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Sensitive and selective gas chromatography-tandem mass spectrometry method for the detection of nitrobenzene in tobacco smoke

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ABSTRACT

Nitrobenzene, a potentially harmful compound found in tobacco smoke, has been largely excluded from prior analysis due to difficulties with quantification. Quantifying harmful compounds in cigarette smoke is useful to compare products, to examine the impact of design parameters on delivery, and to help estimate exposures. A sensitive high-throughput method has been developed for quantifying nitrobenzene in machine-generated mainstream cigarette smoke using isotope dilution gas chromatography-tandem mass spectrometry (ID-GC-MS/MS). This method has sufficient sensitivity to measure vapor phase nitrobenzene concentrations in the low nanogram range, with a 418 pg/cig method limit of detection. Precision estimates from two quality control cigarette products resulted in percent relative standard deviations of 11.5% and 14.9%; product variability estimates from 13 cigarette products resulted in percent relative standard deviations ranging from 2.8% to 16.9%. Nitrobenzene in the machine-generated, mainstream smoke from 15 cigarette products are reported and range from 18 to 38 ng/cig under the Health Canada Intense smoking regimen.

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1. Introduction

Tobacco smoke is a complex mixture of more than 7000 components, including 93 compounds that the United States Food and Drug Administration (FDA) considers to be harmful or potentially harmful constituents (HPHCs) [1,2]. Tobacco smoke is often collected for analysis in two fractions: the particulate phase fraction, also called total particulate matter or TPM, and the vapor phase fraction, comprised primarily of volatile organic compounds (VOCs). One well-represented class of compounds in tobacco smoke are the nitro compounds, which are pyrosynthesized in situ during the smoking process by a reaction between nitrates present in the leaves of the tobacco plant and hydrocarbon radicals formed in the burning zones of the tobacco product [3]. A number of different aromatic and aliphatic nitro compounds are prevalent in cigarette smoke. However, since 1986, only three of these have been discussed with regularity in the context of toxicity, biological activity, and carcinogenicity: nitromethane, 2-nitropropane, and nitrobenzene [4,5]. As these compounds are relatively nonpolar and low-boiling, they typically end up in the vapor phase fraction and may be counted among the volatile or semi-volatile organic components [6]. Detection of nitrobenzene in cigarettes is important because it is a possible human carcinogen, as indicated in animal, metabolic, and structure-activity relationship studies [7–15]. Previous attempts to include nitrobenzene in multi-analyte VOC methods for cigarette smoke failed, as they lacked the necessary sensitivity to quantify the low levels of this compound found in smoke [6,16]; therefore, a separate method was developed.

To our knowledge, there are two reports dealing specifically with detection of nitrobenzene compounds in cigarettes. Hoffmann and Rathkamp published an elaborate method using impingers, distillation, liquid-liquid extraction, and column chromatography to collect, clean up, and concentrate different nitrobenzene compounds in mainstream smoke extracts before quantitation via a gas chromatograph interfaced with an electron capture detector (GC-ECD) [3,17]. This method, while appropriate for the technology available in 1970, is impractical and unnecessarily complex for present-day analysts and is certainly at odds with the mod-

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ern desire for high-throughput methods. In the second method, reported by Xie et al., only the nitrobenzene content in the TPM was considered, which required that the authors smoke 20–80 cigarettes onto a single filter pad in order to generate sufficient nitrobenzene content for detection. A single solid-phase extraction (SPE) step was used for cleanup, followed by a 50-fold concentration step using a nitrogen blow down evaporator prior to GC-ECD analysis. To further separate the sample, the authors employed a heart-cutting step in the chromatographic analysis, in which they employed a Deans' switch [18] to selectively capture desired portions of eluent from the first column and redirect it to a second analytical column for further separation prior to detection. In this manner, they could attain substantially greater separation between analytes that were inadequately resolved by the primary column. However, the back-to-back chromatography used in this method results in long analysis times and is impractical as a high throughput method. Also, in the Xie et al. approach only the nitrobenzene content of the TPM was analyzed; the content of the vapor phase fraction of smoke was not measured [9].

Nitrobenzene itself is the only aromatic nitro compound currently considered a harmful or potentially harmful analyte by the FDA [2] and has presented major challenges in previous attempts at quantitation in analytical methods with mixed analyte panels; therefore it is the sole focus of this study. We developed and validated a sensitive and selective "extract and shoot" approach for the detection of nitrobenzene in mainstream smoke. This approach requires minimal solvent, no cleanup step, and a short chromatographic separation time (5.5 min) to optimize output for high-throughput analyses. Gas chromatography (GC) was chosen as the primary means of sample separation due to the semivolatile nature of nitrobenzene, and tandem mass spectrometry (MS/MS) was chosen as the detection method due to its high sensitivity and specificity. Deuterated nitrobenzene (nitrobenzene-d_5) was selected as the internal standard to help account for potential handling losses, aging losses, and matrix effects [16,19].

