Determination of Toxic Carbonyl Compounds in Cigarette Smoke

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Received 2 March 2005; revised 22 July 2005; accepted 28 July 2005

ABSTRACT: Toxic carbonyl compounds, including formaldehyde, malonaldehyde, and glyoxal, formed in mainstream cigarette smoke were quantified by derivatization—solid phase extraction—gas chromatography methods. Cigarette smoke from 14 commercial brands and one reference (2R1F) was drawn into a separatory funnel containing aqueous phosphate-buffered saline. Reactive carbonyl compounds trapped in the buffer solution were derivatized into stable nitrogen containing compounds (pyrazoles for β-dicarbonyl and α,β-unsaturated aldehyde; quinoxalines for α-dicarbonyls; and thiazolidines for alkanals). After derivatives were recovered using C_{18} solid phase extraction cartridges, they were analyzed quantitatively by a gas chromatograph with a nitrogen phosphorus detector. The total carbonyl compounds recovered from regular size cigarettes ranged from 1.92 mg/cigarette^{-1} to 3.14 mg/cigarette^{-1}. The total carbonyl compounds recovered from a reference cigarette and a king size cigarette were 3.23 mg/cigarette^{-1} and 3.39 mg/cigarette^{-1}, respectively. The general decreasing order of the carbonyl compounds yielded was acetaldehyde (1110–2101 μg/cigarette^{-1}) > diacetyl (301–433 μg/cigarette^{-1}), acrolein (238–468 μg/cigarette^{-1}) > formaldehyde (87.0–243 μg/cigarette^{-1}), propanal (87.0–176 μg/cigarette^{-1}) > malonaldehyde (18.9–36.0 μg/cigarette^{-1}), methylglyoxal (13.4–59.6 μg/cigarette^{-1}) > glyoxal (1.93–6.98 μg/cigarette^{-1}).
 Environ Toxicol 21: 47–54, 2006.

Keywords: acetaldehyde; cigarette smoke; dicarbonyl compounds; formaldehyde; glyoxal; malonaldehyde; toxic aldehydes

INTRODUCTION

Cigarette smoking is associated with a wide variety of diseases, including cancer (Kodama et al., 1997), atherosclerosis (Yokode et al., 1995), and pulmonary disease (Jeffery et al., 1984). Many studies have been conducted to pinpoint the specific chemical(s) that cause these diseases. Consequently, vast numbers of chemicals have been reported in cigarette smoke, over 3000 organic compounds (Lu et al., 2003). In addition, the presence of many toxic chemicals, including polynuclear aromatic hydrocarbons (IARC, 1986), *N*-nitrosamines (Hecht et al., 1975; Caldwell and Conner, 1990), reactive carbonyl compounds (Miyake and Shibamoto, 1995), dioxins (Muto and Takazawa, 1989), and acrylamide (White et al., 1990) have been recognized in cigarette smoke.

Some low molecular weight carbonyl compounds such as formaldehyde, acetaldehyde, acrolein, glyoxal, methylglyoxal, and malonaldehyde (MA) are extremely difficult to analyze because they are highly volatile, highly reactive, and highly water soluble. However, the quantification of these toxic aldehydes in cigarette smoke is of great importance because tobacco smoke is one of the major sources of toxic aldehydes contamination in indoor air (Feinnan, 1988). For example, formaldehyde that is present in side stream cigarette smoke can mean considerable exposure for the nonsmoker through passive smoking (NRC, 1980).

Because direct trace analyses of these reactive carbonyl compounds are almost impossible, many stable derivatives have been prepared. The most commonly used derivative for volatile aldehydes is 2,4-dinitrophenylhydrazine (2,4-DNP) derivative, which is detected by gas chromatography

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Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.20153

or HPLC. For example, formaldehyde (25–69 μg/cigarette^{-1}) and acetaldehyde (752–1234 μg/cigarette^{-1}) were analyzed in mainstream cigarette smoke using this derivative (Houlgate et al., 1989). However, the preparation of 2,4-DNP requires a low pH and a high temperature (95°C), which may cause alteration in the sample of interest. Recently, we developed a derivative, which can be prepared at mild conditions of neutral pH and room temperature (Yasuhara and Shibamoto, 1989; Miyake and Shibamoto, 1993). This method involves derivatization of volatile aldehydes with cysteamine to yield stable thiazolidines, which are subsequently determined by a gas chromatograph (GC) equipped with a fused silica capillary column and a nitrogen phosphorus detector (NPD). The volatile aldehydes (C_1–C_8) formed in mainstream cigarette smoke from 26 commercial brands were analyzed by this method, and the total amount of aldehydes recovered ranged from 2.37 to 5.14 mg/cigarette^{-1} (Miyake and Shibamoto, 1995).

