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Characterization of Canadian Cigarettes Using Multi-Stable Isotope Analysis by Gas Chromatography−Isotope Ratio Mass Spectrometry

MARIE-JOSÉE BINETTE,* PIERRE LAFONTAINE, MICHELINE VANIER, AND LAY-KEOW NG

Laboratory and Scientific Services Directorate, Canada Border and Services Agency, 79 Bentley Avenue, Ottawa, Ontario K2E 6T7, Canada

A reliable method for measuring $^{15}N/^{14}N$ and $^{2}H/^{1}H$ ratios of nicotine in cigarette tobacco has been developed. It involves a simple procedure for extracting nicotine from tobacco using methanol. The extract is directly analyzed on a gas chromatography isotope ratio mass spectrometer (GC−IRMS). The method is reproducible with 4% RSD for $\delta^{2}H$ and $\delta^{15}N$. Brand name cigarettes manufactured in Canada ($N = 47$) and in China ($N = 23$) have been analyzed. The results show that nicotine from Canadian cigarettes has a higher $^{2}H/^{1}H$ ratio and a lower $^{15}N/^{14}N$ ratio than the Chinese cigarettes. The $\delta^{2}H$ values for Canadian cigarettes range from −232.7‰ to −203.4‰ with an average of −222.1‰; the $\delta^{2}H$ values for Chinese cigarettes range from −262.6‰ to −219.9‰ with an average of −243.8‰. The $\delta^{15}N$ values for Canadian cigarettes range from −7.7‰ to −6.3‰ with an average of −7.1‰; the $\delta^{15}N$ values for Chinese cigarettes range from −7.6‰ to −5.7‰ with an average of −6.3‰. The combined measurements of $^{2}H/^{1}H$ and $^{15}N/^{14}N$ have been shown to be useful in identifying counterfeits of Canadian cigarettes analyzed in this study.

KEYWORDS: Tobacco; counterfeit; nicotine; GC−IRMS; stable isotope; $^{15}N/^{14}N$ ratio; $^{2}H/^{1}H$ ratio

INTRODUCTION

Smuggling and illegal sale of counterfeit cigarettes have increased in recent years in Canada due mainly to the higher taxation on brand name cigarettes and to the ease of access to the illicit commodity. Such activities are a violation of the Canadian *Criminal Code (R. S., 1985, c. C-46)* and result in the loss of significant revenue in taxation. The development of methods that can distinguish between authentic and counterfeit products is a necessity. The Laboratory and Scientific Services Directorate (LSSD) is responsible for providing analytical services to the Canadian government to support the administration of tax, border and trade policies and protect Canadian society against the illegal movement of goods.

Detection of counterfeit cigarettes is a considerable challenge as producers of such materials have become adept at the preparation of more "sophisticated" imitations. Multiple methods are required to address the problem. Currently, to identify counterfeit cigarettes, our laboratory uses established methods based on chemical profiling of various tobacco extracts by GC/MS (*1*) and by ESI/MS (*2*), in addition to the physical examination of the packaging. The main goal of this paper is to establish a method for the analysis of nicotine by GC−IRMS for the characterization of Canadian cigarettes and then evaluate its applicability to the identification of counterfeit cigarettes. Stable isotope analysis using IRMS has been shown to be a powerful technique for product authentication (*3*−*8*).

In recent years, scientists have responded to the challenge of assessing the origin or authenticity of several goods such as wine (*3*), beer (*4*), pistachio (*5*), olive oil (*6*) and plant materials such as cannabis (*7*) and tobacco (*8*) by stable isotope ratio analysis at natural abundance. In 1997, Jamin et al. published a paper on the multielement and multisite isotopic analysis of purified nicotine extracted from tobacco leaves (*9*). They concluded that carbon and nitrogen isotope composition analyses, using EA−IRMS, and site-specific deuterium content of nicotine could provide information on the geographical origin of tobacco. To our knowledge, there has been no report that describes the characterization of cigarettes by analyzing $^{2}H/^{1}H$ and $^{15}N/^{14}N$ isotopic ratios of nicotine in tobacco using GC−IRMS.

