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Development of a Cigarette Tobacco Filler Standard Reference Material

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Abstract

A new tobacco filler Standard Reference Material (SRM) has been issued by the National Institute of Standards and Technology (NIST) in September 2016 with certified and reference mass fraction values for nicotine, *N*-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and volatiles. The constituents have been determined by multiple analytical methods with measurements at NIST and at the Centers for Disease Control and Prevention, and with confirmatory measurements by commercial laboratories. This effort highlights the development of the first SRM for reduced nicotine and reduced tobacco-specific nitrosamines with certified values for composition.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b02550. Additional details concerning sample preparation and chromatographic methods, figures showing the apparatus used in production, representative chromatographic separations, and detailed plots for stability studies (PDF)

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Notes

The authors declare no competing financial interest.

The composition of tobacco products is influenced by the tobacco varieties used in production, growing conditions, curing methods, and additives included during processing.[1] The accurate characterization of the composition of tobacco and tobacco products is essential to quality control in manufacturing, in clinical research studies, and in regulation. A variety of approaches are used to characterize the composition of tobacco and tobacco products. Nicotine and related alkaloids are commonly determined by gas chromatography with flame ionization detection (GC-FID),[2–5] gas chromatography with mass spectrometric detection (GC-MS),[6–12] and liquid chromatography.[13–16] Related liquid chromatography mass spectrometric methods have also been reported for the determination of nicotine alkaloids in biological fluids.[17–21] Methods for the determination of tobacco-specific nitrosamines (TSNAs) have been reported based on liquid chromatography tandem mass spectrometry (LC-MS/MS).[22–24]

Both the sample preparation and the instrumental method represent potential sources of biases and measurement variability. In previous studies, acidic and basic aqueous extractions have been used for the determination of nicotine in tobacco products, and this is consistent with the high solubility of the free base and nicotine salts in aqueous solvents. Methyl-*t*-butyl ether has been used in combination with 2 mol/L sodium hydroxide for gas chromatographic methods,[6–8] and aqueous solutions of citric acid and acetic acid have been employed for liquid chromatographic methods.[13,14,16,25] Tobacco-specific nitrosamines are typically determined in extracts processed for the measurement of nicotine and related alkaloids. The CORESTA Recommended Method No. 72 specifies the use of 100 mmol/L aqueous ammonium acetate for extraction of TSNAs, with subsequent analysis by LC-MS/MS.[23]

The assessment of volatile constituents in tobacco is dependent on the method employed, since a rigorous definition of the measurand (i.e., volatiles) is lacking. The primary volatile constituent of tobacco is water; however, published methods usually do not distinguish between moisture (water) and nonaqueous volatiles, and consequently ambiguity exists. Most methods determine volatile components under conditions that do not cause chemical decomposition of the plant matrix by pyrolysis. CORESTA has published a comparison of methods that have been used to determine moisture and volatile constituents in tobacco.[26] For smokeless tobacco products, they recommend determination of volatiles from the mass difference after drying at 100 °C ± 1 °C for 3 h ± 0.5 min. In the current study, the results of several methods in common use are reported individually.

