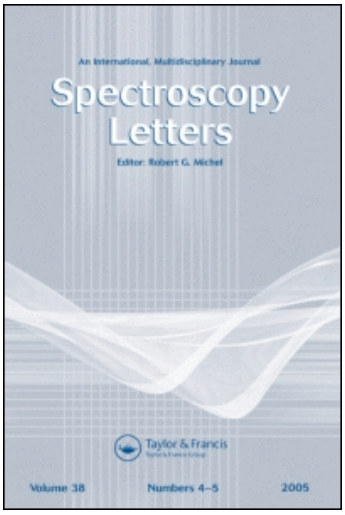

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ABSTRACT A closed-vessel microwave-digestion method was developed for the determination of trace amounts of Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, Tl, and Pb by inductively coupled plasma mass spectrometry and Hg by cold-vapor atomic absorption spectrometry in cigarette tobacco samples. In order to gauge the effectiveness of the digestion procedure, recovery studies were conducted using solutions prepared from National Institute of Standards and Technology Standard Reference Material 1573a Trace Elements in Tomato Leaves and Polish Certified Reference Material Virginia Tobacco Leaves. Limits of detection were below $1\ \mu g\ g^{-1}$ for all elements studied. Samples from two genuine-brand and three counterfeit packs were analyzed. The mean amounts of Be, As, Mo, Cd, Sb, Tl, Pb, and Hg were higher in counterfeit cigarettes, while the amounts of V, Cr, Mn, Co, Cu, Zn, Se, and Ba were comparable among legal and counterfeit cigarettes; the amount of Ni was higher in the legal cigarettes. Evaluation of Be, As, Mo, Cd, Sb, Tl, Pb, and Hg with their potential hazards for smokers is briefly discussed.

KEYWORDS cigarettes, counterfeit, CV atomic absorption spectrometry, inductively coupled plasma mass spectrometry, mercury, tobacco, trace metals

INTRODUCTION

By now, it is well established that cigarette smoking is dangerous to human health. Tobacco-related illnesses remain the leading cause of preventable death, with approximately 430,000 deaths in the USA[1] and approximately four million annual deaths worldwide attributed to tobacco use.[2] Smoking-related health risks include heart disease and stroke, as well as cancers of the lung, mouth, larynx, throat, esophagus, bladder, kidney, pancreas, liver, cervix, colon, and stomach.

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Tobacco smoke consists of mainstream smoke (MSS) and sidestream smoke (SS). MSS is the smoke inhaled by a smoker, and SS is the smoke that is released from the burning tip of a cigarette between puffs. Researchers have found that SS contains higher levels of cancer-causing substances than does MSS per unit mass of smoke particulate material.[3] This is one reason why passive smoking has become such a health concern. A study by Invernizzi et al.[4] investigated the transfer of particulate matter ($PM_{2.5}$) pollution from smoking to nonsmoking coaches in a train. The smoking coaches were separated from the adjacent nonsmoking carriages by automatic sliding doors, and each coach was equipped with a separate HVAC (heat, ventilation, and air conditioning) system. $PM_{2.5}$ concentrations were measured in the coaches in real time (sampling interval of 2 min) for short time periods (<1 h). The results showed that the nonsmoking coaches next to the smoking coaches had $PM_{2.5}$ concentrations that reached 180 $\mu g\,m^{-3}$. Measurements taken in the smoking coach revealed exceedingly high values of $PM_{2.5}$ that reached up to about 250 $\mu g\,m^{-3}$. For comparison, the current United States Environmental Protection Agency ambient air quality standard for $PM_{2.5}$ limit averaged over a 24-h period is 35 $\mu g\,m^{-3}$.[5]

The tobacco plant, *Nicotiana tabacum*, is known to readily absorb trace elements from the soil and to accumulate them in its leaves at unusually high concentrations. Trace elements have important effects on many life processes. Some of these elements are toxic for humans, even at very low levels of intake. Tobacco smoking delivers the stored trace elements to the lungs.[6] The fraction of a particular metal that is transferred to the smoke phase varies substantially, depending on the volatility and other properties of the element.[7–10] For example, 4–6% of the tobacco Cd is transferred to MSS smoke, while about 40% ends up in SS smoke; 16–22% of Pb is transferred to MSS smoke and 18–30% into SS smoke.[7,10] Some of these readily pass into the bloodstream and are accumulated, cause damage to the organs (mainly kidney and liver), and act as tumor promoters in conjunction with carcinogens.[11,12]

Since the tobacco plant concentrates heavy metals from its growing environment,[13–15] in particular, accumulating Cd in its leaves,[16] acidic or contaminated soil is usually avoided for tobacco cultivation. Growing tobacco on strongly acidic (but unpolluted) soils is known to enrich the lower leaves in Cd by as much as five-fold.[17] After this became known, the major manufacturers of genuine cigarette brands tended to avoid such crops, instead seeking sources of tobacco cultivated on less acidic soils. A possible consequence is that the rejected crops find their way into counterfeit production. Between 2000 and 2001, 5% of all cigarettes smoked in Britain were thought to be counterfeits produced in illegal factories in China, and the number has increased in subsequent years.[18,19] Counterfeit cigarettes are also a problem in the United States, according to the Bureau of Alcohol, Tobacco, and Firearms.[19] Research carried out at the University of St. Andrews, UK, showed that counterfeits being sold in the United Kingdom can contain five times as much Cd and six times as much Pb as genuine-brand cigarettes.[19] Smokers of counterfeit cigarettes are exposed to higher concentrations of heavy metals, including the human carcinogens Cd, Ni, and As.[20]