Stable isotope fractionation in a plant depends on several factors such as photosynthetic pathway, i.e. the Calvin cycle pathway (C3), the Hatch−Slack pathway (C4) or the crassulacean acid metabolism (CAM) pathway, environmental factors (i.e., soil type), climatic factors (e.g., rainfall, temperature and sunshine) and agricultural practices (e.g., use of fertilizer and pesticide). There are various tobacco types used for cigarette manufacture, namely, Virginia, burley, Maryland and Oriental. For example, Canadian and Chinese cigarettes are mainly Virginia blends, while American products are typically blends of three tobacco types: Virginia, burley and a lesser amount of

* Author to whom correspondence should be addressed. E-mail: Marie-Josee.Binette@cbsa-asfc.gc.ca.

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Oriental. The choice of a particular blend is driven by consumer satisfaction and is often the result of a manufacturer's proprietary knowledge, physical and chemical properties of the tobacco and also on the availability and cost of tobacco from different growing regions. In our particular application, it is very difficult to try to establish a relationship between the stable isotope measurements and factors affecting the isotopic fractionation in the tobacco plant (e.g., soil type, climatic or agricultural practices) without having information on the growing region and environmental history of the cigarette tobacco. The purpose of this study is not to identify the growing country of origin of the tobacco but, rather, to determine if Canadian cigarettes give a characteristic stable isotope signature which can be used to differentiate them from the counterfeit cigarettes.

Cigarettes that are seized and sent to our laboratory for analysis are often suspected of being imitations of Canadian products. The majority of these cigarettes are from shipments originating from China. This is why most of our efforts in this work are focused on investigating the difference in isotopic composition of nicotine extracted from cigarettes from Canada and China. Although we do not know the growing region of the tobacco for each brand of cigarette in the study, we are hoping that the meticulous leaf selection and tight quality control of manufacturing for authentic brand name cigarettes will separate them from the counterfeits.

Nicotine was selected as the probe for this study. It is the principal alkaloid in tobacco and has been identified as a positive attribute to tobacco quality (*10*). Nicotine can range in concentration from 0.5 to 8% in the major cultivated tobacco species (*10*). Due to its chemical composition, nicotine can be characterized by three stable isotope ratios: $^{2}H/^{1}H$, $^{13}C/^{12}C$ and $^{15}N/^{14}N$. It can be readily extracted from tobacco using various extraction procedures such as Soxhlet extraction, microwave assisted extraction technique (*11*) or a sonification-based extraction as presented in the sample preparation part of this paper.

MATERIALS AND METHODS

Samples. Samples of pure Virginia tobacco ($N = 2$), pure burley tobacco ($N = 1$) and pure Oriental ($N = 1$) were purchased from Imperial Tobacco. Authentic cigarettes were grouped based on their country of origin. Canadian cigarettes of different brands ($N = 47$ for extraction in methanol and $N = 6$ for extraction in isooctane) were purchased from reliable local retailers. Chinese cigarettes of different brands ($N = 23$ for extraction in methanol and $N = 6$ for extraction in isooctane) were sampled from marine containers originating from China. Cigarettes from the United States ($N = 3$ for extraction in methanol and $N = 6$ for extraction in isooctane) were provided to us from Health Canada. Samples of counterfeited Canadian brand name cigarettes ($N = 5$) and of counterfeited American brand name cigarettes ($N = 2$) were also analyzed; these samples were seized suspicious cigarettes that had been identified as not legitimate based on chemical analysis and physical examination of the packaging.

Cigarette Sample Preparation. The tobacco from cigarettes was separated from the other components. The tobacco was ground in batches of 15 g (approximately 20 cigarettes) for 30 s at a speed of 6500 rpm, using a Retsch laboratory knife mill (Grindomix GM 200, VWR Canlab, Montreal, Canada), to obtain a homogeneous sample with particle size smaller than 0.6 mm.