The development and validation of robust analytical methods, and assurance of analytical results, is facilitated by the availability of suitable quality control materials.[27] Cigarette and ground tobacco matrix reference materials have been produced by several research laboratories including the University of Kentucky Center for Tobacco Reference Products (CTRP), the Institute of Nuclear Chemistry and Technology, the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA), the North Carolina State University Tobacco Analytical Services Laboratory (TASL), and the National Institute of Standards and Technology (NIST). The CTRP currently has two certified reference cigarettes available designated 1R6F and 3R4F.[28] The composition of smoke, resulting from ISO and Health Canada smoking regimes for these reference materials is characterized, as well as the heavy metal and alkaloid content of the tobacco filler. Other than 1R6F and 3R4F, three more ground tobacco reference materials, designated RT2, RTDFC, and 1R5F, are also produced by CTRP, with blended and single-variety tobacco source materials with nicotine content ranging from ~18.9 mg/g to ~35.5 mg/g of nicotine. These materials are characterized for alkaloids, TSNAs, and moisture content. The Institute of Nuclear Chemistry and Technology has developed two reference materials for inorganic trace analysis of tobacco, designated Oriental Tobacco Leaves (CTA-OTL-1) and Virginia Tobacco Leaves (CTA-VTL-2).[29] Both materials consist of dried, ground, and sieved tobacco leaves and are certified for elemental composition. CORESTA and TASL have produced four Reference Products (CRPs) to support the determination of pH, moisture, nicotine and TSNAs in smokeless tobacco products.[30] In addition, NIST has issued two Standard Cigarettes for Ignition Strength and Ignition Resistance Testing (SRM 1082 and SRM 1196); however, these reference materials are not characterized for chemical composition.

The National Institute of Standards and Technology has collaborated with the Center for Tobacco Products, U.S. Food and Drug Administration to develop a Standard Reference Material (SRM) to support the analysis of tobacco products. This SRM is intended primarily for use in evaluating the accuracy of procedures for the determination of nicotine, TSNAs (*N*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)), and moisture in reduced nicotine and reduced TSNA tobacco. It is also intended for use in validating working or secondary reference materials.

EXPERIMENTAL SECTION

Material Acquisition and Preparation

Approximately 2500 kg of air-cured, very low nicotine tobacco (VLN, variety designated Vector 21–41, formerly Type 31-V under 7 CFR 30.38(b))[31] was procured by FDA, Center for Tobacco Products (CTP) with the following nominal specifications: <0.3 mg/g nicotine, <13% water, <1000 ng/g of NNN, and <250 ng/g of NNK. The procurement and processing was coordinated by Research Triangle Institute (Research Triangle Park, NC). Tobacco was supplied by Goodrich Tobacco Company, LLC (Williamsville, NY) from a single crop of very low nicotine tobacco harvested in 2011. The tobacco was stemmed in 2011, and the strips were processed at Kentucky Cut Rag, LLC (Lexington, KY) in October 2013, using normal procedures for the production of cigarette tobacco filler. Except for water, no additional ingredients were added during processing. The dried and chopped leaves (referred

to as “cut rag”; 30 cuts per inch) were blended and enclosed in plastic bags that were placed in cardboard cartons. Blending was carried out using a tobacco silo (Griffin and company, model C6008), a rectangular storage bin into which material was introduced from above by a set of oscillating belts that spread the material in layers from side to side and front to back in a horizontal plane. A total of 37 boxes, each containing approximately 68.2 kg (150 lbs) of tobacco were stored at −20 ° C upon receipt.

SRM Production

The prototype was developed using the bulk cigarette tobacco filler as received, without additional processing. A unit of SRM 3222 Cigarette Tobacco Filler was configured as a box containing 20 jars of cigarette tobacco filler, with each jar nominally containing 10 g of the bulk material. Approximately 750 kg of tobacco filler material was packaged from 14 of the original 37 boxes; the remaining material has been stored for use in the production of SRM reissues. A mechanical device was fabricated to facilitate the filling process (see Figures S1 and S2 of the Supporting Information, SI). Tobacco was introduced into the device through a stirred hopper, and a measured volume of material was delivered into each jar. Jar contents typically ranged from 10 g to 14 g of tobacco.

Reagents

Nicotine ditartrate dihydrate (NIC; Lot # 15-WG-100-1), rac-*N*′-nitrosonornicotine (NNN; Lot # 1-SGP-117-6), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; Lot # 6-RCD-13-2), rac-*N*′-nitrosonornicotine-d_4 (d_4-NNN; Lot # 4-WHH-69-1), and 4-(methyl)-d_3-nitrosamino)-1-(3-pyridyl)-1-butanone (d_3-NNK; Lot # 18-AZC-81-1) were obtained from Toronto Research Chemicals, (North York, ON). Nicotine-2,4,5,6-d_4 (d_4-NIC, Lot B609P26) was obtained from CDN Isotopes, (Quebec, CA).