Trace elements are present in tobacco as a result of a combination of both air deposition and soil uptake, with the physiology of the plant determining the importance of the particular source. Numerous factors influence the trace element concentrations found in tobacco, including genotype, soil type and pH, stalk position, and application of metal-containing pesticides to leaves. A thorough discussion of metal concentrations and sources in counterfeit tobacco products has been given by Stephens et al.[20] In brief, there is no obvious reason that counterfeiters deliberately add these metals to their products, but by the way they are manufactured, toxic chemicals enter into the tobacco products.[20] China is the world's largest producer of counterfeit cigarettes, mostly in the southern provinces of Guangdong and Fujian. It is understood that China accounts for 80% of the known supplies of counterfeit cigarettes; in 2006, the Chinese government seized over nine billion counterfeit cigarettes.[21]

The elemental contents of environmental samples have been measured by various methods. Nondestructive methods, such as X-ray fluorescence (XRF),[22,23] polarized energy dispersive XRF (EDPXRF),[20] proton-induced X-ray emission (PIXE),[23] and instrumental neutron activation analysis (INAA),[24,25] are able to measure the solid samples directly. For these techniques, no sample digestion is needed, and thus,

the risk of contamination during sample preparation is lower. INAA, in particular, can be used as a reference method. However, these methods sometimes have high detection limits and are often time consuming and require high sample amounts and powerful irradiation facilities. More commonly applied techniques include atomic absorption spectroscopy (AAS) methods,[26] both flame and electrothermal, and inductively coupled plasma (ICP) techniques. However, matrix effects can cause major interferences in XRF and PIXE. AAS (Graphite Furnace, Flame, or Hydride Generation AAS) can give accurate data, but a separate determination has to be made for each element. ICP atomic emission spectrometry (ICP-AES) is a rapid, multi-element technique that exhibits few matrix or chemical interferences,[27] but it lacks the sensitivity, and often the selectivity, to accurately determine many of the less abundant but more important elements in environmental samples.

ICP mass spectrometry (ICP-MS) has become one of the most attractive detection systems for the determination of trace and ultra-trace elements in environmental samples. The ICP-MS technique provides excellent detection limits, wide linear dynamic range, multi-element capability, the capability to measure isotope ratios, high sample throughput, and in-house analysis capability, all at a relatively low cost. While modern ICP-MS instruments are more stable over longer time periods than earlier models, for quantitative measurements, it is necessary to correct for instrument drift using internal standards, frequent calibration checks, or a combination of both.

While ICP-MS can be applied to solid samples, for example, with the use of laser ablation, it is most sensitive and quantitative when used with liquid sample introduction via solution nebulization.[28] The application of this technique to environmental samples, therefore, requires dissolution of samples prior to analysis. The dissolution is usually accomplished via wet digestion procedures, in which various combinations of mineral acids, such as HNO_3, $HNO_3 + HCl$, $HNO_3 + HCl + HF$, and $HNO_3 + HClO_4$, are used.[29–31] Wet digestion procedures are usually slow and tedious, however, needing constant monitoring, and they are subject to possible contamination and potential loss of some volatile elements.

The use of closed-vessel systems for acid digestion, especially with microwave ovens, has now become routine.[32–36] Microwave-assisted digestion has the advantages of reduced time for sample dissolution, fewer possibilities for technical errors caused by the spilling of hot digestion solutions, lower consumption of reagents, and lower losses of volatile metals.[33] In addition, modern microwave ovens are safer and simpler and provide more controlled and reproducible conditions than does a hot plate or block digester.[32]

In this article, a microwave digestion method used prior to the measurement of trace amounts of Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, Tl, and Pb by ICP-MS and Hg by cold-vapor AAS in cigarette tobacco samples is described. In order to gauge the effectiveness of the digestion procedure, recovery studies were conducted using solutions prepared from the National Institute of Standards and Technology (NIST) Standard Reference Material 1573a Trace Elements in Tomato Leaves and Virginia Tobacco Leaves (CTA-VTL-2), a certified reference material from Poland. To demonstrate the practicality of microwave digestion for the pretreatment of cigarette tobacco samples and analysis for trace elements, samples from two genuine and three counterfeit cigarette packs obtained by Wadsworth Center from the New York State Department of Taxation and Finance were analyzed.

MATERIALS AND METHODS

Samples

Five sets of cigarettes were received by the Wadsworth Center from the New York State Department of Taxation and Finance. Two sets were genuine-brand cigarettes: (1) Marlboro Gold Virginia Stamped and (2) Marlboro Red Virginia Stamped, and three sets were cigarette samples identified as counterfeit (1) Marlboro Red stamp H0631, (2) Marlboro Gold-wrapped, and (3) Marlboro Gold-unwrapped.