The difficult task of conducting trace analyses of reactive dicarbonyl compounds (MA, glyoxal, and methylglyoxal) has received much attention because these chemicals are implicated in various diseases (Yin and Brunk, 1995; Okado-Matsumoto and Fridovich, 2000). These chemicals are also derivatized into stable compounds for trace analysis. β-Dicarbonyl compounds (MA) or α,β-unsaturated aldehyde (acrolein) were derivatized into pyrazoles or pyrazolines, respectively, and then analyzed by a GC with NPD (Dennis and Shibamoto, 1990; Tamura and Shibamoto, 1991).

In the present study, genotoxic carbonyl compounds formed in the mainstream cigarette smoke from various commercial brands were quantitatively analyzed using the above method with some modification.

MATERIALS AND METHODS

Cigarette Samples and Chemicals

All cigarette samples were bought from local markets and were stored in sealed packages until used. A reference cigarette 2R1F (University of Kentucky) was a gift from Dr. Pinkerton at the University of California, Davis. Cysteamine hydrochloride, malonaldehyde tetrabutylammonium salt, 1,2-phenylenediamine hydrochloride, *N*-methylhydrazine, potassium phosphate, sodium chloride, and potassium chloride were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals were bought from reliable commercial sources.

Sample Preparations

A 50 mL of 0.1 N phosphate-buffered saline (pH 7.4) was placed in a 1000 mL separatory funnel. The headspace of the separatory funnel was evacuated at 8.4 mmHg for 5 min. Immediately after a cigarette was lit, about 1 mm of the other end of the cigarette was inserted in the tip of the separatory funnel. The cock of the separatory funnel was opened gradually to draw the mainstream cigarette smoke into the separatory funnel. It took 20 s to draw the smoke from one cigarette completely. After smoke was drawn into the separatory funnel, the funnel was shaken vigorously for 3 min to produce a cigarette smoke extract. Blank sample was prepared according to the same procedure except the ambient air was introduced to the separatory funnel instead of cigarette smoke.

Quantitative Analysis of MA and Acrolein

MA and acrolein were analyzed after they were derivatized into 1-methylpyrazole (1-MP) and 1-methyl-2-pyrazoline, respectively, by a method previously reported, with some modifications (Dennis and Shibamoto, 1990; Tamura and Shibamoto, 1991; Miyake and Shibamoto, 1995). The derivatizing agent *N*-methylhydrazine (10 μL) was added to 5 mL of the cigarette smoke extract in a vial with a phenolic cap. The reaction mixture was stirred with a magnetic stirrer for 30 min. The reaction solution was placed in a C_{18} SPE cartridge (Varian, Harbor City, CA, USA) and then eluted with 5 mL of ethyl acetate under reduced pressure using a vacuum manifold (Alltech Associates, Derield, IL, USA). The SPE cartridge was preconditioned by rinsing with one volume each of methanol and deionized water alternately. After 10 μL of 2-methylpyrazine solution (10 mg/mL ethyl acetate) was added as a GC internal standard, the volume of the eluent was brought to exactly 5 mL with ethyl acetate. MA and acrolein were analyzed as 1-MP and 1-methyl-2-pyrazoline, respectively, by a GC with a NPD. A typical gas chromatogram of ethyl acetate extract obtained from this experiment is shown in Figure 1. A GC with a mass spectrometer (MS) was used to confirm the identity of the 1-MP and 1-methyl-2-pyrazoline. The mass spectral data of 1-MP are m/z^{-1} (relative intensity): 82 (M^+ 100), 81 (46), 54 (38), and 53 (13), and those of 1-methyl-2-pyrazoline are m/z^{-1} (relative intensity): 84 (M^+ 59), 82 (100), 56 (24), and 42 (59).