Sample Preparation

Different approaches were used to process samples for the determination of nicotine and nitrosamines to achieve independence in the analytical procedures. Sample preparation methods designated PREP1 to PREP5 involve different extraction solvents, extraction approaches (e.g., shaking, vortex mixing, or sonication), grinding protocols, and extraction times. For all methods, samples were selected across the production lot using a stratified random sampling scheme. Specific details are compiled in Table 1 and in the SI.

Sample Preparation (Volatiles/Moisture)

Different approaches were also used to characterize samples for volatile components. These methods are designated DRY1 to DRY5, and involve approaches that utilize drying over desicants (DRY1), forced air oven drying (DRY2-DRY4, DRY6), and Karl Fischer water analysis (DRY5). The differences in methodology are summarized in Table 1, and the experimental details are provided in the SI.

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS; NIST)

LC-MS/MS was performed using an Agilent (Newark, DE, U.S.A.) model 1290 series LC system equipped with a vacuum degasser, quaternary pump, an autosampler, and column

oven. The LC was connected to an Agilent 6460 Triple Quad system operated in the positive electrospray ionization (ESI) mode. Multiple reaction monitoring (MRM) was used with the Q1 and Q3 quadrupoles set at unit resolution. The source parameters were as follows: gas temperature, 300 °C; gas flow, 15 L/min; nebulizer pressure, 45 psi; sheath gas temperature 250 °C; sheath gas flow 11 L/min; capillary voltage, 3000 V (pos and neg); nozzle voltage, 500 V (pos and neg); source parameters, high pressure RF 150 V (pos and neg), and low pressure RF 60 V (pos and neg). The mass spectrometer parameters were optimized using the Mass Hunter Optimizer software to maximize the intensity of the [M − H]+ ion, and the collision energy was adjusted to optimize the signal for the most abundant product ions to obtain MRM transitions. The ion transitions used for quantification were 163.1 → 130 *m/z* and 167.1 → 134.1 *m/z* for NIC and d_4-NIC, respectively based on peak shape and abundance. For the TSNAs, the ion transitions used for quantification were 208.1 → 122 *m/z* and 211.1 → 122 *m/z* for NNK and d_3-NNK, respectively and 178.1 → 148.1 *m/z* and 182.1 → 152.1 *m/z* for NNN and d_4-NNN, respectively, based on peak shape and abundance.

Several chromatographic methods were used with different columns and conditions to evaluate potential interferences that might result in biases. The reversed-phase methods designated LC1 to LC6 utilize C18 and pentafluorphenyl stationary phases, with different aqueous ammonium acetate and acetonitrile gradient elution conditions. The gradients were developed to include nicotine and the tobacco specific nitrosamines. Specific details of each separation method are provided in the SI, and separation examples are provided in Figures S3–S8 for extracts of SRM 3222.

Gas Chromatography Mass Spectrometry (GC1; CDC)

Nicotine GC-MS analysis was performed on an Agilent 6890 GC with a 5973 Mass Spectrometer equipped with a Leap CTC (Carrboro, NC, U.S.A.) CombiPAL autosampler. The chromatographic separation was achieved using an Ultra-2 GC column (25 m × 0.32 mm × 0.52 μm). The GC inlet was maintained at 230 °C with a constant flow (1.7 mL/min) of ultrapure helium as the carrier gas. An injection split ratio of 75:1 was used, with 1 μL injections. The GC oven ramp used the following sequence: hold at 175 °C for 1 min; ramp at 5 °C/min to 180 °C; ramp at 35 °C/min to 240 °C. Total GC run time was 3.7 min. The heated transfer line from the GC oven to the MS ion source was maintained at 280 °C. Selected ion monitoring (SIM) parameters (mass and dwell time) were: quinoline; 102 *m/z* (10 ms; internal reference), and nicotine; 133 *m/z* (10 ms; quantitation), 162 *m/z* (35 ms; confirmation). Two additional confirmation ions were included in case of matrix interference: quinoline; 129 *m/z* (10 ms) and nicotine; 161 *m/z* (35 ms). Additional details have been published.[8]

