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Simultaneous determination of volatile organic compounds and carbonyls in mainstream cigarette smoke using a sorbent cartridge followed by two-step elution

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ABSTRACT

We developed a simple method for the simultaneous determination of volatile organic compounds (VOCs) and carbonyls in the mainstream cigarette smoke using a sorbent cartridge at ambient temperature without the traditional cryogenic impinger. A sorbent cartridge is installed between intake filter and the pump of the smoking machine. Collection of cigarette mainstream smoke is performed according to the Canadian Intense regime or the ISO regime. As adsorbent, Carboxen 572 (CX-572) is the most suitable for collection of VOCs and carbonyls in the mainstream cigarette smoke. Elution of VOCs and carbonyls from CX-572 is performed by the two-step elution with carbon disulfide and methanol. VOCs are eluted by first elution with carbon disulfide and carbonyls are eluted by second elution with methanol. For VOCs, a portion of eluate is analyzed by gas chromatography–mass spectrometry. For carbonyls, a portion of eluate is derivatized with enriched 2,4-dinitrophenylhydrazine solution and analyzed by high-performance liquid chromatography. Measurement values by CX-572 cartridge method are very close to those obtained by traditional impinger method except for 2-butanone. Impinger methods use 2,4-dinitrophenylhydrazine solution containing 50% water and 2-butanone-DNPhydrazone may be hydrolyzed with water. In the CX-572 method, the hydrolysis of 2-butanone is prevented because the eluate solution contains no water. CX-572 method can measure cigarette smoke resulting from not only one whole cigarette but also from one puff volume because of its high sensitivity and simple operation.

1. Introduction

Cigarette smoking is associated with a variety of pulmonary and cardiovascular disorders including emphysema, atherosclerosis and cancer [1–5], and causes 30% of all cancer deaths. Cigarette smoke, which can be divided into gas phase and particulate matter, is a complex mixture consisting of more than 5000 chemicals and at least 50 of these are carcinogenic [6,7]. Volatile organic compounds (VOCs) in gas phase cigarette smoke include hazardous substances, specifically, benzene, 1,3-butadiene, isoprene and acrylonitrile that are carcinogenic and prevalent toxins [8,9]. Benzene induces leukemia both in humans with occupational exposures [10,11] and in experimental animals [12,13]. In International Agency for Research on Cancer (IARC) monographs [14], benzene was classified as a Group-1 compound (*carcinogenic to humans*), citing additional evidence of an increased incidence of acute nonlymphocytic leukemia in workers exposed to benzene. 1,3-Butadiene is known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans, including epidemiological and mechanistic studies [15]. Similarly to benzene, 1,3-butadiene was currently classified as a Group-1 carcinogen by IARC [16]. Isoprene has been identified as a carcinogen in humans and experimental animals [17] and was classified as a Group 2B carcinogen (*possibly carcinogenic to humans*) on the basis of sufficient evidence for carcinogenicity at multiple organ sites in both mice and rats, especially male mice, exposed by inhalation [18]. Acrylonitrile was found to be carcinogenic to rats with tumors reported in the central nervous system, mammary gland, and a few other rare tumor sites. IARC classified acrylonitrile in 1999 as Group 2B, based on evidence from experimental animals [19]. Similarly to VOCs, carbonyls such as aldehydes and ketones in cigarette smoke, have received much attention as hazardous substances in studies of environmental and biological chemistry. Long-term exposure to relatively high levels of formaldehyde is known to increase the risk to humans [20–23]. In 2004, IARC reclassified formaldehyde as a Group 1 human carcinogen that causes nasopharyngeal cancer and also concluded that there is a "strong but not sufficient evidence for a causal association between leukemia and occupational exposure to formaldehyde" [24]. Acetaldehyde may be responsible

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for increased risk of head and neck cancer and esophageal cancer and was classified as a Group 2B substance [25–27]. Acrolein is not currently a suspected human carcinogen as, to date, no studies have been conducted to observe its carcinogenic effects on human cells. However, studies in rats have shown an increase in cancerous tumors from ingestion but not inhalation, and Feng et al. [28] have recently suggested a connection between acrolein in cigarette smoke and an increased risk of lung cancer.

Therefore, it is very important to measure VOCs and carbonyls in cigarette smoke and evaluate the effect of smoking on human health. At the present time, measurement of VOCs and carbonyls in the mainstream cigarette smoke is performed by two different collection methods and two analytical instruments. In the VOCs analysis, generally, measurement is performed under Health Canada Intense Regime (HCI) T-116 [29] or CORESTA Recommended Method (CRM) No. 74 [30]. In these regulations, VOCs are collected by passing the mainstream smoke of two or five cigarettes through a glass fiber filter disk and into the cryogenic impinger containing 10 mL methanol cooled to below −70 °C by using dry-ice/isopropanol bath. Then, the impinger solutions are injected onto a gas chromatograph/mass spectrometer (GC/MS) for quantitation. In the carbonyl analysis, generally, measurements are performed under HCI T-104 [31] or CRM No. 70 [32]. In these regulations, carbonyls are collected by passing the mainstream smoke of two or five cigarettes into the impinger containing 80 mL or 35 mL DNPH solution. Then, the impinger solutions are injected onto a high performance liquid chromatography (HPLC) for quantitation.

Thus, these traditional impinger methods need large sampling devices, cryogenic bath cooled to below −70 °C for VOCs, and very complicated operation. Therefore, we developed the simple method for the measurement of VOCs in the mainstream cigarette smoke using a sorbent cartridge at ambient temperature without the cryogenic impinger.

