Notes

Determination of Carbonyl Compounds Generated from the E-cigarette Using Coupled Silica Cartridges Impregnated with Hydroquinone and 2,4-Dinitrophenylhydrazine, Followed by High-Performance Liquid Chromatography

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Carbonyl compounds in E-cigarette smoke mist were measured using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. A total of 363 E-cigarettes (13 brands) were examined. Four of the 13 E-cigarette brands did not generate any carbonyl compounds, while the other nine E-cigarette brands generated various carbonyl compounds. However, the carbonyl concentrations of the E-cigarette products did not show typical distributions, and the mean values were largely different from the median values. It was elucidated that E-cigarettes incidentally generate high concentrations of carbonyl compounds.

Keywords E-cigarette, carbonyl compounds, acrolein, glyoxal, methylglyoxal, glycerol, propylene glycol

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Introduction

E-cigarettes (electronic cigarettes or e-cigs) are battery-powered devices designed to deliver nicotine to a smoker in the form of a vapor, and were first introduced into the Chinese market in 2004. Currently, they are widely used around the world. In the United States, as of 2011, approximately 21% of adults who smoked traditional cigarettes had used electronic cigarettes, which was an increase from 10% in 2010, according to a study released by the Centers for Disease Control and Prevention.[1] Overall, approximately 6% of all adults have tried E-cigarettes, and these estimates are nearly double those from 2010.[1] It was reported in the news media in 2013 that electronic cigarettes were beginning to gain cultural acceptance, and sales were growing rapidly.[2]

An electronic cigarette contains three essential components: a plastic cartridge that serves as a mouthpiece and a reservoir for a liquid, an "atomizer" that vaporizes the liquid, and a battery. The liquid used to produce the vapor in electronic cigarettes is a solution of propylene glycol and/or glycerin and/or polyethylene glycol mixed with concentrated flavors and, optionally, a variable percentage of liquid nicotine concentrate. These base liquids have been widely used as food additives, as base solutions in personal care products, such as toothpaste, and in medical devices, such as asthma inhalers. However, there are few reports on chemical compounds in E-cigarette smoke mist; moreover, the health effects of inhaling nicotine vapor into the lungs are uncertain.

We have developed a new method (the HQ-DNPH method) for the determination of acrolein and other carbonyl compounds in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine[3] (DNPH), and we reported that E-cigarettes sometimes accidentally generate various carbonyl compounds, such as formaldehyde, acetaldehyde, acrolein, glyoxal, and methyl glyoxal.[3,4] In these previous studies, we concluded that ethylene glycol was oxidized to formaldehyde and glyoxal; propylene glycol was oxidized to formaldehyde, acetaldehyde, and methylglyoxal; and glycerol was oxidized to formaldehyde, acrolein, glyoxal, and methylglyoxal.[4] In this study, we determined the concentration of various carbonyl compounds generated from a total of 363 E-cigarettes (13 brands). The results are presented herein.

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Experimental

Apparatus and reagents

An HPLC system (Shimadzu, Kyoto, Japan) with two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photodiode array detector, was used. The analytical column was an Ascentis Express RP-Amide (2.7 μm particle size, 150 mm × 4.6 mm i.d., Supelco Inc., Bellefonte, PA). The column temperature was 40°C, and the injection volume was 10 μL. Solution A of the mobile phase mixture was composed of acetonitrile/water (40/60 v/v) containing 5 mmol/L ammonium acetate; solution B was composed of acetonitrile/water (75/25 v/v). HPLC elution was carried out with 100% A for 8 min, followed by a linear gradient from 100% A to 100% B in 37 min, and then maintained constant for 15 min using 100% B. The flow rate of the mobile phase was 0.7 mL/min.

An LM1/PLUS (Borgwaldt Technik GmbH, Hamburg, Germany) smoking machine was used for the collection of cigarette smoke.

The water used for the HPLC analysis and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) was obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), ethanol (>99.5%), hydroquinone (>99%), phosphoric acid (85% solution in water), and ammonium acetate (99.999%) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). The silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was acquired from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan).

The DNPH-impregnated silica cartridge (DNPH-cartridge) and the hydroquinone-impregnated silica cartridge (HQ-cartridge) were prepared according to previous reports.[3,4]

Collection and analysis of E-cigarette smoke

Before collecting smoke from the E-cigarettes, an HQ-cartridge and a DNPH-cartridge were connected. The coupled cartridges were then connected between the mouthpiece of the E-cigarette and the smoking machine, and the smoke from the E-cigarette was drawn into the coupled cartridges from the HQ-cartridge to the DNPH-cartridge according to the Canadian intense regimen;[5] (55 mL puff volume, 2-s puff duration, 30-s puff interval, and 10 puffs). After collection, the coupled cartridges were extracted using acetonitrile containing 1% phosphoric acid in a direction opposite to the air sampling direction until the total volume of the solution was 4.5 mL. After 10 min, ethanol (0.5 mL) was added to the eluate, and the solution was analyzed by HPLC. If the extraction was not performed immediately, the HQ-DNPH cartridge set was decoupled, and the individual cartridges were capped with stoppers.