Commercial Laboratory Analyses

Samples were analyzed by commercial laboratories for comparison with measurements performed at NIST and CDC. Except for method DRY4, the data were not statistically combined with other data sets for purposes of value assignment. The following laboratories provided analytical results: Enthalpy Analytical, Inc. (Richmond, VA), Global Laboratory Services, Inc. (Wilson, NC), and Microbac Laboratories, Inc. (Wilson, NC).

Homogeneity

To assess between-jar variability the entire contents of each jar were ground to a fine powder and 1 g subsamples were analyzed (sample preparation methods PREP2a, PREP2b, and PREP3). Samples processed in this way were presumed to be representative of the bulk characteristics for that jar. Jars were selected by a stratified random selection scheme across the fill order of the production lot. To assess within-jar variability, the contents of a single jar was divided into 1 g subsamples (sample preparation method PREP5). Each subsample was individually ground prior to extraction.

Stability Analyses

Samples were analyzed by Labstat International Inc., (Kitchener, Ontario, Canada) to assess the stability of the NIST Standard Reference Material over a 12-month period. A total of 640 g of the SRM 3222 and 640 g of the University of Kentucky 3R4F tobacco reference material filler were initially stored at −20 °C upon receipt. Containers of both the NIST Standard Reference Material and the Kentucky 3R4F tobacco reference material were stored at −20 °C, 4 °C, and 25 °C, and analyses of nicotine, NNN, NNK, pH, and moisture were performed at 0 months, 1 month, 3 months, 6 months, 9 months, and 12 months. Samples of tobacco were removed from each container at the specified temperature and time frame to determine nicotine, NNN, NNK, pH, and moisture. Seven replicate analyses were performed for each sample/temperature/time/analyte.

Freeze-Thaw Analyses

Samples were analyzed by Labstat International Inc., to assess the effect of freeze—thaw cycles on the level of nicotine, TSNAs, moisture, and pH for the two tobacco reference materials. A total of 320 g of SRM 3222 and 320 g of the University of Kentucky 3R4F tobacco reference material filler were stored at −20 °C upon receipt. Both the NIST Standard Reference Material and Kentucky 3R4F tobacco reference material were analyzed for nicotine, NNN, NNK, pH, and moisture at ten separate times/cycles, with measurements occurring in increments of 2 to 7 d following the previous freeze–thaw cycle. Samples were allowed to warm up to ambient temperature for 2 h, and subsamples were removed for analysis of nicotine, NNN, NNK, pH, and moisture. Seven replicate measurements were performed for each sample/cycle/analyte.

Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or other governmental agency, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

RESULTS AND DISCUSSION

Nicotine, TSNAs, and moisture levels were certified in SRM 3222 using multiple analytical methods with measurements performed at NIST and at CDC for measurement independence. This approach is commonly utilized in the certification of reference materials to afford confidence in the integrity of the measurements. Potential biases that might

originate from the use of a single method are likely to become apparent through comparison with measurements produced from alternate methods performed by independent analysts, with different instrumentation, and/or at different laboratories. Statistical combination of the results thus provides an indication of the reliability of the data with a realistic assessment of measurement uncertainty, typically expressed as a confidence interval.

Several approaches were compared for the determination of nicotine and TSNAs. Details of each approach are provided in the SI and are summarized in Table 1. Sample processing methods were employed with different extraction solvents, mixing techniques, and solvent contact times, using ground and unground samples. Unrelated instrumental methods were also utilized at different laboratories. In addition to the measurements performed by NIST and CDC, data were provided by three commercial laboratories specializing in the analysis of tobacco. The laboratories were asked to use the in-house methods they currently employ for nicotine, TSNAs, and moisture.