Quantitative Analysis of Glyoxal, Methylglyoxal, and Diacetyl

α-Dicarbonyl compounds were derivatized into corresponding quinoxalines with 1,2-phenylenediamine—quinoxaline for glyoxal, 2-methylquinoxaline for methylglyoxal, and 2,3-dimethylquinoxaline for diacetyl—by a method previously reported, with some modifications (Niyati-Shirkhodaee and Shibamoto, 1993). Derivatizing agent 1,2-phenylenediamine (100 μL of 10 mg/mL^{-1} aqueous solution) was added to 5 mL of the cigarette smoke extract in a vial with a phenolic cap. The reaction mixture was stirred with a

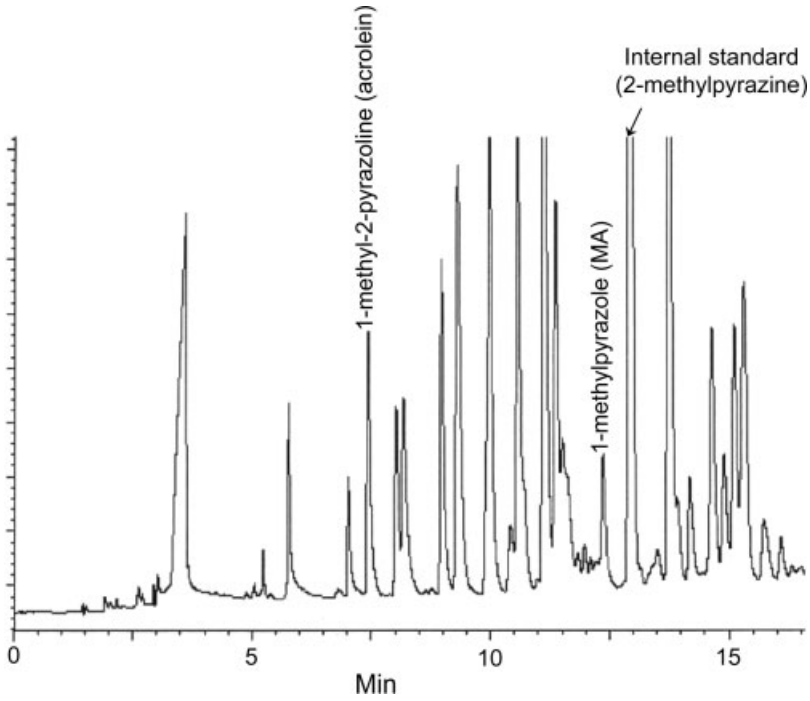

Fig. 1. A typical gas chromatogram of cigarette smoke extract treated with *N*-methylhydrazine (refer to *Instrumentation* for GC conditions).

magnetic stirrer for 30 min. The reaction solution was placed in a C_{18} SPE cartridge (Varian, Harbor City, CA, USA) and then eluted with 5 mL of ethyl acetate under reduced pressure using a vacuum manifold (Alltech Associates, Derield, IL, USA). The SPE cartridge was preconditioned by rinsing with one volume each of methanol and deionized water alternately. After 50 μL of 2-methylpyrazine solution (0.2 mg/mL^{-1} ethyl acetate) was added as a GC internal standard, the volume of the eluent was brought to exactly 5 mL with ethyl acetate. Quinoxaline (glyoxal), 2-methylquinoxaline (methylglyoxal), and 2,3-dimethylquinoxaline (diacetyl) were analyzed by a GC with a NPD. A typical gas chromatogram of ethyl acetate extract obtained from this experiment is shown in Figure 2. A GC with a MS was used to confirm the identity of quinoxaline, 2-methyl quinoxaline, and 2,3-dimethylquinoxaline. The mass spectral data of quinoxaline are as follows: m/z^{-1} (relative intensity) = 130 (M^+ 100), 103 (54), 76 (49), and 50 (16). The mass spectral data of 2-methylquinoxaline are as follows: 144 (M^+ 100), 117 (74), 76 (41), and 50 (19). The mass spectral data of 2,3-dimethylquinoxaline are as follows: 158 (M^+ 78), 117 (100), 76 (31), and 50 (16).