Results and Discussion

Analysis of E-cigarette smoke by the HQ-DNPH method

Various types of carbonyl compounds were detected in the E-cigarette smoke. Figure 1 shows a representative chromatogram of a sample eluate by HPLC analysis with UV (360 nm) detection. In the HQ-DNPH method, it is possible to analyze C1 – C10 carbonyl compounds, and C1 – C3 carbonyl compounds, such as formaldehyde, acetaldehyde, acetone, acrolein, propanal, glyoxal, and methylglyoxal, were detected.

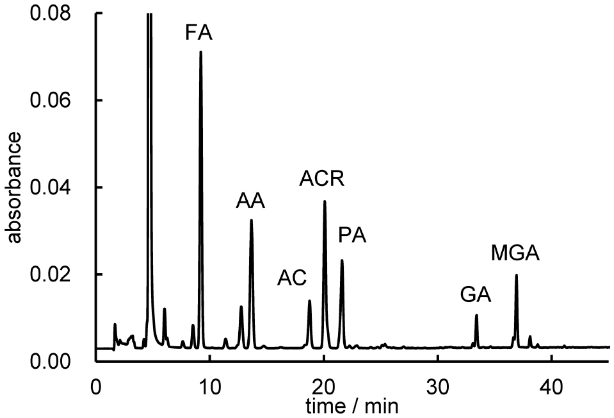

Fig. 1 Representative chromatogram of carbonyl DNPhydrazones derivatized from DNPH with carbonyls found in E-cigarette smoke. FA, formaldehyde; AA, acetaldehyde; AC, acetone; ACR, acrolein; PA, propanal; GA, glyoxal; MGA, methylglyoxal.

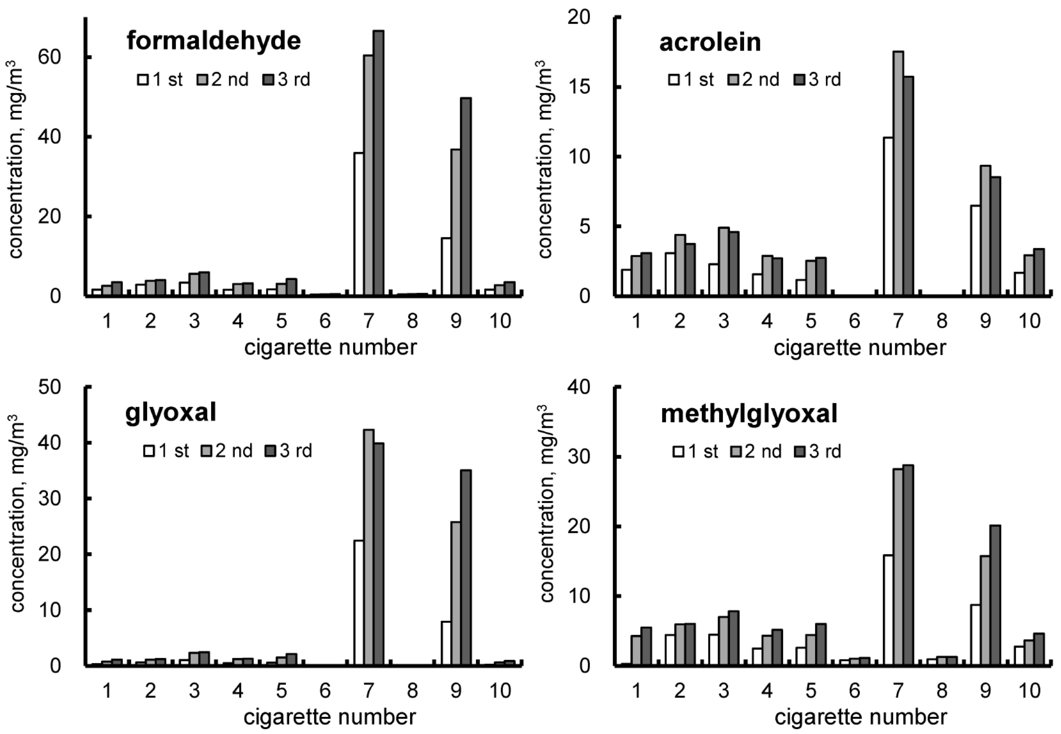

Fig. 2 Concentrations of formaldehyde, acrolein, glyoxal, and methylglyoxal generated from 10 different E-cigarettes of the same brand. Reproduced with permission from Fig. 3 in Ref. 4.