Standards and Reference Materials

The standards used for recovery studies were Standard Reference Material (SRM) 1573a Tomato Leaves from NIST (NIST 1573a; Gaithersburg, Maryland, USA) and CTA-VTL-2 Virginia Tobacco Leaves, a Certified Reference Material (CRM) from the Institute of Nuclear Chemistry and Technology

(INCT CTA-VTL-2; Warsaw, Poland). Calibration standards were from Spex Certiprep (Metuchen, New Jersey, USA), and independent calibration verification standards were from Inorganic Ventures (Lakewood, New Jersey, USA). Hydrofluoric acid 47–51%, environmental grade plus from Alfa Aesar (Ward Hill, Massachusetts, USA), nitric acid 70%, Ultrex II ultra pure reagent grade from J.T. Baker (Phillipsburg, New Jersey, USA), and hydrogen peroxide 30–32%, from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA) were used to digest the samples. Reagent-grade sodium borohydride, sodium hydroxide, potassium iodide, and ascorbic acid from Aldrich were used for hydride generation. Reagents used for Hg analysis were: potassium permanganate, 5% solution; potassium persulphate, 5% solution; and hydroxylamine hydrochloride/sodium hydrochloride, 12% solution from J.T. Baker; nitric acid, 70%; and sulfuric acid, concentrated, AR select grade from Mallinkrodt (Phillipsburg, New Jersey, USA).

Microwave Digestion System

The microwave oven used to digest samples was a CEM Model MARS 5 (CEM Corporation, Matthews, North Carolina, USA). It has a 2450-MHz microwave power system with operator selectable output of 0–1200 W; a fluoropolymer-coated microwave cavity; a cavity exhaust fan and tubing to vent fumes; a digital computer programmable for 100 programs consisting of up to five stages each; a 14-position, alternating rotation turntable; 100-mL Teflon vessels with 355° pressure release valves resistant up to 350 psi and 210°C; pressure and temperature sensors; and three-door safety interlocks and an interlock monitoring system to prevent microwave emission when the door is open. A microcomputer controls and monitors the operation.

Prior to their use, the Teflon vessels were first soaked in Liquinox detergent solution (Alconox, White Plains, New York, USA) and placed in an ultrasonic bath for 30 min, then soaked in 35% HNO_3 overnight. After being rinsed thoroughly with deionized distilled water (DDW), the vessels were filled with 10 mL of 0.1-M nitric acid and treated in the microwave oven under the same conditions as used for the sample digestions. After being cooled, they were flushed once more with DDW.

Sample Digestion

As mentioned above, several different acid combinations are in use for the digestion of solid samples for ICP analysis. In general, it is desirable to use the minimum amount of reagents necessary to effect total digestion of the sample in order to limit interferences and improve sensitivity. With this in mind and from knowledge from previous work,[35,36] the choice of reagents was limited to HNO_3, H_2O_2, and HF for the digestion procedure. Some botanical materials contain silica and require HF for total digestion. Of particular interest were the toxic metals Be, As, Se, Cd, Sb, Tl, Hg, and Pb, and the selection of the method parameters was influenced by this factor. Also included were several less toxic metals in order to demonstrate the utility of the method for trace metals analysis. Since the intent of this study was to use ICP-MS to measure the concentration of these analytes, HCl was not included in the reagents used for digestion in order to avoid the ArCl interference on As and other chloride interferences. We also avoided the use of boric acid (typically used to remove excess HF) so as to eliminate interferences with V and other transition metals; instead the ICP-MS spectrometer was equipped with an inert sample introduction system. These interferences may not be of concern on newer ICP-MS instruments equipped with a collision or reaction cell.

Six digestion reagent mixtures (termed Methods 1–6) were tested with various combinations of HNO_3, HF, and H_2O_2 (Table 1). In order to provide acceptable recovery statistics, five portions (nominally 250 mg) of each of the reference materials were placed in digestion vessels with 2.0 mL of HNO_3. One set (Method 1) used HNO_3 only. The other methods also entailed the use of H_2O_2 (4.0 mL) and concentrated HF (0.0, 0.1, 0.2, 0.3, and 0.5). The vessels were capped, placed in the

TABLE 1 Digestion Reagents

Method	HNO_3 (ml)	H_2O_2 (ml)	HF (ml)	H_2O (ml)
1	2	–	–	4
2	2	4	–	–
3	2	4	0.1	–
4	2	4	0.2	–
5	2	4	0.3	–
6	2	4	0.5	–

microwave system, and digested. In initial tests, the vessels developed high pressures, so a two-step procedure was used in order minimize the chance of venting through the pressure release valves. The digestions were performed according to the following procedure:

Step 1. The temperature was ramped to 110°C (maximum pressure 300 PSI) within 20 min with the application of 600 W power, followed by a dwell time of 5 min at 110°C and an initial cooling in the microwave oven for 5 min. The vessels were then removed from the oven and cooled in a freezer at −20°C for 1 h, after which they were vented and opened.