Typical separations of nicotine, nitrosamines, and deuterated analogs for extracts of SRM 3222 are shown in Figure 1 (see Figures S3–S8 for chromatograms by each of the separation methods LC1–LC6). No interferences were apparent in the chromatograms; however, peak shape was adversely affected for samples extracted with methanol. Peak fronting was attributed to injection solvent mismatch, which results when the sample extract is stronger than the initial gradient composition. In this application, quantitation was not significantly affected by nonideal peak shape (see Table 1, PREP2a and PREP2b), although the issue could be eliminated by the addition of a solvent exchange step.

In the following sections, the analytical results are discussed for the three analyte groups. These data are plotted in Figure 2 for each of the individual methods.

Nicotine

Data sets from five distinct methods (see Table 1 method 1, methods 2 and 3, methods 4 and 5, method 6, and method 7) were combined as the equally weighted mean of means to determine the certified level for nicotine. Data from methods 2 and 3 (also methods 4 and 5) represent reanalysis of the same sample extracts with different LC methods, and these data were combined as a mean. The five method means ranged from 0.095 mg/g to 0.137 mg/g, with RSD ranging from about 3% to 17%. Statistical analysis of the data produced an as-received certified mass fraction value for nicotine of 0.117 mg/g ± 0.018 mg/g, where the uncertainty is an expanded uncertainty about the mean to cover the measurand with approximately 95% confidence. The uncertainty of the combined mean is estimated using a bootstrap procedure based on a Gaussian random effects model for the between-method effects.[32–35] Data from the commercial laboratories (methods 17, 20, and 21; see Table 1) were significantly more disperse than data from NIST or CDC, with RSDs ranging from about 32% to 36% (see Figure 2). Nicotine levels reported by commercial laboratories (methods 20 and 21) were significantly higher than the certified value. One of the laboratories documented the measurements as a deviation from control limits, since the level was significantly lower than the routine calibration range. For another commercial laboratory, the nicotine level was near the quantitation limit for their method.

TSNAs

Three sets of data were combined as the equally weighted mean of means (methods 9, 10 and 11; 12 and 13) to determine the certified level for NNN and NNK. As with nicotine, data from methods 10 and 11, as well as 12 and 13, were obtained from reanalysis of the same sample extracts with different LC methods, and the data were averaged. The method means were somewhat less variable than the method means for nicotine. For NNN, the method means ranged from 1380 ng/g to 1520 ng/g, with RSDs ranging from about 2.4% to 6.5%. For NNK, the method means ranged from 29.6 ng/g to 33.7 ng/g, with RSDs ranging from about 6.1% to 10.6%. The as-received certified mass fraction value for NNN is 1440 ng/g ± 90 ng/g, and for NNK, the certified mass fraction value is 31.3 ng/g ± 2.5 ng/g, where the uncertainty is an expanded uncertainty about the mean to cover the measurand with approximately 95% confidence.

Volatiles

The mass fraction of volatile components was determined using two critically evaluated methods at NIST (i.e., methods 14 and 15) to result in a certified mass fraction value. These methods represent gentle conditions that have been evaluated to minimize biases from sample decomposition. The certified mass fraction of volatiles is 0.115 g/g ± 0.002 g/g, where the uncertainty is an expanded uncertainty about the mean to cover the measurand with approximately 95% confidence. Several additional methods were used to assess the mass fraction of volatile components. The resulting values are reported as reference values, and may be method specific. For example, the mass fraction of volatile components determined by forced air drying at 80 °C is 0.116 g/g ± 0.001 g/g, whereas forced air drying at 100 °C produced a value of 0.121 g/g ± 0.006 g/g (mean of two data sets methods 16 and 18). A similar approach using a Hearson Tobacco oven (method 17) produced a value of 0.119 g/g ± 0.001 g/g. Somewhat lower levels were determined using a Karl Fischer method (method 19) 0.106 g/g ± 0.009 g/g. This level is attributed to the moisture content of the tobacco filler, and does not include other volatile constituents.

