Hoffmann Analyte, GothiaTek® Analyte	3, 4
Chromium (Cr)	Hoffmann Analyte, GothiaTek® Analyte	3, 4
Mercury (Hg)	Hoffmann Analyte	3
Nickel (Ni)	Hoffmann Analyte, GothiaTek® Analyte	3, 4
Lead (Pb)	Hoffmann Analyte, GothiaTek® Analyte	3, 4
Selenium (Se)	Hoffmann Analyte	3
Nitrate	Supplemental product information	6
Nitrite	GothiaTek® Analyte	4
Nitrososarcosine	WHO TobReg Report 951, IARC	5, 8
N-Nitrosodimethylamine (NDMA)	GothiaTek® Analyte	4
Moisture (oven volatiles)	CDC Analyte	2
Benzo(a)pyrene	Hoffmann Analyte, GothiaTek® Analyte	3, 4
Polonium-210	IARC	8
N-Nitrosoanabasine (NAB)	Hoffmann Analyte	3
N-Nitrosoanatabine (NAT)	Hoffmann Analyte	3
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	Hoffmann Analyte	3
N-Nitrosornicotine (NNN)	Hoffmann Analyte	3
Total TSNAs <sup>1</sup>	Hoffmann Analyte, GothiaTek® Analyte	3, 4
Ammonia	Hoffmann Analyte	3
Glycerol	Supplemental product information	6
Propylene glycol	Supplemental product information	6
Triethylene glycol	Supplemental product information	6

1. Total TSNAs: sum of NAB, NAT, NNK, and NNN

## APPENDIX II: CRP Composition

### A- CRP1 – Snus Smokeless Tobacco

CRP1 is a Swedish-style Snus smokeless tobacco product, without any flavourings added, packaged in a plastic can that contains 24 off 1g pouches.

The target product composition as supplied by the manufacture on a wet weight basis (wwb) was as follows:

<u>Component</u>	<u>% (wwb)</u>
Dark air-cured lamina	25.5
Dark air-cured stem	20.8
Sodium carbonate	2.4
Propylene glycol	3.0
Sodium chloride	3.6
Water	44.7
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Total	100.0

The values of the following parameters were estimated at the time of manufacture:

Moisture content	52%
pH	8.5
Nicotine content	0.8% (wwb)

### B- CRP2 – Moist Snuff

CRP2 is an American-style loose moist snuff smokeless tobacco product packaged in a plastic can that contains 34 grams.

The target product composition as supplied by the manufacture on a wet weight basis (wwb) was as follows:

<u>Component</u>	<u>% (wwb)</u>
Dark-fired tobacco	26.3
Air-cured tobacco	8.0
Sodium chloride	7.2
Burley stem	3.8
Sodium carbonate	0.3
Water	54.4
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Total	100.0

The values of the following parameters were estimated at the time of manufacture:

Moisture content	54.6%
pH	7.7
Nicotine content	1.2% (wwb)



### C- CRP3 – Dry Snuff

CRP3 is an American-style loose dry snuff smokeless tobacco product packaged in a plastic can that contains 34 grams.

The target product composition as supplied by the manufacture on a wet weight basis (wwb) was as follows:

<u>Component</u>	<u>% (wwb)</u>
Dark-fired tobacco	16.4
Air-cured tobacco	51.2
Burley stem	21.4
Water	8.0
Sodium carbonate	2.0
Sodium chloride	1.0
	<hr/>
Total	100.0

The values of the following parameters were estimated at the time of manufacture:

Moisture content	8.0%
pH	6.9
Nicotine content	1.9% (wwb)

### D- CRP4 – Loose Leaf Chewing Tobacco

CRP4 is an American-style loose-leaf chewing tobacco packaged in a foil pouch that contains 85 grams of product.

The target product composition as supplied by the manufacture on a wet weight basis (wwb) was as follows:

<u>Component</u>	<u>% (wwb)</u>
Air-cured tobacco	30.7
Air-cured stem	5.4
Sucrose	25.9
Sodium Chloride	1.9
Glycerine	3.2
Dextrose	2.0
Maltose	1.5
Other corn syrup solids	7.2
Sodium propionate	0.2
Water	22.0
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Total	100.0

The values of the following parameters were estimated at the time of manufacture:

Moisture content	22%
pH	6.0
Nicotine content	1.2% (wwb)