Step 2. The temperature was ramped to 200°C (maximum pressure 350 PSI) within 10 min with the application of 1200 W power, followed by a dwell time of 10 min at 200°C. They were again cooled in the microwave oven for 5 min and in a freezer at −20°C for 1 h. The digestates were then transferred to polyethylene sample tubes and the volume made up to 25 ml with DDW.

ICP-MS

The samples were analyzed on an Agilent 4500 ICP mass spectrometer (Agilent Technologies, Palo Alto, California, USA), equipped with ChemStation Software and a Cetac Technologies ASX 500 autosampler (Cetac Technologies, Omaha, Nebraska, USA). For most elements, a Babington nebulizer, a quartz spray chamber, and an inert (platinum) injector (2.5-mm i.d.) were used. The typical operating conditions are listed in Table 2. The instrument was tuned before each sample run with a combination of a software autotuning procedure and manual tuning, using a solution consisting of 10 $\mu g\,L^{-1}$ each of Li, Y, Ce, and Tl in 1% HNO_3. Typical ion counts were 20 $K\,s^{-1}$, 60 $K\,s^{-1}$, and 40 $K\,s^{-1}$ for Li, Y, and Tl, respectively, with relative standard deviations between 1% and 3%. The doubly charged ion ratio ($^{140}Ce^{2+}/^{140}Ce^{+}$) and oxide ratio ($[^{140}Ce^{16}O]+/^{140}Ce^{+}$) were maintained below 1% and 3%, respectively. The sample solution and the internal standard solution (consisting of 1 $mg\,L^{-1}$ of Sc, Y, In, and Bi) were pumped by a peristaltic pump and mixed in a mixing block before being introduced into the nebulizer. During the sample analysis, three points per mass were sampled for each element, with an integration time of 0.1 s per point for all elements except As and Se, for which an integration time of 1.0 s per point was used.

TABLE 2a Normal Plasma ICP-MS Parameters

RF Power	1350 W
RF Matching	2.0 V
Sample Depth	8.2 mm
Carrier Gas	1.12 $L\,min^{-1}$
Blend Gas	0.05 $L\,min^{-1}$
PeriPump	0.10 rps
Spray Chamber Temperature	2°C

TABLE 2b Cool Plasma ICP-MS Parameters (Hydride Generation)

RF Power	750 W
RF Matching	2.0 V
Sample Depth	6.2 mm
Carrier Gas	0.45 $L\,min^{-1}$
Blend Gas	0.75 $L\,min^{-1}$

Hydride Generation ICP-MS

As and Se both showed large positive interferences when analyzed with the nebulized aqueous sample introduction, possibly due to dissolved carbon in the digestate. Carbon is known to enhance the signal from elements with high first ionization potentials in ICP plasmas, such as As and Se, possibly due to its role as an electron acceptor.[37]

It was decided to analyze these two elements via hydride generation ICP-MS, which separates the analyte from the aqueous matrix by producing a volatile hydride that is introduced directly into the plasma. Hydride generation ICP-MS has fewer interferents and better detection limits than by using nebulization ICP-MS, but requires more sample preparation. Since these two elements require different reducing conditions for hydride generation, they were analyzed separately by hydride generation ICP-MS with the same ICP-MS instrument, using the Agilent ISIS system and shield torch under cool plasma conditions. We followed a pre-reduction and analysis procedure developed by Wilbur.[38] Typical parameters are shown in Table 2. No internal standards were used for these analyses; instead, a check standard was run after every five samples to adjust for instrument drift.

Mercury Analysis

Since Hg was one of the elements interest, it was decided to analyze the Hg content of the samples using CVAAS. While Hg analysis by ICP-MS is possible and is becoming increasingly popular, Hg not currently measured by ICP-MS in this laboratory. The CVAAS method is robust for many different sample matrices and has excellent detection limits. Three 6-mL aliquots of microwave digestates were redigested for Hg analysis for each method. The samples were placed in polyethylene vessels with 900 µL potassium permanganate, 150 µL concentrated nitric acid, 300 µL concentrated sulfuric acid, 480 µL potassium persulphate, and 360 µl hydroxylamine hydrochloride. Calibration standards were prepared in a similar manner. The standards and samples were then digested for 2 h at 95°C in a water bath (Precision Model 265 from Thermo Electron Corporation, Winchester, Virginia, USA). Hg levels were determined on a Leeman Labs Hydra AA cold vapor spectrophotometer (Thermodyne Leeman Labs, Hudson, NH) according to EPA method 245.1, which used a dual-beam detection system with a 254-nm emission source.

RESULTS AND DISCUSSION

Proper sample preparation is the first step required for successful analysis of elemental components of plant tissue samples. The digestion procedure must dissolve the analytes of interest in a matrix suitable for the type of analysis with minimal sample loss and contamination. In order to check the effectiveness of the digestion methods in accomplishing this task, analyzed reagent blanks, spiked reagent blanks, and two certified reference materials, NIST 1573a and INCT CTA-VTL-2 were analyzed.