Table 1 Concentrations (mg/m³) of major carbonyl compounds generated from 13 brands of E-cigarettes

Product	N_{high} / N_{low}	FR	Formaldehyde	Acetaldehyde	Acrolein	Propanal	Glyoxal	Methylglyoxal
A	16	31	61 ± 64	48 ± 51	7.5 ± 6.9	16 ± 19	4.6 ± 6.5	5.3 ± 5.7
	35		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B	6	20	44 ± 19	0.3 ± 0.1	12 ± 4.3	2.0 ± 1.2	29 ± 12	20 ± 7.8
	24		2.6 ± 1.6	n.d.	2.2 ± 1.6	n.d.	n.d.	3.7 ± 2.2
C	8	27	40 ± 28	1.7 ± 2.5	9.7 ± 10	6.1 ± 6.3	18 ± 9.5	22 ± 10
	22		3.1 ± 2.6	n.d.	1.1 ± 1.1	n.d.	1.3 ± 1.4	2.1 ± 1.9
D	12	24	28 ± 12	25 ± 12	36 ± 18	24 ± 19	7.7 ± 4.1	11 ± 7.5
	37		1.5 ± 1.8	n.d.	n.d.	n.d.	n.d.	n.d.
E	14	40	31 ± 14	27 ± 11	34 ± 12	27 ± 15	8.2 ± 4.4	8.6 ± 7.9
	21		1.3 ± 1.5	n.d.	1.2 ± 1.7	n.d.	n.d.	n.d.
F	2	40	12 ± 1.7	2.8 ± 0.2	2.0 ± 0.1	0.7 ± 0.1	2.8 ± 0.7	5.8 ± 0.9
	3		3.6 ± 3.1	1.6 ± 0.4	1.2 ± 0.5	n.d.	n.d.	1.6 ± 1.5
G	1	4	53	19	19	6.3	17	37
	25		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H	5	17	19 ± 8.9	8.3 ± 4.3	8.1 ± 4.0	n.d.	4.6 ± 0.9	8.4 ± 5.7
	25		1.7 ± 2.6	n.d.	n.d.	n.d.	n.d.	n.d.
I	6	20	5.8 ± 1.9	11 ± 5.9	11 ± 4.2	14 ± 4.1	n.d.	n.d.
	24		2.8 ± 2.6	4.8 ± 5.2	5.0 ± 4.9	6.0 ± 6.2	n.d.	n.d.
J	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	4		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
K	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	13		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The upper line indicates the mean value for the high-concentration group, and the lower line indicates the mean value for the low-concentration group. Indices N_{high} and N_{low} indicate the number of E-cigarettes that generated high and low concentrations of carbonyl compounds, respectively. FR indicates the failure rate, which was calculated using the following equation: $FR = N_{high}/(N_{high} + N_{low}) \times 100$. Values are mean ± SD. n.a., not available; n.d., not detected.

Concentration of carbonyl compounds in E-cigarette smoke

The concentration of carbonyl compounds in the smoke mist from 13 brands of E-cigarettes sold in Japan was determined by the HQ-DNPH method. The analysis of these actual brands of E-cigarettes revealed very large variations in the carbonyl concentrations among not only different brands, but also different examples of the same products. Typical distributions of the carbonyl concentrations were not observed for any of the E-cigarettes tested, and the mean values were largely different from the median values. These concentration variations were not caused by the analytical method, because the HQ-DNPH method has good reproducibility (RSD less than 2.1%).[3] We previously reported that the smoke mist generated from E-cigarettes unexpectedly contains carbonyl compounds.[4] This conclusion is based on the fact that for the same E-cigarette products, it was found that some E-cigarettes generated high concentrations of carbonyl compounds, while others did not. Figure 2 shows the concentrations of formaldehyde, acrolein, glyoxal, and methylglyoxal generated from 10 electronic cigarettes of the same brand. These results represent triplicate measurements for 10 samples. As can be seen in the figure, the number 7 and 9 E-cigarettes generated peculiarly high concentrations of carbonyl compounds. Therefore, the resulting data were divided into two groups based on the formaldehyde concentration (10 mg/m³): a high concentration group and a low concentration group. Table 1 shows the concentrations of the major carbonyl compounds generated from 13 brands of E-cigarettes. In the table, the top entry in each cell indicates the mean value for the high-concentration group, and the lower entry indicates the mean value for the low-concentration group. The indices N_{high} and N_{low} indicate the number of E-cigarettes that generated high and low concentrations of carbonyl compounds, respectively. FR indicates the failure rate, which was calculated by the following equation: $FR = N_{high}/(N_{high} + N_{low}) \times 100$.