## APPENDIX III: Data Summary

**Table 1. CRP1**

Analyte	units	Range	Observations	Labs
Aflatoxin B1,B2,G1,G2	ppb	<0.18 to 0.26	6	2
Ochratoxin A	ppb	1.80 to 4.62	9	3
Nicotine	%	0.880 to 1.126	21	7
Nornicotine, anatabine, anabasine	%	0.022 to 0.042	12	4
Total sugars	%	0.79 to 1.10	12	4
Acetaldehyde	ppm	0.46 to 3.07	6	2
Acrolein	ppm	0.031 to 0.036	3	1
Crotonaldehyde	ppm	<0.010	3	1
Formaldehyde	ppm	0.249 to 0.753	6	2
pH		7.75 to 8.09	15	5
Ash	%	7.24 to 16.53	11	4
Calcium (Ca)	%	0.88 to 1.48	11	4
Potassium (K)	%	0.73 to 1.78	11	4
Sodium (Na)	%	1.26 to 2.79	8	3
Arsenic (As)	ppm	0.060 to 1.46	9	3
Cadmium (Cd)	ppm	0.229 to 0.900	11	4
Chromium (Cr)	ppm	0.310 to 0.658	9	3
Mercury (Hg)	ppm	<0.01 to 0.62	8	3
Nickel (Ni)	ppm	0.460 to 0.657	11	4
Lead (Pb)	ppm	0.130 to 3.40	11	4
Selenium (Se)	ppm	0.060 to 0.150	6	2
Nitrate	%	0.63 to 0.75	14	5
Nitrite	ppm	<0.66 to 1.69	9	3
Nitrososarcosine	ppb	7.96 to 13.5	3	1
N-Nitrosodimethylamine (NDMA)	ppb	0.261 to 12.3	12	4
Moisture (oven volatiles)	%	50.73 to 56.1	18	6
Benzo[a]pyrene	ppb	<0.5 to <2	9	3
Polonium-210	pCi/g	<0.71 to 0.207	6	3
N-Nitrosoanabasine (NAB)	ppm	0.018 to 0.06	25	6
N-Nitrosoanatabine (NAT)	ppm	0.420 to 0.730	25	6
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	ppm	0.182 to 0.240	25	6
N-Nitrosornicotine (NNN)	ppm	0.478 to 0.910	25	6
Total TSNAs <sup>1</sup>	ppm	1.111 to 1.920	25	6
Ammonia	ppm	584 to 700	9	3
Glycerol	%	<0.005 to 0.12	15	6
Propylene glycol	%	2.54 to 2.97	15	6
Triethylene glycol	%	<0.005 to <0.01	4	2

1. Total TSNAs: sum of NAB, NAT, NNK, and NNN

**Table 2. CRP2**

Analyte	units	Range	Observations	Labs
Aflatoxin B1,B2,G1,G2	ppb	<0.18 to 0.26	6	2
Ochratoxin A	ppb	<0.5 to <0.4	9	3
Nicotine	%	1.173 to 1.449	21	7
Nornicotine, anatabine, anabasine	%	0.0386 to 0.0648	12	4
Total sugars	%	<1 to 0.58	12	4
Acetaldehyde	ppm	0.1432 to 1.29	6	2
Acrolein	ppm	0.069 to 0.076	3	1
Crotonaldehyde	ppm	0.006 to 0.014	3	1
Formaldehyde	ppm	0.274 to 1.100	6	2
pH		7.47 to 7.85	15	5
Ash	%	5.86 to 16.35	11	4
Calcium (Ca)	%	1.01 to 2.70	11	4
Potassium (K)	%	1.33 to 1.83	11	4
Sodium (Na)	%	2.29 to 3.37	8	3
Arsenic (As)	ppm	0.079 to 0.177	9	3
Cadmium (Cd)	ppm	0.539 to 1.10	11	4
Chromium (Cr)	ppm	0.250 to 0.441	9	3
Mercury (Hg)	ppm	<0.09 to 0.170	8	3
Nickel (Ni)	ppm	0.590 to 3.95	11	4
Lead (Pb)	ppm	0.150 to 3.12	11	4
Selenium (Se)	ppm	0.040 to 0.060	6	2
Nitrate	%	1.329 to 1.620	14	5
Nitrite	ppm	3.61 to 5.57	9	3
Nitrososarcosine	ppb	28.7 to 58.4	3	1
N-Nitrosodimethylamine (NDMA)	ppb	0.98 to 3.89	12	4
Moisture (oven volatiles)	%	53.10 to 54.93	18	6
Benzo[a]pyrene	ppb	8.3 to 49.4	12	4
Polonium-210	pCi/g	<0.58 to 0.273	3	3
N-Nitrosoanabasine (NAB)	ppm	0.12 to 0.21	25	6
N-Nitrosoanatabine (NAT)	ppm	1.46 to 2.23	25	6
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	ppm	0.37 to 0.58	25	6
N-Nitrosornicotine (NNN)	ppm	1.44 to 2.12	25	6
Total TSNAs <sup>1</sup>	ppm	3.38 to 5.00	25	6
Ammonia	ppm	1870 to 2093	9	3
Glycerol	%	<0.005 to <0.5	13	5
Propylene glycol	%	<0.005 to <0.5	13	5
Triethylene glycol	%	<0.005 to <0.01	4	2