Quantitative Analysis of Formaldehyde, Acetaldehyde, and Propanal

Volatile alkyl aldehydes were derivatized into corresponding thiazolidines with cysteamine—thiazolidine for formaldehyde, 2-methylthiazolidine for acetaldehyde, and 2-ethylthiazolidine for propanal—by a method previously reported, with some modifications (Yasuhara and Shibamoto, 1989; Miyake and Shibamoto, 1993). Derivatizing agent cysteamine (1 mL of 0.3 M aqueous solution) was added to 4 mL of the cigarette smoke extract in a vial with a phenolic cap. The pH of the cysteamine solution was adjusted to 8 with a 6 N NaOH solution prior to use. The reaction mixture was stirred with a magnetic stirrer for 30 min. The reaction solution was placed in a C_{18} SPE cartridge and then eluted with 5 mL of ethyl acetate under reduced pressure using a vacuum manifold. The SPE cartridge was preconditioned by rinsing with one volume each of methanol and deionized water alternately. After 10 μL of 2,4,5-trimethylthiazole solution (50 mM in ethyl acetate) was added as a GC internal standard, the volume of the eluent was brought to exactly 5 mL with ethyl acetate. Thiazolidine (formaldehyde), 2-methylthiazolidine (acetaldehyde), and 2-ethylthiazolidine (propanal) were analyzed by a GC with a NPD. A typical gas chromatogram of ethyl acetate extract obtained from this experiment is shown in Figure 3. A GC with a MS was used to confirm the identity of thiazolidine, 2-methylthiazolidine, and 2-ethylthiazolidine. The mass spectral data of thiazolidine are as follows: m/z^{-1} (relative intensity) = 89 (M^+ 100), 88 (38), 59 (19), and 45 (18). The mass spectral data of 2-methylthiazolidine are as follows: 103 (M^+ 90), 88 (100), 56 (90), and 44 (51). The mass spectral data of 2-ethylthiazolidine are as follows: 117 (M^+ 17), 88 (100), 70 (25), and 56 (16).

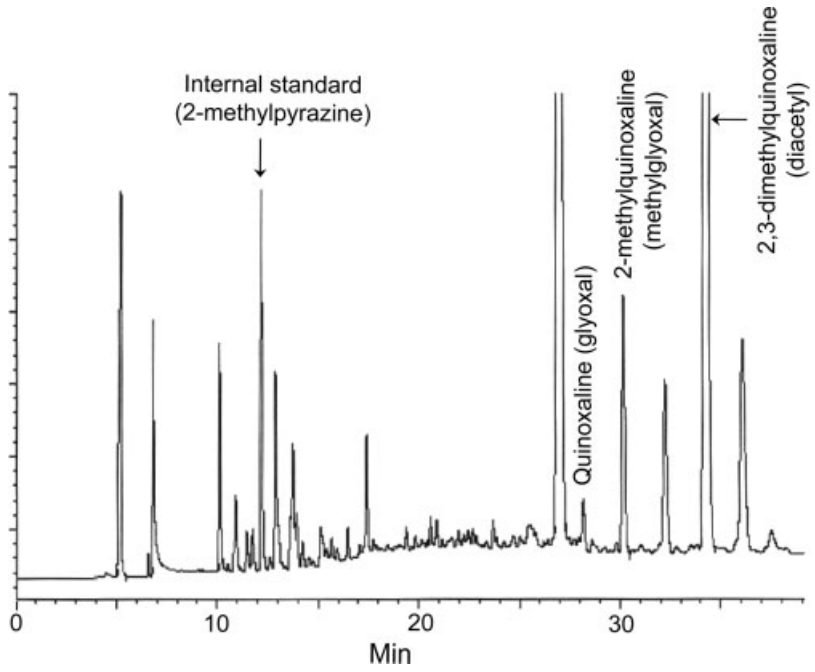

Fig. 2. A typical gas chromatogram of cigarette smoke extract treated with 1,2-phenylenediamine (refer to *Instrumentation* for GC conditions).

Recovery Efficiency Tests on MA from Various SPE Cartridges

Recovery efficient test on MA from seven different commercial SPE cartridges (C18, C8, CH, PH, PPL, ENV, and LMS—all from Varian, Harbor City, CA, USA) were conducted. A phosphate-buffered saline (5 mL, 0.1 N, pH 7.4) containing 100 μL of malonaldehyde tetrabutylammonium salt solution (1 mM) and 10 μL of *N*-methylhydrazine was stirred using a magnetic stirrer at room temperature for 30

