Journal of Chromatography A, 1426 (2015) 48–55

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of nicotine, tar, volatile organic compounds and carbonyls in mainstream cigarette smoke using a glass filter and a sorbent cartridge followed by the two-phase/one-pot elution method with carbon disulfide and methanol

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ARTICLE INFO

Article history:
Received 25 July 2015
Received in revised form
16 November 2015
Accepted 17 November 2015
Available online 19 November 2015

Keywords:
Mainstream cigarette smoke
Volatile organic compounds
Carbonyl compounds
Nicotine
Tar
One-pot elution

ABSTRACT

We have developed a new analytical method for the determination of nicotine, tar, volatile organic compounds and carbonyls in main-stream cigarette smoke using a sorbent cartridge packed with Carboxen 572 (CX-572) and a Cambridge filter pad (CFP) followed by the two-phase/one-pot elution method. A CX-572 cartridge is installed between the intake of the CFP and the pump of the smoking machine. Gaseous compounds collected with the CX-572 cartridge and total particulate matter (TPM) collected with the CFP are coeluted simultaneously in the same vial and then analyzed by high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC/MS) and gas chromatograph–thermal conductivity detector (GC/TCD). Carbonyl compounds are determined by adding derivatizing reagent (2,4-dinitrophenylhydrazine, DNPH) to the eluate followed by HPLC analysis. VOCs and nicotine are determined by GC/MS, and water is determined by GC/TCD. The same sample eluate solution is used for HPLC, GC/MS and GC/TCD analyses. As a result of measuring main-stream cigarette smoke generated from reference cigarettes, almost all carbonyl compounds and VOCs except formaldehyde were passed through a CFP and trapped in a CX-572 cartridge. 100% of nicotine, tar and TPM were trapped in a CFP. 50% of water and 53% of formaldehyde were trapped in a CFP. The one-pot data is almost equal to the sums of CFP (particulate matter) and CX-572 (gaseous compounds) data. The two-phase/one-pot elution method can simultaneously measure nicotine, tar, volatile organic compounds and carbonyl compounds in cigarette smoke with simple operation and small amounts of reagents.

1. Introduction

Cigarette smoke can be a cause of serious health-related problems [1]. Preventing exposure to cigarette smoke, either active or passive, should be a fundamental priority of all clinical and public health practice. While the association between inhalation of mainstream smoke and diseases has been established for many years, the realization that exposure to passive smoking adversely affects human health is more recent [2,3]. 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 [4–6]. The World Health Organization (WHO) has brought the tobacco epidemic to international awareness stating that "The tobacco epidemic is one of the biggest public health threats the world has ever faced, killing nearly six million people a year. More than five million of those deaths are the result of direct tobacco use while more than 600,000 are the result of non-smokers being exposed to second-hand smoke. Approximately one person dies every 6 s due to tobacco, accounting for one in 10 adult deaths" [7]. Up to half of current users will eventually die of a tobacco-related disease [8]. Therefore, it is very important to measure chemical compounds in cigarette smoke and evaluate the effect of smoking on human health.

At the present time, measurements of volatile organic compounds (VOCs), carbonyls and nicotine in mainstream cigarette smoke are performed by three different collection methods

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http://dx.doi.org/10.1016/j.chroma.2015.11.058
0021-9673/

