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 10^10 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 an 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 1RF and 3RF are currently available and have been extensively characterized for nicotine, TSAs, moisture, and other harmful and potentially harmful contaminants (HHCs). Using specific smoking regimes from the ISO and Health Canada, the tobacco smoke produced by these products have been analyzed for HHCs as well.

Recently, NIST has collaborated with the Center for Tobacco Products (CTP) at the Food and Drug Administration (FDA) to develop a Cigarette Filter Tobacco Standard Reference Material (SRM 3222, Figure 1) to support the analysis of tobacco products. The primary 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 HHCs in its smoke condensate.

Experimental

1. Source and Preparation of SRM 3222

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

2. Certification of SRM 3222

Mass fraction values were assigned to nicotine and TSAs in the certification of SRM 3222 based on isotopic dilution liquid chromatography with tandem mass spectrometry (LC-MS/MS) and different sample preparation approaches. Detailed information on the methods used for nicotine, TSAs, 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.

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Columns</th>
<th>Carrier Gas Flow</th>
<th>Oven Program</th>
<th>MS Temperatures</th>
<th>Full Scan Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>SLB-PHPhes, 50% phenyl phase (Supelco, Bellefonte, PA)</td>
<td>1.0 mL/min of helium</td>
<td>Isothermal at 70 °C for 1 min and 5 °C/min to 350 °C for 19 min</td>
<td>Ion Source 230 °C, Transfer Line 350 °C, and Quadrupole 150 °C</td>
<td>Full Scan 100 – 500</td>
</tr>
</tbody>
</table>

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

The certified mass fraction values for Nicotine, NNN, and NNK 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.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>As-Received</th>
<th>Dry Mass Basis</th>
</tr>
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<tbody>
<tr>
<td>Nicotine</td>
<td>0.117 ng/g ± 0.018 ng/g</td>
<td>0.132 ng/g ± 0.021 ng/g</td>
</tr>
<tr>
<td>NNN</td>
<td>1440 ng/g ± 90 ng/g</td>
<td>1630 ng/g ± 110 ng/g</td>
</tr>
<tr>
<td>NNK</td>
<td>31.3 ng/g ± 2.5 ng/g</td>
<td>35.4 ng/g ± 2.8 ng/g</td>
</tr>
<tr>
<td>Volatiles</td>
<td>0.115 g/g ± 0.002 g/g</td>
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</table>

*Values are reported on a dry-mass basis using the certified value for volatiles as a conversion factor.

Conclusions

A new Cigarette Tobacco Filter (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


Disclaimer

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