Homogeneity

Homogeneity was assessed from samples taken within a single container for comparison with samples distributed across the production lot. Within-jar and between-jar homogeneity were assessed for 1 g subsamples. The RSD for the within-jar homogeneity experiment was 6.6% (see method 8 in Table 1). The RSD for between-jar homogeneity experiments ranged from 2.8% to 5.7% (see methods 2 to 5 in Table 1). No trends were observed in data plotted as a function of fill order (see Figure S9). Within-jar and between-jar variability have been taken into account in the expanded uncertainty for 1 g subsamples from the ground contents of individual jars.

Stability

Tobacco samples consisting of NIST SRM 3222 and the University of Kentucky 3R4F tobacco reference material were analyzed over a 12 month period at 0, 1, 3, 6, 9, and 12 mo, at 0 °C, −20 °C, 4 °C, and 25 °C (Figures S10–S21 and S28–S39). At each time period, nicotine, NNN, and NNK, pH, and moisture content levels were determined in the two

samples stored at different temperatures. Nicotine values in SRM 3222 were below detectable limits (BDL) for the in-house method used by Labstat; levels were measurable in the University of Kentucky 3R4F reference material. The 3R4F material exhibited some variability at different temperatures, over the 12-mo period. Levels of NNN in SRM 3222 ranged from 1100 to 1300 ng/g for samples stored at −20 °C, 4 °C, and 25 °C, compared to the University of Kentucky reference sample with NNN levels that ranged from 2100 to 2500 ng/g for the same storage conditions. Measurement variability for NNN at each of these time points was similar for both the NIST and University of Kentucky reference materials. However, levels of NNK in SRM 3222 remained unchanged (50 ng/g) at −20 ° C, 4 °C, and 25 ° C, compared with NNK levels that ranged from 800 ng/g to 1000 ng/g in 3R4F. Overall, the yields for nicotine, NNN, and NNK remained consistent at the various temporal conditions for SRM 3222. The pH values for the NIST and Kentucky reference materials were consistently stable at 8.5 and 5.5, respectively, at all temperatures during the 12-mo study. The moisture content and dry matter remained stable at 12% and 90%, respectively, throughout the 12-mo study period for the University of Kentucky 3R4F reference material and NIST SRM 3222.

Freeze-Thaw Analysis

The stability of NIST SRM 3222 and the University of Kentucky 3R4F reference material were studied for 10 unique freeze–thaw cycles (Figures S22–S27 and S40–S45). Nicotine was below detectable limits in the 10 freeze–thaw cycles for SRM 3222; however, in the University of Kentucky 3R4F reference material nicotine levels ranged from 15 mg/g to 17 mg/g in the freeze–thaw cycles. Levels of NNN ranged from 1200 ng/g to 1500 ng/g compared to 2000 ng/g to 2800 ng/g for the Kentucky 3R4F reference material, and no trends were apparent. The measured yields for NNK in SRM 3222 remained unchanged at 50 ng/g compared to the 700 ng/g to 950 ng/g observed in the University of Kentucky 3R4F reference material. The pH for the NIST and University of Kentucky 3R4F reference materials were consistent at 8.5 and 5.5, respectively, with a 12% moisture content and 85% in dry matter over the 10-unique freeze–thaw cycles.