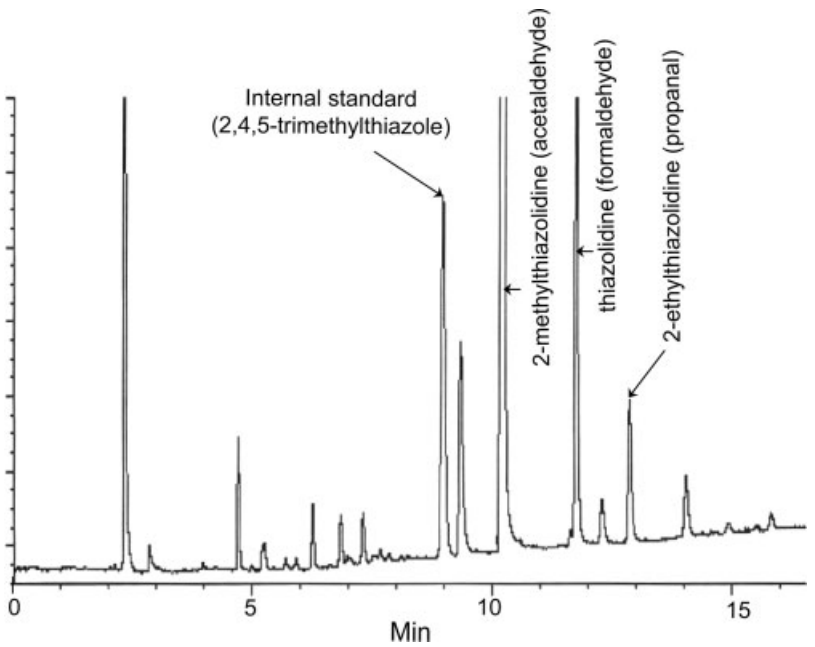

Fig. 3. A typical gas chromatogram of cigarette smoke extract treated with cysteamine (refer to *Instrumentation* for GC conditions).

TABLE I. Amounts of carbonyl compounds determined in the main stream of cigarette smoke from various brands of cigarettes

	Amounts (μg/cigarette^{-1})							
Brand	Malonaldehyde	Acrolein	Glyoxal	Methylglyoxal	Diacetyl	Formaldehyde	Acetaldehyde	Propanal
A[a,b]	28.8 ± 0.60	431 ± 13.0	1.93 ± 0.01	13.4 ± 0.10	433 ± 11.0	116 ± 5.00	2040 ± 16.0	167 ± 1.00
B[b,e]	18.9 ± 2.20	220 ± 9.00	2.09 ± 0.1	45.3 ± 0.90	308 ± 19.0	127 ± 7.00	1110 ± 21.0	87.0 ± 3.00
C[b]	28.9 ± 0.90	423 ± 1.00	2.99 ± 0.18	29.1 ± 1.70	335 ± 29.0	194 ± 17.0	1978 ± 16.0	164 ± 1.00
D[b]	26.7 ± 2.00	315 ± 19.0	3.19 ± 0.17	24.1 ± 1.70	349 ± 13.0	114 ± 5.00	1784 ± 49.0	149 ± 5.00
E[b]	29.0 ± 1.10	391 ± 4.00	3.39 ± 0.10	53.5 ± 2.20	359 ± 23.0	165 ± 5.00	1788 ± 25.0	150 ± 2.00
F[b]	28.3 ± 1.40	238 ± 6.00	4.78 ± 0.14	34.2 ± 0.70	355 ± 17.0	121 ± 9.00	1518 ± 63.0	132 ± 6.00
G[b]	29.0 ± 1.00	411 ± 11.0	2.95 ± 0.11	35.0 ± 0.90	303 ± 9.00	135 ± 5.00	1877 ± 39.0	155 ± 2.00
H[b,d]	26.2 ± 0.10	405 ± 5.00	2.76 ± 0.23	23.6 ± 1.70	320 ± 14.0	149 ± 5.00	1788 ± 20.0	148 ± 1.00
I[b,d]	24.4 ± 0.80	419 ± 27.0	2.94 ± 0.11	27.0 ± 2.60	311 ± 16.0	153 ± 1.00	1709 ± 22.0	141 ± 1.00
J[b]	24.2 ± 0.80	288 ± 4.00	2.61 ± 0.11	20.4 ± 0.70	307 ± 8.00	87.0 ± 3.00	1511 ± 31.0	123 ± 4.00
K[b,d]	21.0 ± 0.80	321 ± 10.0	3.05 ± 0.07	30.6 ± 0.60	345 ± 12.0	149 ± 5.00	1573 ± 24.0	129 ± 2.00
L[b,d]	28.7 ± 0.60	418 ± 32.0	2.21 ± 0.10	27.8 ± 0.50	357 ± 8.00	135 ± 10.0	2013 ± 81.0	161 ± 6.00
M[b,e]	19.3 ± 0.90	285 ± 22.0	2.47 ± 0.19	25.4 ± 0.40	331 ± 12.0	120 ± 3.00	1727 ± 23.0	105 ± 3.00
N[c]	27.9 ± 3.20	439 ± 28.0	3.06 ± 0.02	40.4 ± 0.20	325 ± 15.0	174 ± 3.00	1832 ± 33.0	148 ± 3.00
O[c,e]	36.0 ± 0.50	468 ± 17.0	6.98 ± 0.38	59.6 ± 2.30	301 ± 24.0	243 ± 11.0	2101 ± 28.0	176 ± 4.00