2. Experimental

2.1. Chemicals and materials

Nitrobenzene (CAS# 98-95-3, 99% extra pure grade), methylene chloride (CAS# 75-09-2, HPLC grade), and hexanes (CAS# 110-54-3, HPLC grade) were obtained from Thermo Fisher Scientific, (Waltham, MA, USA). Deuterated internal standard nitrobenzene-d_5 (ISTD, CAS# 4165-60-0, isotopic purity: 99.7% atom % D, 99.9% chemical purity) was obtained from Crescent Chemical Company (Islandia, NY, USA). Methanol (MeOH, CAS# 67-56-1, CHROMASOLV HPLC grade, ≥99.9%) and 1 L Tedlar PLV gas sampling bags with Thermogreen LB-2 septa were purchased from Sigma Aldrich (St. Louis, MO, USA).

Thirteen different popular American cigarette products were acquired through The Lab Depot, Inc. (Dawsonville, GA, USA) representing three major domestic cigarette manufacturers: Philip Morris (six products), R.J. Reynolds (six products), and Lorillard (one product). University of Kentucky 3R4F reference cigarettes (Lexington, KY, USA) and CORESTA Monitor #6 (CM6) test pieces were used as "quality control" (QC) materials.

Prior to smoking, received cigarette products were labeled and stored in a -20 °C freezer (maintained at or below −16 °C) within 10 days of receipt in their original packaging in accordance with International Organization for Standardization (ISO, Geneva, Switzerland) guidance document ISO 3402:1999. If opened, cigarette packs returned to storage were stored in sealed bags in a -20 °C freezer within 10 days of opening. Prior to sampling, cigarette samples and Cambridge filter pads were placed in the temperature- and humidity-controlled smoking chamber and conditioned at 22 ± 1 °C and 60 ± 3% relative humidity for at least 48 h and no more than 10 days.

2.2. Instrumentation and method conditions

Cigarette smoking was conducted on Cerulean SM450 20-port smoking machines (Cerulean, Richmond, VA), which were located and operated inside a temperature- and humidity-controlled smoking chamber (Parameter Generation & Control Inc., Black Mountain, NC, USA). Cigarette filter holders (44 mm) were purchased from Cerulean (Molins PLC, Milton Keynes, UK) and fitted with 44 mm Cambridge filter pads (Borgwaldt, Hamburg, Germany). A soap bubble meter obtained from Borgwaldt (Hamburg, Germany) was used to verify smoking machine puff volume. The vapor phase portion of cigarette smoke was collected using 1 L Tedlar collection bags attached to the puff engine exhaust ports using 3.5-inch lengths of PVC tubing. Cigarettes were smoked under ISO conditions (at 22 ± 1 °C and 60 ± 3% relative humidity). Two cigarettes were smoked per sample according to a modified Health Canada Intense (HCI) regimen. The intense regimen prescribes a 55 mL puff volume with a 2 s puff duration every 30 s, with 100% filter ventilation blockage. A total of three clearing puffs were collected after all cigarette coals were extinguished at the end of the smoke collection. Quality control (3R4F and CM6) cigarettes were smoked in parallel with the cigarette samples during each smoking run. Quality control samples were accepted or rejected based on a modified set of Westgard rules [20,21]. Note that for cigarettes requiring longer smoke times, 2 L Tedlar bags can be used instead of 1 L bags without significant changes in the recovery.

Volumetric and positive-displacement repeating pipettes were obtained from Eppendorf Corporation (Hauppauge, NY, USA). Bag shaking was carried out with the help of an Eberbach 6010 fixed speed, reciprocal shaker (Eberbach Corporation, Ann Arbor, MI, USA).

An Agilent 7890B GC system interfaced to an Agilent 7000C tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and a Gerstel MPS autosampler rail (GERSTEL GmbH & Co. KG, Mülheim an der Ruhr, North Rhine-Westphalia, Germany) were used for analysis. The GC inlet was fitted with an Agilent ultra-inert universal gooseneck inlet liner with glass wool and maintained at 250 °C. An 11 psi injection and a 25:1 split ratio were employed in constant flow mode. The carrier gas was research grade helium (Airgas, Inc., Radnor, PA, USA). The column was a 30 m Agilent J&W HP-5MS Ultra Inert capillary column with a 250 μm I.D. and a 0.25 μm film thickness. During chromatography, the oven was held at 110 °C for 2 min, then ramped to 150 °C at a rate of 20 °C/min, then to 300 °C at a rate of 100 °C/min. Mass spectrometry was carried out using electron ionization and multiple reaction monitoring (MRM), with the source heated to 230 °C and both the MS1 and MS2 quadrupoles heated to 150 °C. Ultra-high purity grade nitrogen (Airgas) was used as the collision cell gas. Two MRM transitions were selected based on abundance, with the primary (quantitation) transition selected as the transition from the molecular ion to the most abundant fragment and the secondary (confirmation) transition selected as the transition to the second most abundant fragment. A third MRM transition was monitored for the internal standard. For all transitions, the MS1 and MS2 resolution was set to "wide" and a dwell time of 80 ms was employed. The transitions and collision energies employed are summarized in Table 1. Data acquisition and analysis were carried out using Agilent MassHunter Workstation software. Analyte concentrations were calculated from the ratio of the analyte peak area to the ISTD peak area.