Blanks and Limits of Detection

A reagent blank was run with each set of reference materials or real sample digestion experiment. The reagent blanks were treated and analyzed in exactly the same way as the real samples. The limit of detection (LOD) is most often expressed as the lowest concentration of an element that can be determined to be statistically different from an analytical blank. It is here defined as three times the standard deviation of measurements from 10 digestion blanks. As the blank contained all of the digestion reagents, but not the sample matrix, the LOD obtained here is a hydride detection limit rather than a full method detection limit. Hg was analyzed by cold vapor AAS, As and Se were analyzed by hydride generation ICP-MS, and the other metals were analyzed by normal nebulization ICP-MS. LODs for the six methods are listed in Table 3 and are below 1 $\mu g\,g^{-1}$ for all of the elements studied.

TABLE 3 Detection Limits

Element	Method 2 ($\mu g\,g^{-1}$)	Method 6 ($\mu g\,g^{-1}$)
Be	0.003	0.005
V	0.018	0.018
Cr	0.032	0.032
Mn	0.090	0.090
Co	0.007	0.007
Ni	0.130	0.028
Cu	0.142	0.142
Zn	0.040	0.040
As[a]	0.024	0.135
Se[a]	0.050	0.060
Sr	0.050	0.013
Mo	0.003	0.004
Cd	0.003	0.004
Sb	0.050	0.004
Ba	0.029	0.029
Tl	0.005	0.005
Pb	0.021	0.021
Hg[b]	0.020	0.020

[a]Analyzed by hydride generation ICP-MS.
[b]Analyzed by CVAAS.

Sample Analysis

The proposed digestion method was validated by (1) evaluation of the recovery from the microwave digestion procedure of a multi-elemental standard solution spiked into a reagent blank, and (2) analysis of two reference materials (NIST SRM 1573a and INCT CTA-VTL-2). The digestion method proposed was used to analyze the tobacco in legal and counterfeit cigarettes.

For assessment of the instrumental recovery of dissolved analytes, spike solutions were prepared at the 20 $\mu g\,L^{-1}$ level by the addition of a multi-element standard solution to reagent blanks, and the proposed microwave digestion procedures and elemental analyses were performed to examine the recovery of the elements of interest. Methods 1 and 2, which do not use any HF, have good recoveries for most of the elements analyzed, with Method 2

having slightly better overall recoveries and somewhat lower standard deviations. The methods that employ HF decrease the spike recoveries for the first-row transition metals V, Cr, Mn, Co, Ni, Cu, and Zn, as well as for As and Se. Most of these elements are known to form volatile or insoluble compounds with HF.[39] As the HF concentration is increased, the recoveries for these elements increase somewhat, with Method 6 having the best overall recoveries among the methods employing HF, possibly due to increased complexation of the metal cations (Table 4). Method 6 has better recoveries for Co, Ni, Cu, and Zn than does Method 2, as well as comparable recoveries for the heavier elements Mo, Cd, Sb, Ba, Tl, and Pb; Hg recoveries are excellent for all six methods (100–109%).

Further increasing the amount of HF used might improve the recoveries of some of the elements, but it may decrease the recoveries of elements that form volatile fluoride compounds. In past experiments, it has been seen that increasing the HF concentration results in losses for Se and As,[35] two of the elements of interest in this study, so this route was not pursued further in this study.

For determination of accuracy and precision of the proposed dissolution method for tobacco product samples, NIST SRM 1573a and INCT CTA-VTL-2 materials were used as references in each of the six methods. Table 5 shows the recoveries of V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, and Hg in the NIST 1573a SRM from all six methods. Be, Tl, and Pb are not listed in the table because there is no certified value in this SRM. Method 2 seems to have better overall percent recovery for most elements (V, Mn, As, Se, Mo, Sb, and Ba); values were between 95% and 115% with good precision (standard deviations between 1% and 5%). Cu recovery was low (49–59%), possibly due to the formation of insoluble fluoride compounds, such as Ni (56–69%), from all six methods; both have very low standard deviations (1–5%). Cr, Co, Zn, and Hg recoveries were between 82% and 93% with low standard deviation (between 1% and 5% for Cr, Co, and Zn and 12% for Hg).

Table 6 shows the recoveries of V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, Pb, and Hg from all six methods in the INCT CTA-VTL-2 CRM. Be and Tl are not listed in the table because there is no certified value in the standard. Again, Method 2 seems to have a better overall percent recovery for most elements (Mn, As, Se, Mo, Cd, Ba, Pb, and Hg); values were between 94% and 105%, with good precision (standard deviations between 1% and 7%). Sb recovery was 99% with a standard deviation of 12%, while V, Cr, Co, Ni, Cu, and Zn had average recoveries between 64% and 87% with good precision (standard deviations between 1% and 4%).