[a] Reference cigarette 2R1F.
[b] Regular size.
[c] King size.
[d] Menthol.
[e] No additive.

min. The reaction solution was placed in a SPE cartridge and then eluted with 5 mL of ethyl acetate under reduced pressure using a vacuum manifold. The SPE cartridge was preconditioned by rinsing with one volume each of methanol and deionized water alternately. After 10 μL of 2-methylpyrazine solution (10 mg/mL^{-1} in ethyl acetate) was added as a GC internal standard, the volume of the eluent was brought to exactly 5 mL with ethyl acetate. MA was quantified as 1-MP by a GC with a NPD.

Instrumentation

An Agilent Technologies Model 6890 GC equipped with a NPD and a 30 m × 0.25 mm i.d. (d_f = 1 μm) ZB-WAX bonded phase fused silica capillary column (Phenomenex, Torrance, CA, USA) was used for quantitative analysis of derivatives. The injector and detector temperatures were 200°C and 300°C, respectively. The linear velocity of helium carrier gas was 30 cm/sec^{-1} with a split ratio of 21:1. The oven temperature was programmed from 60 to 130°C at 3°C/min^{-1} for pyrazole derivatives. The oven temperature was held at 80°C for 3 min and programmed to 180°C at 4°C/min^{-1} and held for 10 min for quinoxaline and thiazolidine derivatives. The quantitative analysis was conducted according to the internal standard method reported previously (Ettre, 1967).

A HP Model 5890 series II GC interfaced to a HP 5971 MS was used to confirm the derivatives in the samples. The GC conditions were the same as for the above GC. The mass spectra were obtained by electron impact ionization at 70 eV and an ion source temperature of 250°C.

RESULTS

The recovery efficiencies of MA from the seven SPE cartridges were 101 ± 4.2% from C_{18}, 96.1 ± 0.4% from C_8, 76.7 ± 1.8% from CH, 90.0 ± 2.1% from PH, 98.7 ± 5.6% from PPL, 99.3 ± 2.4% from ENV, and 92.7 ± 2.1% from LMS. The values are mean ± standard deviation (n = 3). All SPE cartridges exhibited satisfactory recovery efficiency. However, C_{18} cartridge was chosen for further experiments because of its large capacity of lipid adsorption. The limit of quantitation (LOQ) of MA was 8.7 pg, equivalent to 14.8 pg 1-MP in the present study.

Table I presents the results of the quantitative analysis of volatile carbonyl compounds. The values are mean ± standard deviation (n = 3). Data were corrected using blank values.

Figure 1 shows a typical NPD gas chromatogram of the ethyl acetate extract from the cigarette smoke extract derivatized with *N*-methylhydrazine. All peaks in this chromatogram contain one or more nitrogen atoms. Acrolein formed in mainstream cigarette smoke from regular size cigarettes ranged from 220 (**B**) to 431 μg/cigarette^{-1} (**A**) and average was 366 μg/cigarette^{-1}. The LOQ of acrolein was 7.1 pg, equivalent to 13.0 pg 1-methyl-2-pyrazoline in the present

study. Neither MA nor acrolein was detected in the blank sample.

Figure 2 shows a typical NPD gas chromatogram of the ethyl acetate extract from the cigarette smoke extract derivatized with 1,2-phenylenediamine. The LOQ of each α-dicarbonyl compound were 9.2 pg for glyoxal, 13.0 pg for methylglyoxal, and 17.0 pg for diacetyl in the present study. The levels of glyoxal found in mainstream cigarette smoke from regular size cigarettes ranged from 1.93 (**A**) to 4.78 μg/cigarette^{-1} (**F**). The level of glyoxal in mainstream cigarette smoke from king size cigarette **O** was 6.98 μg/cigarette^{-1}, which was greater than those in mainstream cigarette smoke from regular size cigarettes. The level of methylglyoxal in mainstream cigarette smoke from king size cigarette **O** was 59.6 μg/cigarette^{-1}, which was also greater than those in regular size mainstream cigarette smoke. The levels of diacetyl in mainstream cigarette smoke from regular size cigarettes ranged from 307 (**J**) to 433 μg/cigarette^{-1} (**A**). The level of diacetyl in mainstream cigarette smoke from a king size cigarette **O** was the lowest (301 μg/cigarette^{-1}) among the 15 brands of cigarettes. None of glyoxal, methylglyoxal, and diacetyl were detected in the blank sample.