CONCLUSIONS

SRM 3222 Cigarette Tobacco Filler has been developed to support measurement quality in the characterization of tobacco products. Good agreement was obtained for measurements and methods utilized by NIST and CDC. No significant changes were apparent in the levels of nicotine, NNN, NNK, pH, and moisture determined for samples stored at different temperatures over a 12-mo storage period, or for samples exposed to 10 freeze–thaw cycles. SRM 3222 is intended primarily for use as a control material in the characterization of the chemical composition of tobacco filler, and may also be useful in the development of new analytical methods by the tobacco industry and the research community.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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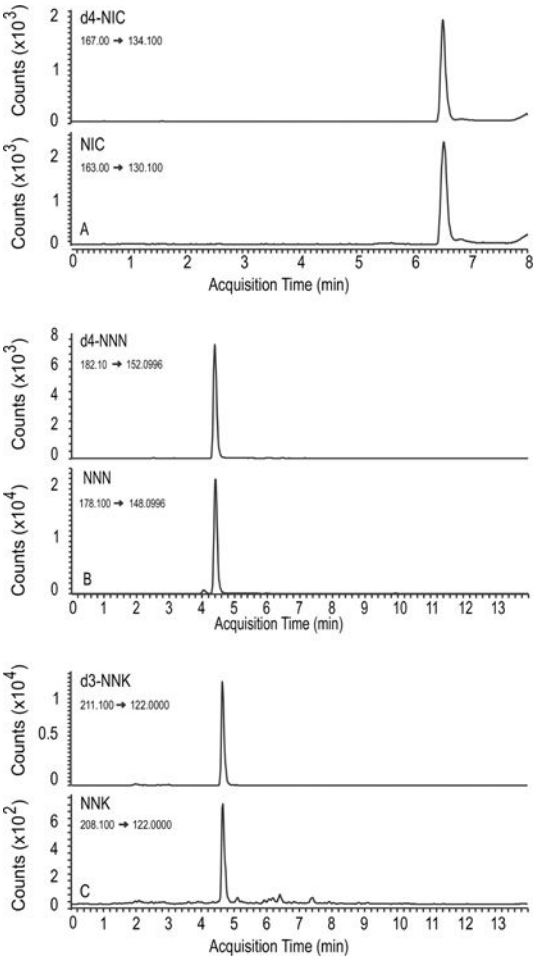

Figure 1.

Typical separations of nicotine, NNN, NNK, and corresponding deuterated internal standards. (A) Method 4 (PREP2b/LC1); (B and C) method 12 (PREP2b/LC5).