TABLE 4 Spiked Digestion Blank Recovery (%)

	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
Be	106	97	69	54	74	68
V	106	106	90	79	75	89
Cr	103	102	86	74	71	84
Mn	113	118	90	118	86	110
Co	103	103	83	71	66	82
Ni	95	102	55	62	73	70
Cu	97	108	52	58	83	69
Zn	108	88	57	55	68	83
As[a]	103	104	63	72	80	79
Se[a]	105	105	61	73	88	78
Mo	106	107	115	102	109	105
Cd	107	104	93	88	90	92
Sb	107	105	103	13	92	99
Ba	110	108	104	131	92	94
Tl	108	103	92	88	86	88
Pb	107	105	89	102	94	86
Hg[b]	105	103	109	102	106	100

[a]Analyzed by hydride generation ICP-MS.
[b]Analyzed by CVAAS.

TABLE 5 Trace Metals Recovery – NIST SRM 1573a

	Method 1		Method 2		Method 3		Method 4		Method 5		Method 6	
	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd
Be	[a]		[a]		[a]		[a]		[a]		[a]	
V	124	10.6	106	2	105	3	91	3	87	2	85	3
Cr	85	5.1	82	2	82	1	75	1	75	1	77	3
Mn	98	4.7	95	2	76	43	103	2	94	3	102	2
Co	81	5.1	83	5	91	1	92	2	99	3	117	6
Ni	69	2.0	66	3	56	1	66	2	68	2	67	5
Cu	59	1.3	49	5	51	1	59	2	59	1	59	5
Zn	49	1.4	52	2	49	1	51	2	54	1	58	6
As[b]	75	7	107	3	83	15	100	16	99	5	87	3
Se[b]	117	4	95	3	236	15	171	6	101	12	229	117
Mo	108	5.6	115	4	106	2	107	3	103	3	100	1
Cd	80	4.4	88	1	87	2	85	1	87	1	90	2
Sb	79	6.3	95	5	108	8	28	1	13	4	102	18
Ba	95	6.3	95	1	96	1	93	2	95	2	106	2
Tl	[a]		[a]		[a]		[a]		[a]		[a]	
Pb	[a]		[a]		[a]		[a]		[a]		[a]	
Hg[c]	106	5	93	12	101	13	98	18	76	4	76	6

[a]No value listed for these elements in reference material.
[b]Analyzed by hydride generation ICP-MS.
[c]Analyzed by CVAAS.

Comparison with Literature Results

There have been several previous studies of trace metals in tobacco products that also used the INCT CTA-VTL-2 CRM, or a similar one, Oriental Tobacco Leaves (CTA-OTL-1), from the same vendor to assess recoveries. Table 7 shows a comparison of our results

TABLE 6 Trace Metals Recovery – INCT CRM CTA-VTL-2

	Method 1		Method 2		Method 3		Method 4		Method 5		Method 6	
	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd	Rec (%)	sd
Be	[a]		[a]		[a]		[a]		[a]		[a]	
V	94	1	87	1	82	3	83	2	79	2	79	2
Cr	75	2	74	2	71	1	73	3	67	2	71	3
Mn	102	1	98	2	98	2	102	2	95	1	104	2
Co	75	1	73	1	80	2	88	1	91	2	117	10
Ni	68	3	67	3	63	3	68	3	79	3	75	4
Cu	73	2	67	2	65	2	70	2	77	1	76	2
Zn	57	3	64	4	60	1	57	1	69	3	70	2
As[b]	96	10	100	3	124	25	102	8	112	7	102	5
Se[b]	78	2	102	4	126	12	81	5	51	16	115	2
Mo	101	3	98	2	103	2	100	2	106	2	110	9
Cd	85	1	94	2	94	1	92	1	93	2	99	1
Sb	88	7	101	13	103	10	16	1	72	8	110	12
Ba	104	3	103	3	116	2	116	3	114	1	129	1
Tl	[a]		[a]		[a]		[a]		[a]		[a]	
Pb	103	4	105	4	100	3	98	9	93	2	101	2
Hg[c]	108	5	105	7	105	9	95	10	97	7	103	195

[a]No value listed for these elements in reference material.
[b]Analyzed by hydride generation ICP-MS.
[c]Analyzed by CVAAS.

TABLE 7 Comparison of Recovery Results (%)

Analysis CRM	This work ICP-MS VTL	Dombovari et al.[40] ICP-MS OTL	Borkowska-Burnecka et al.[41] ICP-OES VTL	Dombovari[c] et al.[42] ICP-MS OTL
V	87	–	50	–
Cr	74	91	40	
Mn	98	100	95	101
Co	117	–	–	–
Ni	75	92	111	
Cu	76	110	107	102
Zn	70	–	102	106
As[a]	100	–	–	–
Se[a]	102	–	–	–
Mo	98	–	–	–
Cd	99	98	104	–
Sb	101	–	–	–
Ba	103	110	87	–
Pb	101	0	100	–
Hg[b]	105	–	–	–

[a]Analyzed by hydride generation ICP-MS.
[b]Analyzed by CVAAS.

with the findings of three previous works.[40–42] The results for Mn, Cd, Ba, and Pb are comparable among all of the studies. Our recovery for V was significantly better than that of Borokowska-Burnecka et al. (87% vs. 50%) This study showed a 74% recovery for Cr compared to 91% for Dombovari and 40% for Borokowska-Burnecka et al. The proposed method had much lower recoveries for Ni, Cu, and Zn than did those of the other authors; because this study was primarily interested in those metals that pose the most significant health concerns, there was no attempt to improve the recoveries for these elements. There were also good recoveries for Co, As, Se, Mo, Sb, and Hg, which were not measured in the earlier studies, and good spike recoveries for Be and Tl, which had no listed values in any of the reference materials.