Figure 3 shows a typical NPD gas chromatogram of the ethyl acetate extract from the cigarette smoke extract derivatized with cysteamine. The LOQ of each alkanal were 3.1 pg for formaldehyde, 7.6 pg for acetaldehyde, and 13.0 pg for propanal in the present study. The levels of three alkanals in mainstream cigarette smoke from 13 brands of regular size cigarettes in the present study ranged from 87 (**J**) to 194 μg/cigarette^{-1} (**C**) for formaldehyde; from 1110 (**B**) to 2040 μg/cigarette^{-1} (**A**) for acetaldehyde; and from 87 (**B**) to 167 μg/cigarette^{-1} (**A**) for propanal. The levels of alkanals in blank sample were 9.90 $\pm$ 0.27 μg/cigarette^{-1} for formaldehyde and 25.7 $\pm$ 1.77 μg/cigarette^{-1} for acetaldehyde. Propanal was not detected in the blank sample.

DISCUSSION

Derivatives were previously recovered by liquid–liquid continuous extraction from an aqueous reaction solution. The recovery efficiency of derivatives by this method was satisfactory (Miyake and Shibamoto, 1995). However, the process was tedious and the solvent dichloromethane had to be exchanged with ethyl acetate for GC analysis because any chlorinated solvent can damage the NPD. These drawbacks were solved using the SPE method in the present study.

There have been numerous reports on the constituents of cigarette smoke (Lu et al., 2003). However, there is no report on MA in the mainstream cigarette smoke prior to the present study. MA formed in mainstream cigarette smoke from regular size cigarettes ranged from 18.9 (**B**) to 29.0 μg/cigarette^{-1} (**G**). MA formed from two king-size cigarettes **N** and **O** were 27.9 and 36.0 μg/cigarette^{-1}, respectively.

MA, formaldehyde, acrolein, acetaldehyde, and glyoxal are major reactive carbonyl compounds resulting from the oxidation of biological membranes (Vaca et al., 1988) and animal blood plasma (Miyake and Shibamoto, 1998). Also, these reactive carbonyl compounds directly cross-link to proteins and bind covalently to nucleic acids (Lam et al., 1986) and consequently cause biological complications, including carcinogenesis (Furihata et al., 1989), aging, and atherosclerosis (Halliwell and Gutteridge, 1989). Therefore, MA has been widely used as a biomarker of lipid peroxidation, which is associated with these diseases. For example, serum MA was monitored to assess oxidative damage caused by active cigarette smoking (Altuntas et al., 2002).

It is interesting that the reference cigarette (**A**) yielded the highest level of acrolein among regular size cigarettes. Acrolein formed from king size cigarettes (439 μg/cigarette^{-1} from **N** and 468 μg/cigarette^{-1} from **O**) was significantly greater than that from regular size cigarettes.

Acrolein has been known as a toxic volatile organic compound and there are many comprehensive reviews on acrolein toxicity (Ghilarducci and Tjeerdema, 1995). Acrolein is highly toxic by chronic inhalation. For example, all of the salt-induced hypertension rats exposed to 4.0 ppm acrolein died within the first 11 days (Kutzman et al., 1986). Therefore, some pathological damage caused by cigarette smoke may be partially due to the presence of acrolein in mainstream cigarette smoke.

The exact amount of glyoxal in mainstream cigarette smoke has never been reported prior to the present study. The levels of methylglyoxal in mainstream cigarette smoke from regular size cigarettes ranged from 13.4 (**A**) to 53.5 μg/cigarette^{-1} (**E**). The presence of glyoxal, methylglyoxal, and diacetyl in mainstream cigarette smoke has been known since the early 1980s (Moree-Testa and Saint-Jalm, 1981). However, quantitative analysis of these three α-dicarbonyl compounds in mainstream cigarette smoke has never been reported prior to the present study.