Extraction of Nicotine from Cigarette Tobacco in Methanol. This extraction procedure is a modification of one used in our laboratory for the quantification of nicotine and related alkaloids from whole tobacco using gas chromatography coupled with a nitrogen phosphor detector, GC−NPD. In that method, the extraction solution consists of a 0.05 N potassium hydroxide solution in methanol. For our application, only methanol is used. Nicotine extracts were obtained by placing 50 mg of ground tobacco and 1 mL of HPLC grade methanol into a 2 mL microcentrifuge tube. The microcentrifuge tubes were placed in a plastic rack that can hold up to 48 samples, which was placed in an ultrasonic bath (Aquasonic Model 75D, VWR Scientific Products, Montreal, Canada). Samples were sonicated for four hours in four intervals of one hour each. At the end of each interval, the microcentrifuge tubes were shaken, and the water in the ultrasonic bath was replaced with fresh water. The microcentrifuge tubes were then centrifuged for 5 min at 13200 rpm (Centrifuge 5415D, Eppendorf, Eppendorf Canada, Mississauga, Canada). The supernatant was transferred into a 2 mL autosampler vial for analysis on the GC−IRMS. Final solutions were stored in the freezer until analyzed. Solutions were stable for at least six months. Concentrations of final solutions typically ranged from 800 to 1200 ppm of nicotine ($N = 20$ cigarettes). Since isotopic analysis was performed by GC−IRMS, there was no need to purify the extract prior to analysis.

Microwave-Assisted Extraction of Nicotine from Cigarette Tobacco in Isooctane (2,2,4-Trimethylpentane) (*11*). Approximately 5 g of ground tobacco was placed on a weighing dish and put in the humidity chamber (VWR International from Sheldon manufacturing, Cornelius, OR, USA) at 85% relative humidity and at 25 °C overnight, to bring the moisture level of the tobacco to approximately 20%. 1 g of ground tobacco was mixed with 15 mL of isooctane in a microwave extraction quartz tube. The mixture was irradiated with microwave at 200 W for six minutes in a Prolabo Soxwave 3.6 (Fontenay-Sous-Bois, France), which can prepare three samples at once. After extraction, the mixture was allowed to stand for 10 min. The supernatant was decanted into a 40 mL volumetric flask and allowed to stand for an additional 20 min. The solution was transferred into five 2 mL centrifuge tubes and centrifuged and stored as described in the methanol procedure. Concentrations of final solutions typically ranged from 300 to 500 ppm of nicotine ($N = 20$ cigarettes).

Nicotine Preparation. In-house reference material and control material of nicotine consisted of commercially available nicotine purchased from Fluka and SIGMA, respectively. Solutions of 1000 ppm nicotine in methanol were prepared for analysis of nicotine extracted from cigarettes using methanol. Solutions of 400 ppm nicotine in isooctane were prepared for analysis of nicotine extracted from cigarettes using isooctane.

Quality Control. Three quality control materials were used: the SIGMA nicotine preparation and two nicotine extracts of cigarettes, one Canadian brand name cigarette and the reference cigarette produced by Kentucky Tobacco Research and Development Center, 2R4F. The main constituents of 2R4F tobacco are 32.5% flue-cured, 19.9% burley and 11.1% Oriental. The quality control materials were analyzed in nonconsecutive triplicate during each sequence of sample analysis. The quality control materials were also used to establish the method's reproducibility (intraday and interday).

Instrumentation. All $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratio analyses were made using a GC-C-IRMS system consisting of an Agilent 6890N gas chromatograph (Thermo Finnigan, Bremen, Germany) equipped with a split/splitless injector and with a combustion furnace (ceramic tube, Al_2O_3, packed with CuO, NiO and Pt wires, 320 mm × 0.5 mm i.d.). The chromatographic separation was done on a PTA-5 fused silica capillary column, base deactivated, 30 m × 0.25 mm × 0.5 μm. Isotope ratios were measured on an instrument consisting of a GC-Combustion Interface (Thermo Finnigan GC Combustion III, Thermo Finnigan, Bremen, Germany) equipped with a reduction furnace (ceramic tube, Al_2O_3, packed with three twisted pure Cu wires, 320 mm × 0.5 mm i.d.), a Nafion membrane dryer and an open split; and an isotope ratio mass spectrometer (Thermo Electron Delta V Plus, Thermo Electron Corporation, Bremen, Germany). The combustion furnace temperature was set to 980 °C and the reduction furnace temperature to 630 °C.