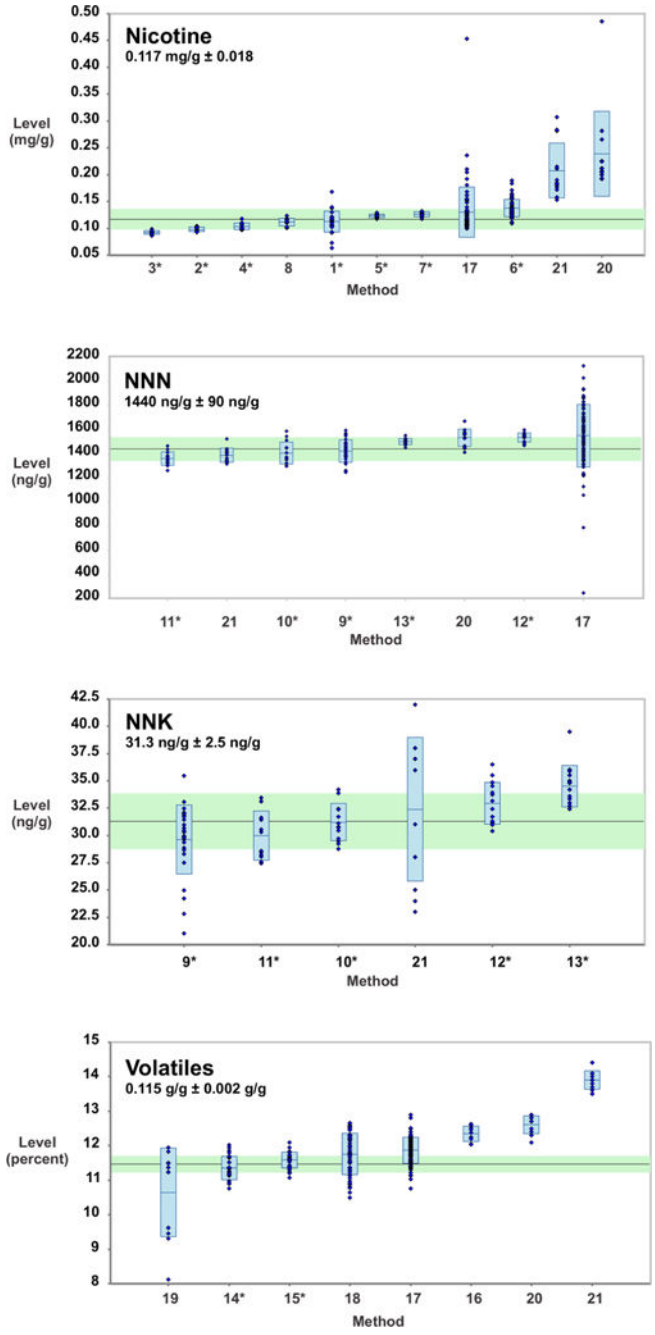

Figure 2.

Results for the determination of nicotine, nitrosamines, and volatiles plotted as a function of method number (see Table 1). Data points are individual measurements, and the box plot represents the mean and standard deviation. The band represents the certified value and expanded uncertainty at the approximately 95% level of confidence. Method data labeled with asterisks were used in assignment of certified values.

Table 1

Summary of Methods and Measurements Used in the Determination of Nicotine, Nitrosamines, and Oven Volatiles

method no.	instrumentation	preparation method	analytical method	nicotine; $\overline{X}$ mg/g	σ	N	NNN; $\overline{X}$ ng/g	σ	N	NNK; $\overline{X}$ ng/g	σ	N	volatiles; $\overline{X}$ g/g	σ	N
certified values[a]				**0.117**	**0.018**		**1440**	**90**		**31.3**	**2.5**		**0.115**	**0.002**	
1*	LC-MS/MS	PREP1	LC1	0.113	0.020	28									
2*	LC-MS/MS	PREP2a	LC1	0.096	0.004	14									
3*	LC-MS/MS	PREP2a	LC3	0.091	0.003	14									
4*	LC-MS/MS	PREP2b	LC1	0.102	0.006	14									
5*	LC-MS/MS	PREP2b	LC3	0.121	0.003	14									
6*	GC-MS (CDC)	PREP4	GC1	0.137	0.016	56									
7*	LC-MS/MS	PREP3	LC1	0.126	0.004	14									
8	LC-MS/MS	PREP5	LC6	0.112	0.007	14									
9*	LC-MS/MS	PREP1	LC2				1420	90	28	29.6	3.2	28			
10*	LC-MS/MS	PREP2a	LC5				1410	90	14	31.2	1.7	14			
11*	LC-MS/MS	PREP2a	LC4				1360	50	14	30.0	2.3	14			
12*	LC-MS/MS	PREP2b	LC5				1540	40	14	32.9	1.9	14			
13*	LC-MS/MS	PREP2b	LC4				1500	30	14	34.5	1.9	14			
14*	desiccator		DRY1										0.114	0.003	28
15*	oven		DRY2										0.116	0.002	28
16	oven		DRY3										0.124	0.002	9
18	oven (CDC)		DRY6										0.118	0.006	56
19	Karl Fischer (oven)		DRY5										0.106	0.013	11
17	commercial lab 1		DRY4	0.130	0.047	73	1540	240	114				0.119	0.003	114
20	commercial lab 2		DRY3[b]	0.238	0.079	13	1530	70	13				0.126	0.003	12
21	commercial lab 3		DRY6[c]	0.188	0.061	13	1390	60	13	32.4	6.6	13	0.139	0.003	10

[a]The uncertainties provided with each certified value are an expanded uncertainty about the mean to cover the measurand with approximately 95% confidence. Asterisk denotes data used in assignment of certified values.

[b]3 g sample size.

[c]5 g sample size.