Determination of Nicotine, NNN, and NNK in a New Cigarette Tobacco Filler Standard Reference Material (SRM 3222) and Its Smoke

Walter B. Wilson, Jeanita S. Pritchett, and Lane C. Sander

Chemical Sciences Division, Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

Introduction

The United States is the fourth largest tobacco producing country in the world, with an annual production of approximately 800 million pounds (3.63 x 10^6 kg). An estimated 42.1 million Americans currently smoke cigarettes. Studies have shown that the levels of nicotine and other constituents in tobacco and tobacco smoke lead to addiction and adverse health effects from smoking. A variety of approaches are used to characterize the composition of tobacco and tobacco smoke. Concentration levels of nicotine, nicotine derivatives, and tobacco specific nitrosamines (TSNAs) are considered of high importance in these characterizations. The availability of suitable reference materials is a important part in the development and validation of robust analytical methods.

The University of Kentucky Center for Tobacco Reference Products (CTRP) has been the primary reference material producer in the U.S. for cigarette and ground tobacco materials. Reference material 1R6F and 3R4F are currently available and have been extensively characterized for nicotine, TSNAs, moisture, and other harmful and potentially harmful contaminants (HPHCs). Using specific smoking regimes from the ISO and Health Canada, the tobacco smoke produced by these products have been analyzed for HPHCs as well.

Recently, NIST has collaborated with the Center for Tobacco Products (CTP) at the Food and Drug Administration (FDA) to develop a Cigarette Filler Tobacco Standard Reference Material (SRM 3222, Figure 1) to support the analysis of tobacco products. The primarily use of the SRM is for the validation of current and new analytical methodologies for the determination of nicotine, NNN, NNK, and moisture in a low nicotine tobacco sample. In addition, some preliminary studies have been conducted on the analysis of these analytes and other HPHCs in its smoke condensate.

Figure 1: One unit of SRM 3222.

Experimental

1. Source and Preparation of SRM 3222

The tobacco source is an air-cured, low nicotine tobacco that was processed using normal procedures used for the production of cigarette tobacco filler. The leaves were dried, chopped, and blended prior to storage at -20 °C prior to packaging. Four ounce jars were filled to capacity with tobacco, without additional processing. A unit of SRM 3222 contains 20 jars of the cigarette tobacco filler, each containing ≈ 10 g of the material.

2. Certification of SRM 3222

Mass fraction values were assigned to nicotine and TSNAs in the certification of SRM 3222 based on isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS) and different sample preparation approaches. Detail information on the methods used for nicotine, TSNAs, volatiles, and moisture have been reported [1, 2].

3. Smoke Analysis of SRM 3222

The tobacco smoke was collected using the apparatus shown in Figure 2. A detailed video on the collection process can be found elsewhere [3]. The tobacco smoke condensate was qualitatively characterized by GC/MS using the operating parameters in Table 1 with a specific interest on nicotine and nicotine derivatives.

Table 1: GC/MS Operating Parameters for Smoke Analysis of SRM 3222.

Inlet	Cool on-column
Columns	SLB-PAHms, 50% phenyl phase (Supelco, Bellefonte, PA)
	60 m x 0.25 mm i.d. x 0.25 µm film
Carrier Gas Flow	1.2 mL/min of helium
Oven Program	Isothermal at 70 °C for 1 min and 5 °C/min to 350 °C for 19 min
MS Temperatures	Ion Source 230 °C; Transfer Line 350 °C, and Quadrupole 150 °C
Full Scan Mode	*m/z* 100 – 500