Analysis of Counterfeit and Legal Cigarettes

Since Method 2 (2 mL $HNO_3 + 4$ mL H_2O_2) has the best overall recoveries for the majority of the elements that were of interest in this study, this method was used for the digestion of the legal and counterfeit cigarettes and analysis by ICP-MS. For the analysis of other plant materials with higher silicate content, or where other elements, especially crustal elements, are the analytes, Method 6 might yield better recoveries. The concentrations of 17 metals (Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, Tl, Pb, and Hg) in the cigarette tobacco from legal and counterfeit cigarettes are shown in Table 8a as μg metal per g of tobacco; Table 8b shows the concentrations expressed as μg per cigarette in order to facilitate the assessment of health effects. The weight of tobacco in a legal cigarette ranged between 0.52 g and 0.66 g with a mean of 0.61 g and relative standard deviation (RSD) of 6.6%, while the weight of a counterfeit cigarette ranged between 0.57 g and 0.78 g with a mean of 0.70 g and RSD of 6.8%. Table 8a shows that the amounts of Be, As, Mo, Cd, Sb, Tl, Pb, and Hg are higher in counterfeit cigarettes, while the amounts of V, Cr, Mn, Co, Cu, Zn, Se, and Ba, are comparable among legal and counterfeit cigarettes; unexpectedly, Ni is several-fold (mean 2.7-fold) higher in the legal ones.

A brief discussion of the concentrations and potential health implications of Be, Ni, As, Mo, Cd, Sb, Tl, Pb, and Hg is presented below. The toxicity of many trace elements varies with the chemical species and other factors, including simultaneous exposure to other trace metals, and is beyond the scope of this article. Further research on the chemical forms of trace metals in both MSS and SS tobacco smoke would be useful in assessing the risks from trace metals exposure from smoking both counterfeit and legal cigarettes. A different extraction procedure would probably be necessary as the microwave

TABLE 8a Trace Metal Concentrations ($\mu g\ g^{-1}$) in Legal and Counterfeit Cigarettes

Cigarettes	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As
Legal	0.016	0.948	1.273	142	0.425	1.180	4.00	18.2	0.250
	0.017	0.896	0.484	118	0.348	1.132	3.49	14.9	0.250
Mean	0.016	0.922	0.878	130	0.387	1.156	3.75	16.6	0.250
Counterfeit	0.034	0.812	0.302	153	0.394	0.358	2.94	13.7	0.370
	0.021	0.492	0.818	161	0.395	0.554	5.79	25.9	1.07
	0.029	1.098	1.586	95.1	0.293	0.372	3.38	9.04	0.420
Mean	0.028	0.801	0.902	136	0.360	0.428	4.04	16.2	0.620

	Se	Mo	Cd	Sb	Ba	Tl	Pb	Hg
Legal	0.230	0.383	0.017	0.050	75.1	0.060	0.604	0.020
	0.250	0.382	0.017	0.040	68.3	0.055	0.607	0.021
Mean	0.240	0.382	0.017	0.045	71.7	0.058	0.606	0.020
Counterfeit	0.340	0.596	0.054	0.080	69.8	0.141	4.60	0.054
	0.230	0.458	0.043	0.190	59.2	0.140	7.93	0.043
	0.180	0.884	0.049	0.080	80.2	0.100	4.54	0.049
Mean	0.250	0.646	0.049	0.117	69.7	0.127	5.69	0.049

digestion procedure is highly oxidizing and would most likely change the chemical form of some of the target elements.

Concentrations of Beryllium, Cadmium, and Thallium

The elements Be, Cd, and Tl are all toxic at low concentrations, and their amounts were higher in the counterfeit cigarettes than in the legal cigarettes with mean values 0.028 versus 0.016, 0.049 versus 0.017, and 0.127 versus 0.058 $\mu g\ g^{-1}$, respectively. According to the International Agency for Research on Cancer, beryllium and beryllium compounds are group 1 carcinogens in both animals and humans.[43] Cd salts are known carcinogens. Overexposure of Cd or Cd compounds has been associated with acute and chronic toxicity. Symptoms of acute poisoning from the inhalation of cadmium dusts or fumes include headache, chest pains, cough, metal fume fever, and weakness. Tl compounds, such as thallium sulfate, were widely used in the past as rat poisons and ant baits, but these uses are no longer allowed in the United States and many other

TABLE 8b Trace Metal Concentrations (μg per cigarette) in Legal and Counterfeit Cigarettes