Four (J, K, L, M) out of the 13 E-cigarette brands did not generate any carbonyl compounds. The other nine E-cigarette brands (A, B, C, D, E, F, G, H, I) generated various carbonyl compounds. The concentrations of carbonyl compounds obtained for the high concentration group were significantly higher than that determined for the low concentration group. The maximum concentrations of formaldehyde, acetaldehyde, acrolein, propanal, glyoxal, and methylglyoxal were 260, 210, 73, 83, 42, and 38 mg/m³, respectively. For a typical cigarette smoking experience of 10 puffs, these values translate to maximum concentrations of 140 μg formaldehyde/cigarette, 120 μg acetaldehyde/cigarette, 33 μg acrolein/cigarette, 46 μg propanal/cigarette, 23 μg glyoxal/cigarette, and 21 μg methylglyoxal/cigarette. Most notably, very high concentrations of formaldehyde were measured in the smoke from the E-cigarettes. Glyoxal and methylglyoxal are peculiar to E-cigarette smoke, and have not been detected in the mainstream smoke from normal cigarettes. Glyoxal is known to be mutagenic to *Salmonella typhimurium* strains TA100, TA102, and TA104.[6,7] It has been shown that glyoxal reacts with guanine residues in DNA.[8] Its tumor promoting activity has also been reported.[9,10] Methylglyoxal, the most mutagenic of all aldehydes, is known to inhibit formaldehyde metabolism, thus

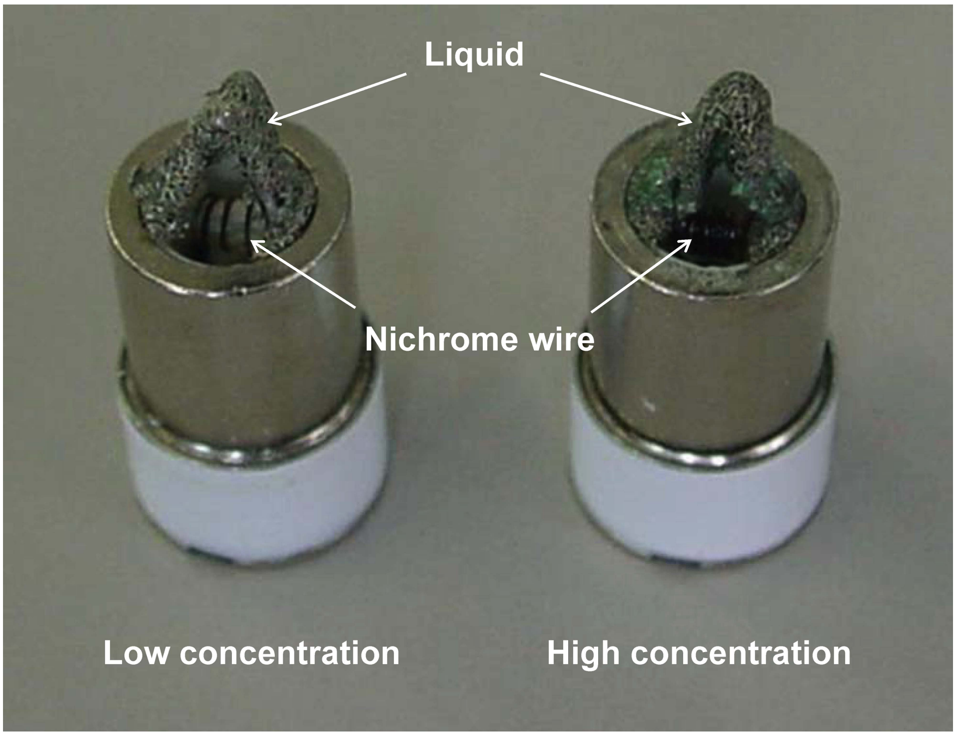

Fig. 3 E-cigarette atomizers that generated low and high concentrations of carbonyl compounds.

enhancing formaldehyde-induced cytotoxicity.[11]

After smoking an E-cigarette, the atomizer that generated the high concentrations of carbonyl compounds was burned black. Figure 3 shows atomizers after smoking 10 puffs. The left atomizer generated a low concentration. The right atomizer generated a high concentration of carbonyl compounds, and the color around Nichrome wire changed from white to black. These results suggest that the compounds in the E-cigarette liquid, such as glycerol and glycols, incidentally touch the heated Nichrome wire and are oxidized to formaldehyde, acetaldehyde, acrolein, glyoxal, and methylglyoxal.

Conclusions

E-cigarettes incidentally generate carbonyl compounds in the E-cigarette smoke mist. A possible cause for carbonyl generation is the oxidation of liquids in the E-cigarette, such as glycerol and glycols, when they incidentally touch the heated Nichrome wire in the atomizer, and are oxidized to formaldehyde, acetaldehyde, acrolein, glyoxal, and methylglyoxal. In some cases, these hazardous compounds are generated with extremely high concentrations. Suppliers and users of E-cigarettes should pay attention to this phenomenon.

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