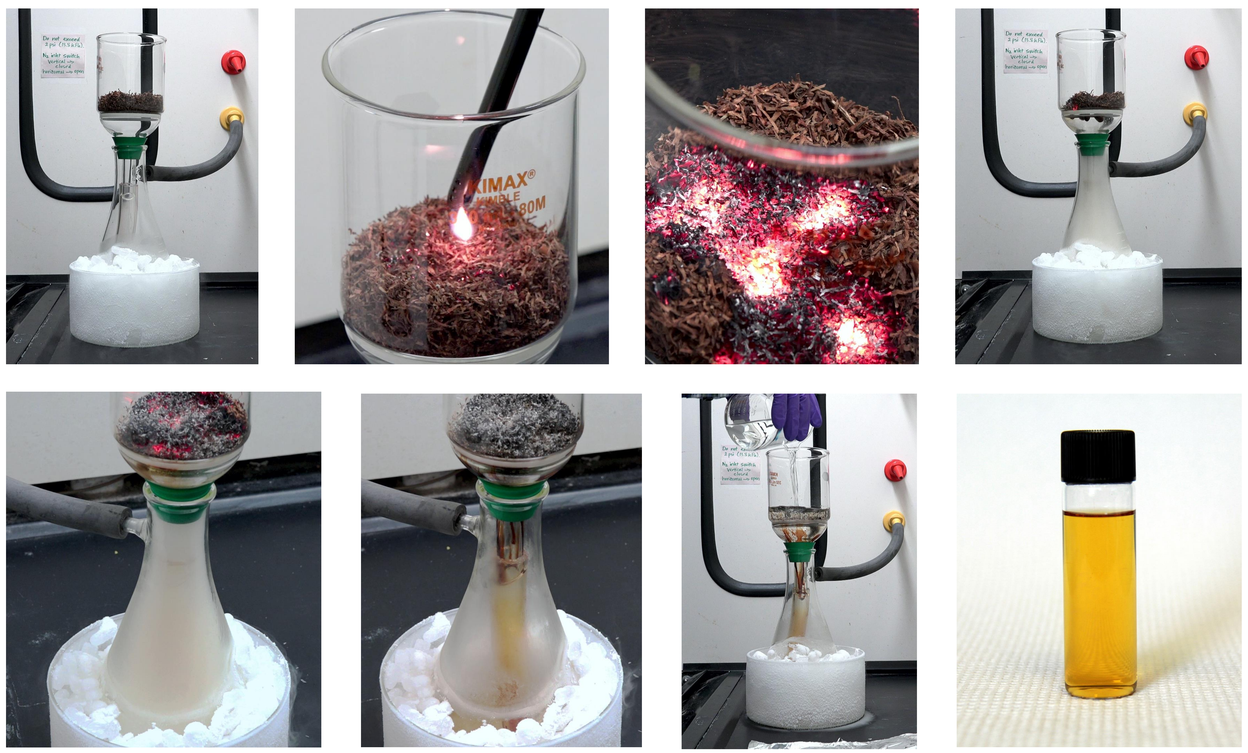

Figure 2: Photographs at different stages during the burning of SRM 3222 and smoke collection to produce the smoke condensate.

Results and Discussion

1. SRM 3222 via ID-LC-MS/MS

The certified mass fraction values for Nicotine, NNN, NNK, and Volatiles in SRM 3222 are summarized in Table 2. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account. The certified values are based on the results of NIST measurements and measurements performed at the U.S. Centers for Disease Control and Prevention (CDC) and commercial laboratories. Only NIST methods are discussed here.

Table 2: Certified Mass Fraction Values for Nicotine, TSNAs, and Volatiles in SRM 3222.

Analytes	As-Received	Dry-Mass Basis
Nicotine	0.117 mg/g ± 0.018 mg/g	0.132 mg/g ± 0.021 mg/g
NNN	1440 ng/g ± 90 ng/g	1630 ng/g ± 110 ng/g
NNK	31.3 ng/g ± 2.5 ng/g	35.4 ng/g ± 2.8 ng/g
Volatiles	0.115 g/g ± 0.002 g/g	

[a] Values are reported on a dry-mass basis using the certified value for volatiles as a conversion factor.
[b] Volatiles are reported based on data obtained for oven drying at 80 °C for three hours and desiccator drying over magnesium perchlorate for 35 days.
[c] The uncertainty listed with each value is an expanded uncertainty about the mean of the results to cover approximately a 95% confidence.

2. Smoke Analysis of SRM 3222 via GC/MS

Preliminary studies have been conducted on the analysis of the smoke produced from the burning of SRM 3222 using the apparatus shown in Figure 2. An important factor in the collection process was the choice of the collection solvent. Methanol, water, n-hexane, and acetone were all investigated. Acetone was selected for the following reasons: (1) provided a homogenous mixture, (2) acceptable solvent for direct analysis via LC-MS/MS or GC/MS, and (3) allowed for the highest number of chromatographic peaks in the analysis. The GC/MS chromatogram obtained in full scan mode for the smoke condensate is shown in Figure 3. The extracted ion chromatograms of the GC/MS analysis are shown in Figure 4 for the *m/z* 84, 98, 118, and 144. The labeled peaks were identified based on the NIST mass spectral database. The mass spectra are shown in Figure 5 for the nicotine peak in the smoke condensate (top) and the reference mass spectra from the NIST database (bottom). Based on similar procedures, three additional nicotine derivatives are identified in Figure 4.