1. Total TSNAs: sum of NAB, NAT, NNK, and NNN

**Table 3. CRP3**

Analyte	units	Range	Observations	Labs
Aflatoxin B1,B2,G1,G2	ppb	<0.18 to 0.26	6	2
Ochratoxin A	ppb	<0.50 to 0.704	9	3
Nicotine	%	1.719 to 2.350	24	8
Nornicotine, anatabine, anabasine	%	0.086 to 0.120	12	4
Total sugars	%	0.47 to 1.4	12	4
Acetaldehyde	ppm	0.381 to 0.565	3	1
Acrolein	ppm	<0.007	3	1
Crotonaldehyde	ppm	0.007 to 0.011	3	1
Formaldehyde	ppm	0.274 to 0.672	6	2
pH		6.70 to 6.86	15	5
Ash	%	10.21 to 27.82	11	4
Calcium (Ca)	%	1.52 to 4.90	11	4
Potassium (K)	%	3.21 to 5.10	11	4
Sodium (Na)	%	0.55 to 1.08	8	3
Arsenic (As)	ppm	0.234 to 0.402	9	3
Cadmium (Cd)	ppm	1.27 to 2.20	11	4
Chromium (Cr)	ppm	2.55 to 7.98	11	4
Mercury (Hg)	ppm	0.01 to 0.65	8	3
Nickel (Ni)	ppm	3.12 to 9.50	11	4
Lead (Pb)	ppm	0.61 to 6.50	11	4
Selenium (Se)	ppm	0.08 to 0.185	6	2
Nitrate	%	4.10 to 5.12	14	5
Nitrite	ppm	8.98 to 14.24	9	3
Nitrososarcosine	ppb	85.3 to 116	3	1
N-Nitrosodimethylamine (NDMA)	ppb	<2 to 4.11	12	4
Moisture (oven volatiles)	%	7.3 to 9.03	18	6
Benzo[a]pyrene	ppb	11.94 to 54.32	12	4
Polonium-210	pCi/g	<0.56 to 0.507	3	3
N-Nitrosoanabasine (NAB)	ppm	0.293 to 0.49	25	6
N-Nitrosoanatabine (NAT)	ppm	4.09 to 6.97	25	6
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	ppm	2.69 to 5.05	25	6
N-Nitrosornicotine (NNN)	ppm	6.358 to 9.718	25	6
Total TSNAs <sup>1</sup>	ppm	14.61 to 21.867	25	6
Ammonia	ppm	3480 to 4146	9	3
Glycerol	%	<0.005 to <0.5	13	5
Propylene glycol	%	<0.005 to <0.5	13	5
Triethylene glycol	%	<0.005 to <0.01	4	2