Glyoxal has potent mutagenic and cytogenic activities and has been reported in various heat-treated foods such as dietary oils stored at accelerated storage conditions (Kasai and Nishimura, 1986; Ueno et al., 1991). For example, a high level of glyoxal formation (12.8 ppm) was observed when salmon oil was heated at 60°C for 7 days (Fujioka and Shibamoto, 2004). Methylglyoxal found in brewed coffee was found to be mutagenic by the Ames test (Kasai et al., 1982). Methylglyoxal was also formed in heated dietary oils such as cod liver oil (2.03 ppm) and tuna oil (2.89 ppm) (Fujioka and Shibamoto, 2004).

Diacetyl has been widely used in flavor compositions, primarily in imitation butter, caramel, coffee, and cream soda, as well as in tobacco (Arctander, 1969). It is also present in natural products such as essential oils and fruits. Diacetyl is included in the USDA-GRAS (United States Department of Agriculture-Generally Recognized As Safe) list (http://vm.cfsan.fda.gov/~dms/eafus.html).

We have reported the analyses of seven alkanals, including the above three alkanals, in mainstream cigarette smoke from 26 commercial brands using the same derivatives as used in the present study (Miyake and Shibamoto, 1995). However, the reference cigarette was not included in the previous study. Therefore, these alkanals were analyzed to examine the difference between the reference cigarette and commercial cigarettes. The levels of three alkanals in mainstream cigarette smoke from 15 brands of regular size cigarettes in the previous study ranged from 73.8 to 265.7 μg/cigarette^{-1} for formaldehyde, from 1706 to 2274 μg/cigarette^{-1} for acetaldehyde, and from 186 to 349 μg/cigarette^{-1} for propanal (Miyake and Shibamoto, 1995). The results indicate that the formation of alkanals from the reference cigarette was comparable to that from commercial cigarettes.

King size cigarette **O** yielded the greatest amount of carbonyl compounds except diacetyl. On the other hand, regular size cigarette **M** (same brand as **O**) produced approximately half the carbonyl compounds produced by **O**. However, the other king size cigarette **N** did not yield significantly higher levels of carbonyl compounds compared with regular size cigarettes, suggesting that different brands play a more important role in carbonyl compound formation than the size of the cigarette. Also, there were no significant differences in carbonyl compounds formation between cigarettes with and without menthol or additives. Moreover, the toxicological study on 150 commonly used tobacco additives, including menthol, using a sensitive tumorigenesis model system did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate (Gaworski et al., 1999).

The precursors and formation mechanisms of these genotoxic carbonyl compounds in the mainstream cigarette smoke are not yet well understood. However, it is well known that these carbonyl compounds are formed from lipids by heat treatment. Tobacco contains certain amounts of lipids (Wassef and Hendrix, 1973) and waxes (Carruithers and Johnstone, 1959), which can be precursors of these carbonyl compounds. Carbonyl compounds—including MA, acrolein, glyoxal, methylglyoxal, diacetyl, formaldehyde, acetaldehyde, and propanal—have reportedly been formed from heat treatment of various lipids via oxidative cleavage of the double bond (Frankel, 1991; Yeo and Shibamoto, 1992). Because the temperature of the lit cigarette is extremely high ($>1000^{\circ}$C), many low molecular weight radicals such as ·OH, ·CHO, ·CH_2CHO, ·CH_3, and ·$COCH_3$ might be formed from lipids upon oxidative pyrolysis, and these radicals combine to form low molecular weight carbonyl compounds (Niyati-Shirkhodaee and Shibamoto, 1993).

CONCLUSIONS

As mentioned above, cigarette smoke contains potent carcinogens, including polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, and dioxins, which are deposited directly into the blood following inhalation. Some carcinogens found in cigarette smoke, such as PAHs, *N*-nitrosamines, and dioxins require enzymatic activation to be toxic. In contrast, reactive carbonyl compounds analyzed in the present study can be directly cross-linked to proteins and bind covalently to nucleic acids (Lam et al., 1986; Matsufuji and Shibamoto, 2004) and consequently cause biological complications, including carcinogenesis (Basu et al., 1984; Nair et al., 1986). Therefore, it is important to determine the amounts of reactive carbonyl compounds to which people are exposed to assess the further health risks associated with cigarette smoke. The method developed in the present study can be applied to monitor toxic carbonyl compounds in the ambient air.

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