All $^{2}H/^{1}H$ ratio analyses were made using a GC-TC-IRMS system consisting of an Agilent 6890N gas chromatograph (Thermo Finnigan, Bremen, Germany) equipped with a split/splitless injector and with a thermo chemical (TC) furnace (ceramic tube (Al_2O_3) 320 mm × 0.5 mm i.d.). The chromatographic separation was done on a DB5 fused silica capillary column, 30 m × 0.25 mm × 0.5 μm. Isotope ratios were measured on an instrument consisting of a GC-TC Interface (Thermo Finnigan GC/TC, Thermo Finnigan, Bremen, Germany) equipped with a nafion membrane dryer and an open split; and an isotope ratio mass spectrometer (Thermo Finnigan Delta^{plus}XP, Thermo

Table 1. δ^2H of Nicotine Extracted in Isooctane and in Methanol from Various Authentic Samples

	δ^2H (‰)			δ^{15}N (‰)		
	i-octane	CH_3OH	Δ[a]	i-octane	CH_3OH	Δ
2R4F	−197.8	−175.5	22.3	−8.5	−6.4	2.1
U.S. brand name	−193.6	−174.6	19.0	−[b]	−6.3	−
Canadian brand name	−242.6	−225.6	17.0	−9.6	−7.0	2.6
pure burley	−206.8	−184.4	22.4	−	−7.4	−
pure Virginia[c]	−250.4	−226.8	23.6	−	−6.7	−
pure Virginia[d]	−245.7	−222.8	22.9	−	−7.1	−
pure Oriental	−225.1	−201.4	23.7	−	−9.0	−

[a] Δ: Difference between δ value in methanol and isooctane. [b] (−): No data. [c] Sampled from top part of the plant. [d] Sampled from bottom part of the plant.

Finnigan, Bremen, Germany). The pyrolysis furnace temperature was set to 1450 °C.

For $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{2}H/^{1}H$ ratios measurements, the injection of the nicotine extract was done with a 10 μL syringe (Hamilton) operated by a liquid autosampler (GC PAL liquid autosampler, Thermo Finnigan, Bremen). The GC injector temperature was set at 270 °C. A 4 mm deactivated gooseneck liner for split/splitless injection, packed with 10 mg of silanized glass wool, was used. The GC carrier gas, helium, was set at a constant flow of 1.9 mL/min. The GC oven temperature was set at 60 °C for 30 s, and then it was increased, at a rate of 20 °C/min, to 300 °C and kept at 300 °C for 1 min.

All stable isotope values are reported in per mil (‰) using the δ notation:

$$\delta^{\text{heavy}}\text{element (‰)} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1\right] \times 1000 \qquad (1)$$

where R is the ratio of heavy to light isotope ($^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{2}H/^{1}H$) in the sample or international standard: Pee Dee Belemnite (PDB) for δ^{13}C, air for δ^{15}N or Vienna Standard Mean Ocean Water (VSMOW) for δ^2H.

RESULTS AND DISCUSSION

The efficiency of the two extraction procedures was verified by recovering residual tobacco after a first extraction and reusing it for a second extraction. The resulting nicotine extracts were analyzed for $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{2}H/^{1}H$ using GC−IRMS and did not contain detectable amounts of nicotine.

Extraction of nicotine from cigarette tobacco in isooctane and in methanol leads to stable solutions; they have been shown to be stable for up to 6 months in the freezer prior to analysis. When kept at room temperature, solutions are stable for at least 24 h. To evaluate stability at room temperature, samples were always analyzed in nonconsecutive triplicates, and the time that elapsed between consecutive analyses of one sample was approximately 6 h. In a typical sequence of analysis, samples could be on the bench for up to 24 h. An indication of the stability of the extracts is provided from relative standard deviation (RSD) of nonconsecutive triplicates. During the measurement of $^{2}H/^{1}H$, the average intraday % RSD of nonconsecutive triplicates measured is 2% for nicotine in isooctane and in methanol. The method's day-to-day reproducibility, evaluated over a six month period, is of comparable magnitude with 4% RSD for δ^2H values for nicotine in methanol and in isooctane and with 4% RSD for δ^{15}N values for nicotine in methanol.