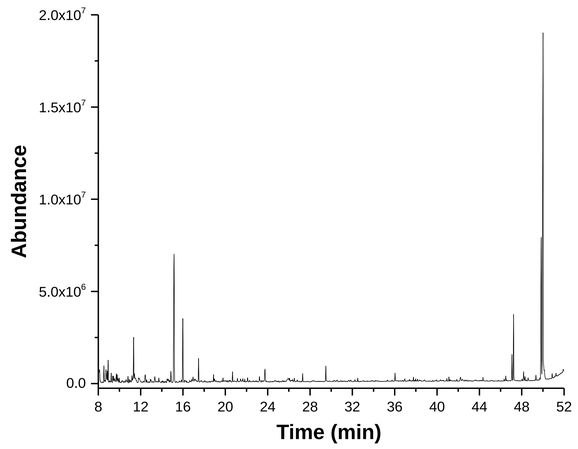

Figure 3: GC/MS chromatogram in full scan mode of the tobacco smoke condensate of SRM 3222 (insert).

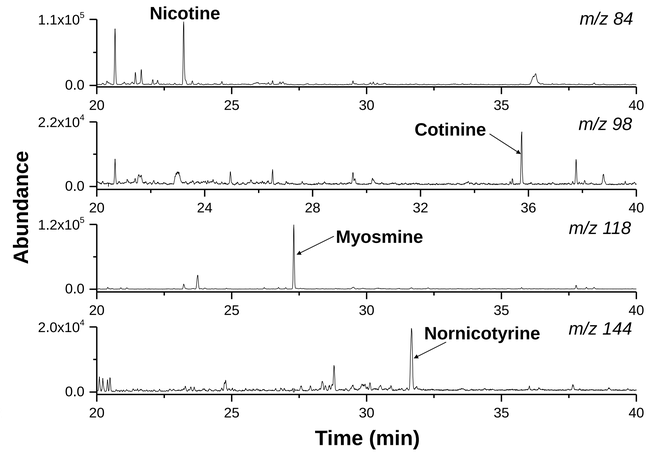

Figure 4: Extracted ion chromatograms of the tobacco smoke condensate for SRM 3222.

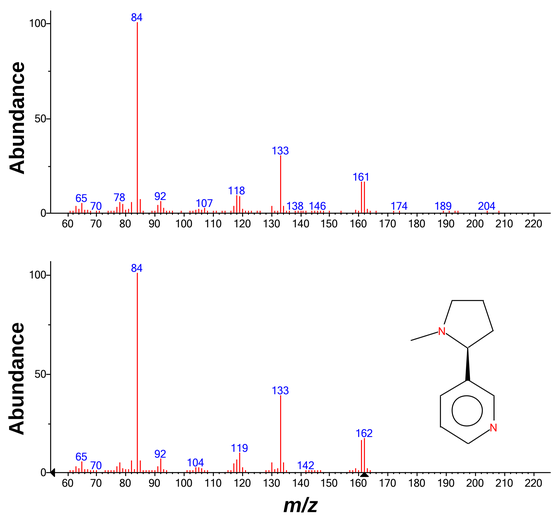

Figure 5: Mass spectra of nicotine in the tobacco smoke condensate (top) and NIST mass spectral database (bottom).

Conclusions

A new Cigarette Tobacco Filler (SRM 3222) has been developed to support the quality of analytical measurements used for tobacco products. Certified mass fraction values were assigned based on measurements performed by NIST and CDC. NIST used a combination of six ID-LC-MS/MS methods and three sample preparation methods for the certification. Preliminary studies have identified nicotine and several nicotine derivatives in the smoke condensate obtained from the burning of SRM 3222.

References

[1] Certificate of Analysis: Standard Reference Material 3222 – Cigarette Tobacco Filler. https://www-s.nist.gov/srmors/view_detail.cfm?srm=3222
[2] L. C. Sander, J. S. Pritchett, Y. C. Daniels, L. Wood, B. Lang, S. A. Wise, J. H. Len, T. L. Johnson, M. Walters, T. Phillips, M Holman, G. Lee, J. Lisko, B. Lane, L. Valentin, and C. Watson, Anal. Chem. (2017) Submitted.
[3] https://www.nist.gov/video/collection-tobacco-smoke-condensate-srm-3222-cigarette-tobacco-filler

Disclaimer

Certain commercial equipment or materials are identified in this poster to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.