Cigarettes	Mass (g)	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As
Legal	0.5920	0.009	0.561	0.754	84.2	0.252	0.699	2.37	10.8	0.148
	0.6570	0.011	0.589	0.318	77.7	0.229	0.744	2.29	9.82	0.164
Mean		0.010	0.575	0.536	81.0	0.240	0.721	2.33	10.3	0.156
Counterfeit	0.6797	0.023	0.552	0.206	104	0.267	0.243	2.00	9.30	0.251
	0.7810	0.017	0.385	0.639	126	0.309	0.433	4.52	20.2	0.836
	0.6771	0.020	0.744	1.07	64.4	0.198	0.252	2.29	6.12	0.284
Mean		0.020	0.560	0.639	98.0	0.258	0.309	2.94	11.9	0.457

		Se	Mo	Cd	Sb	Ba	Tl	Pb	Hg
Legal	0.5920	0.136	0.227	0.010	0.030	44.5	0.036	0.358	0.012
	0.6570	0.164	0.251	0.011	0.026	44.9	0.036	0.399	0.014
Mean		0.150	0.239	0.010	0.028	44.7	0.036	0.378	0.013
Counterfeit	0.6797	0.231	0.405	0.037	0.054	47.4	0.096	3.13	0.037
	0.7810	0.180	0.358	0.034	0.148	46.3	0.110	6.19	0.034
	0.6771	0.122	0.598	0.033	0.054	54.3	0.068	3.08	0.033
Mean		0.178	0.454	0.035	0.086	49.3	0.091	4.13	0.035

countries due to health safety concerns. Thallium and its compounds are very toxic and should be handled with great care.

Concentrations of Arsenic, Molybdenum, and Antimony

As, Mo, and Sb are all toxic elements and their amounts were about two to three times higher in the counterfeit cigarettes than in the legal cigarettes with mean values 0.620 versus 0.250, 0.646 versus 0.382, and 0.117 versus 0.045 $\mu g\,g^{-1}$, respectively. Tobacco may contain a substantial amount of As if arsenical insecticides have been used in the growing process. Arsenic and many of its compounds are especially potent poisons. Overexposure to As has been associated with increased risk of skin, liver, bladder, kidney, and lung cancers. Prolonged exposure to Mo dust can cause irritation to the eyes, nose, throat, and skin.[44] Sb poisoning is clinically very similar to As poisoning. Sb and its compounds have been reported to cause dermatitis, keratitis, conjunctivitis, and nasal septal ulceration by direct contact, or inhalation of fumes or dust.[45]

Concentration of Nickel

Ni is the only element analyzed that had higher concentrations in the legal cigarettes than in the counterfeits, probably due to higher Ni concentration in the soil where the tobacco is grown. Nickel suflide and certain other Ni compounds are suspected carcinogens.[46] Overexposure to Ni compounds can also cause sensitization dermatitis, allergic asthma, and pneumonitis.[45]

Concentrations of Mercury and Lead

Hg and Pb are heavy metals that have both acute and cumulative toxicities. Exposure to either of these elements can cause neurological damage, especially in young children, who can develop learning disabilities at very low exposure levels. The mean Hg concentration in the counterfeit cigarettes was much higher than in the legal ones, 0.049 versus 0.020 $\mu g\,g^{-1}$. Hg and most of its compounds are extremely toxic. It can be inhaled and absorbed through the skin and mucous membranes. Of all the elements analyzed, Pb showed the greatest enhancement in counterfeit cigarettes relative to the legal ones. The amount of Pb in counterfeits was nearly 10 times higher than in legal cigarettes with a mean value of 5.69 versus 0.606 $\mu g\,g^{-1}$. Pb is listed by the International Agency for Research on Cancer as a possible human carcinogen that can damage neural connections (especially in young children) and cause blood and brain disorders. Long-term exposure to Pb or its salts can cause nephropathy and colic-like abdominal pains.[45]

CONCLUSIONS

A microwave digestion method in a closed vessel was developed and optimized for the determination of trace amounts of Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, Tl, and Pb by ICP-MS and Hg by cold-vapor AAS in cigarette tobacco samples. In order to gauge the effectiveness of the digestion procedure, recovery studies were conducted using solutions prepared from NIST Standard Reference Material 1573a and INCT CTA-VTL-2, a certified reference material from Poland, and the results were compared to findings of previous studies. The method that used a mixture of 2 mL HNO_3 and 4 mL H_2O_2 yielded excellent recoveries, between 90% and 120% for most elements. The first-row transition elements V, Cr, Ni, Cu, and Zn had somewhat lower recoveries of 64–73%. The addition of 0.5 mL HF to the digestion mixture improved the recoveries of the latter elements significantly.

Samples were analyzed from two genuine and three counterfeit cigarettes packs. The results show that the concentrations of Be, Mn, As, Se, Mo, Cd, Tl, Pb, and Hg were significantly higher in counterfeit cigarette samples than in genuine-brand cigarette samples. The ratio of the metal concentration in counterfeit cigarettes to legal cigarettes for these elements is 1.93, 1.21, 2.93, 1.18, 1.90, 3.31, 2.53, 10.9, and 2.71, respectively.

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