2. Experimental

2.1. Apparatus and reagents

The GC/MS system (QP 2010 Ultra, Shimadzu, Kyoto, Japan) was used with a fused-silica column (InertCap AQUATIC-2 60 m × 0.25 mm i.d., d = 1.4 μm, GL Sciences, Tokyo, Japan) and operated with temperature programming from 40 °C (held for 6 min) to 250 °C at 6 °C/min, with He as carrier gas at 0.61 mL/min and 70 eV EIMS detection operated in full-scan mode from m/z 40–500. The injection volume is 1 μL (split injection, split ratio 10:1; septum purge 1 mL/min; injector temperature 240 °C. The HPLC system (Prominence LC-20, Shimadzu, Kyoto, Japan) was used with two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photo-diode array detector. The analytical column was an Ascentis RP-Amide, 3 μm particle size, 150 mm × 3 mm i.d. column (Supelco Inc., Bellefonte, PA, USA). Solution A of the mobile phase mixture was acetonitrile/water (50/50, v/v) containing 10 mmol/L ammonium acetate and solution B was acetonitrile/water (80/20, v/v). HPLC elution was carried out with 100% A for 5 min, followed by a linear gradient from 100% A to 100% B in 50 min and then held for 10 min. The flow rate of the mobile phase was 0.7 mL/min. The column temperature was 30 °C and the injection volume was 10 μL.

The cigarette smoke was generated on a single-port piston-type smoking machine Model LM1/PLUS (Heinrich Borgwaldt Hamburg, Germany). Thermal mass flow meter (TSI 4100 Series, TSI Inc.) was used for measuring the smoking machine puff profiles.

Standard 1,3-butadiene solution (2.0 mg/mL in methanol) was purchased from AccuStandard Inc. (New Haven, CT, USA). Benzene-$d6$ (99.95%), Isoprene (95.0%), acrylonitrile (97%) benzene (99.7%), toluene (99.7%), carbon disulfide (99.0%) and methanol (99.8%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Carboxen 563 (CX-563, 20/45 mesh), Carboxen 564 (CX-564, 20/45 mesh), Carboxen 569 (CX-569, 20/45 mesh), Carboxen 572 (CX-572, 20/45 mesh) and activated carbon (AC, 20/45 mesh) were purchased from Sigma–Aldrich Inc. (MO, USA). Anasorb 747 (AS-747, 20/40 mesh) was purchased from SKC Inc. (PA, USA). The water used for HPLC and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA, USA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) was obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). The acetonitrile (HPLC grade, >99.9%), ethanol (>99.5), phosphoric acid (85% solution in water), and ammonium acetate (99.999%) were from Sigma–Aldrich Inc. (MO, USA).

Sample cigarettes used in this study were 3R4F, 1R5F from University of Kentucky (Lexington, KY, USA) and CM6 from Cooperation Center for Scientific Research Relative to Tobacco (CORESTA, Paris, France).

2.2. Preparation of the sorbent cartridge (CX-572 cartridge)

CX-572 particles were weighed into a glass tube and conditioned at 380 °C for 5 h under a flow of purified nitrogen at 50 mL/min using a tube conditioner (TC-20, Markes Int. Ltd., Mid-Glamorgan, UK). After cooling to room temperature, carbon adsorbents were packed into the polyethylene cartridges (Rezorian tube, 1 mL, Supelco Inc., Bellefonte, PA).

2.3. Preparation of the enriched DNPH-solution

Phosphoric acid (25 mL) and DNPH hydrochloride (1 g) are added in a 50 mL volumetric flask, and then this solution is diluted to 50 mL with acetonitrile. This mixture solution is continuously stirred with a magnetic stirrer until a clear solution is obtained and stored in a refrigerator at 4 °C.

2.4. Collection cigarette smoke using a CX-572 cartridge

Test cigarettes are placed at 22 °C temperature and 60% humidity. CX-572 cartridge is installed between intake filter and pump of the smoking machine. Collection of cigarette main-stream smoke is performed according to the HCI regime [33] or the ISO regime [34]. In the HCI regime, mainstream smoke constituents are collected under the conditions of 55 mL puff volume, 2 s puff duration, 30 s puff interval, and 100% blocking of the filter ventilation holes with Mylar adhesive tape. In the ISO regime, mainstream smoke constituents are collected under the conditions of 35 mL puff volume, 2 s puff duration, 60 s puff interval, and no blocking of the filter ventilation holes.

2.5. Elution of CX-572 cartridge by two-step elution and analysis

After collection, CX-572 particles together with the frits are removed from cartridge and deposited into the 15 mL septum-sealed vial. Then, 1 mL of carbon disulfide is slowly added into the vial through the septum using the syringe with needle. After letting the sample stand for 10 min, 4 mL of methanol is added and stirred for 10 s. In the case of VOC analysis, a 1 mL portion of eluate solution is transferred to a 1.5 mL autosampler vial, internal standard (10 mg/mL benzene-$d6$, 8 μL) is added, and then, this solution is analyzed by GC/MS under the conditions described in Section 2.1. In the case of carbonyl analysis, a 0.5 mL of portion of eluate solution is transferred to a 5 mL volumetric flask. Then, 0.1 mL of the enriched DNPH solution is added, and after ten minutes, this solution is diluted to 5 mL with ethanol and analyzed by HPLC.