δ^2H and δ^{15}N values of nicotine vary with the extraction procedure, as shown in **Table 1**. Compared to the methanol extract, the isooctane extract of a given cigarette has a lower $^{2}H/^{1}H$ ratio by approximately 20‰ and a lower $^{15}N/^{14}N$ ratio by approximately 2‰. The similar trend is observed when comparing the mean of δ^2H values for nicotine extracted in methanol with those extracted in isooctane from various cigarettes from each country (**Table 2**); the mean δ^2H value of nicotine extracted in isooctane is lower by 13.6‰ for Canadian cigarettes, by 13.3‰ for Chinese cigarettes and by 16.0‰ for American cigarettes when compared to those of the methanol extracts. When using isooctane for the extraction of nicotine only the free-base form of nicotine makes it into the solvent. Methanol also extracts the free nicotine; however, being a stronger solvent than isooctane, it will also extract various polar components including the salt form of nicotine. In our opinion, the differences in δ^2H values and in δ^{15}N values between the methanol and isooctane extracts indicate that the free form of nicotine has lower $^{2}H/^{1}H$ and $^{15}N/^{14}N$ ratios than its salt counterparts.

Table 2. Mean Value, Standard Deviation (SD), Minima and Maxima of δ^{15}N, δ^2H, and δ^{13}C Values of Nicotine Extracted in Methanol, and δ^2H values of Nicotine Extracted in Isooctane from Various Authentic Cigarettes

	authentic Canadian			authentic Chinese			authentic U.S.	
	δ^{15}N (‰)	δ^2H (‰)	δ^{13}C (‰)	δ^{15}N (‰)	δ^2H (‰)	δ^{13}C (‰)	δ^{15}N (‰)	δ^2H (‰)
				Methanol				
N	47	47	7	23	23	16	3	3
mean	−7.1	−222.1	−31.9	−6.3	−243.8	−32.1	−6.5	−181.0
SD	0.3	5.3	0.2	0.5	12.3	0.4	0.2	6.0
min	−7.7	−232.7	−32.2	−7.6	−262.6	−32.6	−6.6	−186.5
max	−6.3	−203.4	−31.6	−5.7	−219.9	−31.3	−6.3	−174.6
				Isooctane				
N	−[a]	6	−	−	7	−	−	6
mean	−	−235.7	−	−	−257.1	−	−	−197.0
SD	−	3.5	−	−	11.0	−	−	4.1
min	−	−241.3	−	−	−275.3	−	−	−202.5
max	−	−231.4	−	−	−243.1	−	−	−192.0

[a] (−): No data.

Based on the $^{2}H/^{1}H$ isotopic ratio analyses of nicotine from cigarettes from various countries, we concluded that both extraction methods would provide similar information; methanol has the advantage of taking less time to perform. Nicotine results using the two solvents are presented and compared.

Compositional differences have been known to exist among the different types of tobacco (*10*). Based on the limited number of samples analyzed, **Table 1**, there is indication that nicotine extracted from different tobacco types also has different isotopic signatures. Nicotine from burley and that from Virginia tobacco have similar δ^{15}N values that are higher than that of Oriental tobacco. On the other hand, all three tobacco types have relatively different δ^2H values; burley has a higher $^{2}H/^{1}H$ ratio than Oriental tobacco, and Virginia tobacco has the smallest $^{2}H/^{1}H$ ratio. Analysis of more samples would be needed to make definite conclusions.

The identification of counterfeit products relies on the comparison between products suspected of being counterfeit with authentic products. A good representation of authentic products is required. Since the methanol extraction procedure was easier to carry out, a more extensive reference database was built for the extracts of nicotine in methanol. In this study, 47 authentic Canadian cigarettes and 23 authentic Chinese cigarettes were analyzed. A lesser amount of authentic cigarettes from the United States ($N = 3$) and counterfeited cigarettes ($N = 5$) were also analyzed. The results of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{2}H/^{1}H$ ratio analyses are summarized in **Tables 2** and **3**. For comparison, the results of $^{2}H/^{1}H$ ratio analysis of cigarette nicotine extracts in isooctane are also presented in **Tables 2** and **3**.