and three analytical instruments. In the official standard methods, carbonyl compounds (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone and *n*-butyraldehyde) are collected by an impinger containing an acidified solution of 2,4-dinitrophenylhydrazine (DNPH) in acetonitrile. The DNPH solution is then diluted with 1% Trizma base in aqueous acetonitrile and injected onto a high performance liquid chromatography (HPLC) for quantitation [9,10]. VOCs (1,3-butadiene, isoprene, acrylonitrile, benzene, toluene) are collected by a cryogenic impinger containing methanol cooled to below −70 °C using a dry-ice/isopropanol bath. The impinger solution is then spiked with benzene-*d*6 as internal standard and injected onto a gas chromatograph/mass spectrometer (GC/MS) for quantitation [11,12]. Nicotine and tar are collected on a pre-weighed Cambridge filter pad (CFP). The CFP is re-weighed and the difference is calculated as the total particulate matter (TPM). The CFP is then extracted with isopropanol containing internal standards, and the extract is analyzed for nicotine and water by gas chromatography using FID and TCD, respectively. The tar value is determined by subtracting the water and nicotine from the TPM [13]. Thus, these traditional analytical methods need specific devices and operations for each class of target compounds, and moreover, cannot analyze all classes from a single cigarette. Recently, we developed a sorbent cartridge method for the simultaneous measurement of VOCs and carbonyls in mainstream cigarette smoke [14]. A sorbent cartridge packed with Carboxen 572 (CX-572 cartridge) is installed between intake filter and pump of the smoking machine. Elution of VOCs and carbonyls from CX-572 cartridge is performed by the two-step elution with carbon disulfide and methanol. Non-polar compounds such as VOCs are eluted by first elution with carbon disulfide and polar compounds such as carbonyls are eluted by second elution with methanol. The cartridge can be used to measure chemicals not only from one single cigarette, but also from the volume of one single puff because of its high sensitivity and simple operation. However, CX-572 method cannot simultaneously measure nicotine, tar, VOCs and carbonyls. Therefore, we developed a new analytical method for simultaneous determination of nicotine, tar, volatile organic compounds and carbonyls in mainstream cigarette smoke using a glass filter and a sorbent cartridge followed by two-phase/one-pot elution with carbon disulfide and methanol. Particulate matter such as nicotine and triacetin are coeluted with gaseous compounds such as VOCs and carbonyls in a one-pot vial.

2. Experimental

2.1. Apparatus and reagents

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 0.8 mL/min. The column temperature was 30 °C and the injection volume was 10 μL.

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 was 1 μL (split injection, split ratio 10:1), septum purge 1 mL/min, and injector temperature 240 °C.

The GC/TCD system (QP 2010, Shimadzu, Kyoto, Japan) was used with a packed column (Porapack Q 2 m × 3 mm i.d., 80–100 mesh deactivated stainless, GL Sciences, Tokyo, Japan) and operated with oven temperature of 170 °C with He as carrier gas at 30 mL/min. The injection volume was 2 μL, injector temperature 250 °C, and detector temperature 250 °C.

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

A standard 1,3-butadiene solution (2.0 mg/mL in methanol) was purchased from AccuStandard Inc. (New Haven, CT, 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). Benzene-*d*6 (99.95%), Isoprene (95.0%), acrylonitrile (97%), benzene (99.7%), toluene (99.7%) and carbon disulfide (99.0%) were purchased from Wako Pure Chemical Industries, LTD. (Osaka, Japan). Carboxen 572 (CX-572, 20/45 mesh), acetonitrile (HPLC grade, >99.9%), ethanol (>99.5), methanol (anhydrous, 99.8%), phosphoric acid (85% solution in water), (−)-nicotine (≥99%), isoquinolile (97%) and ammonium acetate (99.999%) were purchased from Sigma–Aldrich Inc. (MO, USA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) was purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan).

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). Test cigarettes were used for measurement after being placed at 22 °C temperature and 60% humidity for 2 days.

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

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

2.3. Preparation of the concentrated DNPH-solution

Phosphoric acid (10 mL) and DNPH hydrochloride (1 g) were added to a 50 mL volumetric flask, and diluted to 50 mL with acetonitrile. This mixture solution was then continuously stirred with a magnetic stirrer until a clear solution was obtained. The solution was then stored in a refrigerator at 4 °C until used.

2.4. Preparation of internal standards for GC/MS and GC/TCD analysis

An internal standard mixed solution (ISMS) containing ethanol, benzene-*d*6 and isoquinoline was used for GC/MS and GC/TCD. Ethanol (12.6 mL), benzen-*d*6 (105 μL) and isoquinoline (455 μL) were put into a 20 mL volumetric flask and diluted with methanol. The resulting ISMS contained 500 mg/mL ethanol, 5 mg/mL benzene-*d*6 and 25 mg/mL isoquinoline.

2.5. Collection of cigarette smoke using a CX-572 cartridge

The CX-572 cartridge was installed between the CFP and pump of the smoking machine. Collection of mainstream cigarette smoke was performed according to the Health Canada Intense (HCI) regime [13] or the International Organization for Standardization