1. Total TSNAs: sum of NAB, NAT, NNK, and NNN

**Table 4. CRP4**

Analyte	units	Range	Observations	Labs
Aflatoxin B1,B2,G1,G2	ppb	<0.18 to 0.26	6	2
Ochratoxin A	ppb	<0.4 to <0.5	9	3
Nicotine	%	0.903 to 1.354	21	7
Nornicotine, anatabine, anabasine	%	0.0832 to 0.1185	12	4
Total sugars	%	6.6 to 37.47	12	4
Acetaldehyde	ppm	0.609 to 0.739	3	1
Acrolein	ppm	0.048 to 0.054	3	1
Crotonaldehyde	ppm	0.016 to 0.020	3	1
Formaldehyde	ppm	0.119 to 0.645	6	2
pH		5.88 to 6.10	15	5
Ash	%	4.37 to 12.11	11	4
Calcium (Ca)	%	0.89 to 2.31	11	4
Potassium (K)	%	1.20 to 2.37	11	4
Sodium (Na)	%	0.51 to 1.10	8	3
Arsenic (As)	ppm	0.096 to 0.127	9	3
Cadmium (Cd)	ppm	0.559 to 1.45	11	4
Chromium (Cr)	ppm	0.550 to 0.620	9	3
Mercury (Hg)	ppm	<0.01 to 0.16	8	3
Nickel (Ni)	ppm	0.731 to 9.55	11	4
Lead (Pb)	ppm	0.274 to 3.55	11	4
Selenium (Se)	ppm	0.030 to 0.049	6	2
Nitrate	%	1.20 to 1.48	14	5
Nitrite	ppm	<0.66 to 2.82	9	3
Nitrososarcosine	ppb	12.3 to 22.6	3	1
N-Nitrosodimethylamine (NDMA)	ppb	<0.25 to 2.04	12	4
Moisture (oven volatiles)	%	20.49 to 22.90	18	6
Benzo[a]pyrene	ppb	0.73 to 1.33	9	3
Polonium-210	pCi/g	<0.63 to 0.492	3	3
N-Nitrosoanabasine (NAB)	ppm	0.05 to 0.09	25	6
N-Nitrosoanatabine (NAT)	ppm	0.96 to 1.67	25	6
4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)	ppm	0.34 to 0.51	25	6
N-Nitrosornicotine (NNN)	ppm	1.53 to 2.43	25	6
Total TSNAs <sup>1</sup>	ppm	2.99 to 4.65	25	6
Ammonia	ppm	1890 to 2621	9	3
Glycerol	%	3.850 to 4.84	15	6
Propylene glycol	%	<0.005 to <0.5	13	5
Triethylene glycol	%	<0.005 to <0.01	4	2

1. Total TSNAs: sum of NAB, NAT, NNK, and NNN

## APPENDIX IV: Analytical Method Summary

Aflatoxin B <sub>1</sub> ,B <sub>2</sub> ,G <sub>1</sub> ,G <sub>2</sub> : UHPLC-MS/MS
Ochratoxin A: UHPLC-MS/MS
Nicotine: CRM 62; CDC (Reference Citation #2); Health Canada T-301; Continuous Flow
Nornicotine, anatabine, anabasine: Health Canada T-301; GC-FID
Total sugars: Ion Chromatography; Continuous Flow
Acetaldehyde: HPLC-UV (detected as the 2,4-dinitrophenylhydrazine derivative)
Acrolein: HPLC-UV (detected as the 2,4-dinitrophenylhydrazine derivative)
Crotonaldehyde: HPLC-UV (detected as the 2,4-dinitrophenylhydrazine derivative)
Formaldehyde: HPLC-UV (detected as the 2,4-dinitrophenylhydrazine derivative)
pH: Health Canada T-310; CRM 69; CDC (Reference Citation #2)
Ash: 550°C; 650°C
Calcium: ICP; Flame AAS
Potassium: ICP; Flame AAS
Sodium: ICP; Flame AAS
Arsenic (As): ICP-MS; AAS
Cadmium (Cd): ICP-MS; AAS
Chromium (Cr): ICP-MS; AAS
Mercury (Hg): ICP-MS; AAS
Nickel (Ni): ICP-MS; AAS
Lead (Pb): ICP-MS; AAS
Selenium (Se): ICP-MS; AAS
Nitrate: CRM 36; Health Canada T-308
Nitrite: CRM 36; UV-VIS; Ion Chromatography
Nitrososarcosine: LC-MS/MS
N-Nitrosodimethylamine (NDMA): UHPLC-MS/MS; GC-TEA
Moisture (oven volatiles): CDC(Reference Citation #2); AOAC 966.02
Benzo[a]pyrene: Health Canada T-307; UHPLC-FLD
Polonium-210: Alpha emission spectrometry
NAB, NAT, NNK, NNN, total TSNAs: CRM 72; LC-MS/MS; GC-TEA
Ammonia: Health Canada T-302; Ion Chromatography; Continuous Flow
Glycerol: CRM 60; CRM 61; GC-FID
Propylene glycol: CRM 60; CRM 61; GC-FID
Triethylene glycol: CRM 60

### Abbreviations:

AAS – Atomic Absorption Spectrometry

GC-FID – gas chromatography/ flame ionization detector

GC-TEA – gas chromatography/ thermal energy analyzer

HPLC-UV – high performance liquid chromatography/ ultraviolet detector

ICP – Inductively coupled plasma

ICP-MS - Inductively coupled plasma/ mass spectrometry

LC-MS/MS – liquid chromatography/ spectrometry/mass spectrometry

UHPLC-MS/MS – ultrahigh performance liquid chromatography mass spectrometry/mass spectrometry