Table 3. Comparison between Nicotine Extracted in Methanol and Isooctane from Authentic Samples and Counterfeits of Canadian and American Brand Name Cigarettes

	authentic			counterfeit			
	N	δ^{15}N (‰)	δ^{2}H (‰)	*N*	δ^{15}N (‰)	δ^{2}H (‰)	Δ^{a} δ^{2}H (‰)
			Methanol				
Canadian brand 1	3	−7.3 (0.1)[b]	−228.0 (5.4)	4	−6.7 (0.6)	−250.6 (3.1)	22.5
Canadian brand 2	7	−7.0 (0.3)	−222.6 (3.2)	1	−6.9	−246.7	24.1
U.S. brand 1	1	−6.3	−174.6	2	−5.8 (0.8)	−220.5 (5.3)	45.9
			Isooctane				
Canadian	6	−[c]	−235.7 (3.5)	5	−	−259.4 (6.2)	23.7
U.S.	6	−	−197.0 (4.1)	2	−	−231.3 (10.5)	34.3

[a] Δ: Difference between authentic and counterfeit cigarettes. [b] Values in parentheses correspond to the standard deviation [c] (−): No data.

Nicotine samples extracted from authentic cigarettes from Canada and China were analyzed for their carbon isotope ratio. As shown in **Table 2**, δ^{13}C values are in the range expected for C3 plants (*12*) and varied from −32.2‰ to −31.6‰ for Canadian cigarettes and from −32.6‰ to −31.3‰ for Chinese cigarettes. These results are comparable to those measured in a previous study in which purified extracts of nicotine were analyzed using EA−IRMS (*9*). The range of δ^{13}C values for nicotine extracted from Canadian cigarettes overlaps extensively with the δ^{13}C values measured for Chinese cigarettes. Based on these results, it was concluded that the ^{13}C/^{12}C ratio would not be a useful characterization variable for authenticating Canadian cigarettes.

The ^{2}H/^{1}H and ^{15}N/^{14}N isotopic ratios for nicotine extracts in methanol are presented in **Figure 1** and show that there is grouping of samples based on their country of manufacturing. The hydrogen isotope composition accounts for most discrepancies between cigarettes from Canada and China and therefore plays a very important role in the group formation. Nevertheless, 9 of the 23 authentic Chinese cigarettes are relatively close to the group of authentic Canadian cigarettes. The variation of δ^{15}N and δ^{2}H values among Canadian cigarettes is smaller than that among the Chinese samples. The tighter grouping of Canadian samples could reflect the rigorous control of tobacco leaf selection, grown in particular regions and/or countries, and manufacturing practices of Canadian manufacturers. The range of δ^{15}N values determined for nicotine is slightly negative for Canadian cigarettes, ranging from −7.7‰ to −6.3‰, and for Chinese cigarettes, ranging from −7.6‰ to −5.7‰, and is comparable to those measured in a previous study (*9*). The δ^{2}H values of Canadian samples, ranging from −232.7‰ to −203.4‰, are comparable to those measured for Virginia tobacco. Canadian cigarettes have higher δ^{2}H values when compared to Chinese cigarettes which have δ^{2}H values ranging from −262.6‰ to −219.9‰. Cigarettes from the United States were also analyzed for their δ^{15}N and δ^{2}H values, as shown in **Table 2**. U.S. samples are characterized by relatively higher δ^{2}H values when compared to all other groups of cigarettes (**Figure 1**). This is most likely due to the presence of burley in the tobacco blend of U.S. cigarettes. As shown in **Table 2**, the ^{2}H/^{1}H isotopic ratio of nicotine in isooctane also follows the same pattern for all cigarettes.

Regarding the counterfeits of Canadian brand name cigarettes ($N = 5$), all samples extracted in methanol are relatively different from the authentic Canadian brand name cigarettes and close to the Chinese cigarettes (**Figure 1**). Counterfeits of American brand name cigarettes ($N = 2$) are also very different from the authentic American brand name cigarettes. In both cases, it is

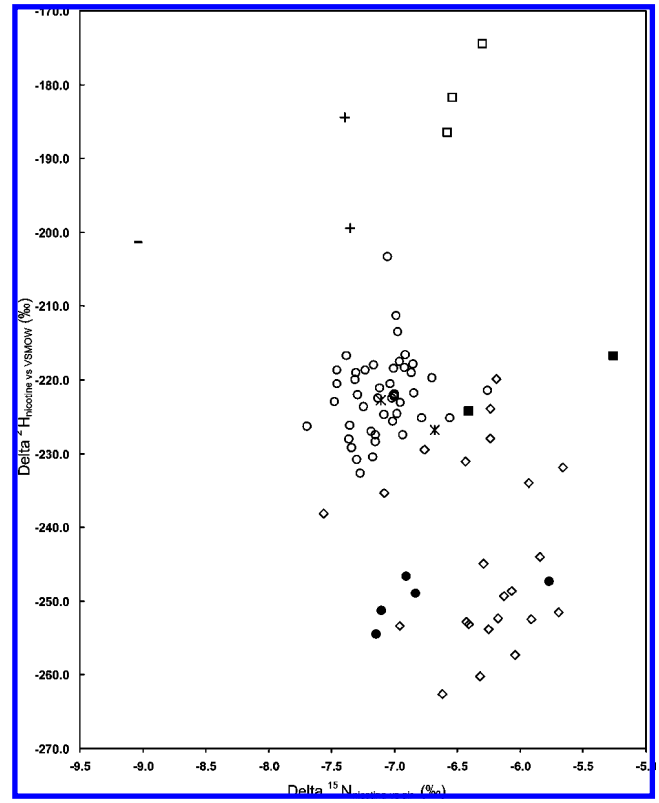

Figure 1. δ^{2}H values versus δ^{15}N values of nicotine extracted in methanol: (∗) Virginia type tobacco; (+) burley type tobacco; (−) Oriental type tobacco; (◇) Chinese cigarettes; (○) Canadian cigarettes; (●) Canadian counterfeit cigarettes; (□) American cigarettes; (■) American counterfeit cigarettes.

the hydrogen isotope composition that accounts for most of the discrepancy between authentic and counterfeit products. Only a limited amount of counterfeited cigarettes was tested; it is possible that some counterfeited cigarettes be indistinguishable from the authentic ones based exclusively on their δ^{15}N and δ^{2}H values.

As presented in **Table 3**, a within brand comparison shows that counterfeits of two popular Canadian brand name cigarettes, labeled as "Canadian brand 1" and "Canadian brand 2", have δ^{2}H values lower by 22.5‰ and 24.1‰, respectively, when compared to the corresponding authentic cigarettes. The difference between counterfeits of one American brand name cigarette and its legitimate counterpart is even more important with an averaged δ^{2}H value that is lower by 45.9‰ for the counterfeit cigarettes. It is likely that the counterfeit cigarettes are Virginia blends. The same can be observed for nicotine extracted in isooctane, **Table 3**, for which the hydrogen isotope composition of nicotine from counterfeits of Canadian ($N = 5$) and American ($N = 2$) cigarettes have δ^{2}H values lower by 23.7‰ and 34.3‰, respectively, when compared to the authentic Canadian ($N = 6$) and American cigarettes ($N = 6$). δ^{2}H values for counterfeits of Canadian brand name cigarettes are also in the range measured for Chinese cigarettes.

In summary, this study demonstrates that the combined ^{2}H/^{1}H and ^{15}N/^{14}N isotope ratio analysis by GC−IRMS of nicotine extracted from tobacco is a good strategy for the characterization of Canadian cigarette tobacco. The sample preparation is simple and fast. The analysis of nicotine extracted from Canadian cigarettes for their hydrogen and nitrogen isotope ratios shows that Canadian cigarettes have a distinct isotopic signature when compared to most cigarettes manufactured in China or in the United States analyzed in this study. As tobacco can be gathered

from different sources, the specific causes of the signature, such as photosynthetic pathway, environmental factors or agricultural practices, cannot be identified; however, the results of this study seem to indicate that the signature can possibly be associated with the country of manufacture. Regarding the difficult task of identifying counterfeit cigarettes, which relies on comparison with authentic samples, this method will be a helpful tool in authenticating Canadian cigarettes providing that a reliable and exhaustive database of Canadian cigarettes is established and that it is used in conjunction with other methods already in place in our laboratory. In addition, as the tobacco inventory is prone to change from year to year, the database will need to be updated on a regular basis. Finally, we are currently working on a method based on inductively coupled plasma/mass spectrometry (ICP/MS) that will complement the IRMS findings presented in this paper.

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