We developed an analytical method for analyzing electronic cigarette (E-cigarette) smoke, and measured the carbonyl compounds and volatile organic compounds generated by 10 brands of second-generation E-cigarettes. A glass filter (Cambridge filter pad) for particulate matter and a solid sorbent tube packed with Carboxen-572 for gaseous compounds were used to collect E-cigarette smoke. These were then analyzed using a two-step elution method with carbon disulfide and methanol, followed by high-performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC/MS). Formaldehyde (FA), acetaldehyde (AA), acetone (AC), acrolein (ACR), propanal (PA), acetal (AT), glyoxal (GO), and methyl glyoxal (MGO) were detected by HPLC in some E-cigarettes. Propylene glycol (PG), glycerol (GLY), and some esters were detected by GC/MS. GO and MGO exist mainly as particulate matter. AA, AC, ACR, PA, and AT exist mainly as gaseous compounds. FA exists as both particulate matter and gaseous compounds. These carbonyl compounds have carbon numbers C1 – C3. The main components of E-liquid are PG (C3) and GLY (C3). Therefore, the oxidation of liquids, such as PG and GLY in E-cigarettes upon incidental contact with the heating element in E-cigarette, is suggested as being a possible cause for carbonyl generation. When the puff number exceeds a critical point, carbonyl generation rapidly increases and then remains constant. The results of this study are now being used to determine the following E-cigarette smoking protocol: puff volume, 55 mL; puff duration, 2 s; and puff number, 30. E-cigarette analysis revealed very large variation in carbonyl concentration among not only different brands, but also different samples of the same product. Typical distributions of carbonyl concentration were not observed in any of the E-cigarettes tested, and the mean values greatly differed from median values.

**Keywords** Electronic cigarette, two-step elution, carbonyls, glyoxal, methyl glyoxal

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(DNPH)-impregnated silica for the derivatization of carboxyls. We analyzed E-cigarette smoke from products sold in Japan using this dual-cartridge method, and found gaseous carbonyl compounds such as FA, AA, and ACR. In the previous study, we tested first-generation E-cigarettes. It is difficult to measure second-generation E-cigarette smoke by the dual cartridge method because of the large amount of smoke produced. Therefore, 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, followed by a two-step elution method. This method can be applied to analyze high concentrations of VOCs and carbonyl compounds. This study used the modified two-step elution method to analyze E-cigarette smoke.

Experimental

Apparatus and reagents

An 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 with a 3-μm particle size and 150 × 3 mm i.d. column (Supelco Inc., Bellefonte, PA, USA). Solution A of the mobile phase mixture was acetonitrile/water (50/50 v/v) that contained 10 mmol/L ammonium acetate; solution B was acetonitrile/water (80/20 v/v). HPLC elution was performed 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.

A gas chromatography mass spectrometry (GC/MS) system (QP 2010 Ultra, Shimadzu, Kyoto, Japan) with a fused-silica column (InertCap AQUATIC-2 60 m × 0.25 mm i.d., d = 1.4 μm, GL Sciences, Tokyo, Japan) was used. GC/MS was operated with temperature programming from 40°C (held for 6 min) to 250°C at 6°C/min with He carrier gas at 0.51 mL/min and 70 eV EIMS detection operated in the full-scan mode from 40 to 500 m/z. The injection volume was 1 μL (split injection, split ratio 10:1; septum purge 1 mL/min; injector temperature 240°C).

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

The water used for HPLC and sample preparations was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA, USA). Carboxen-572 (20/45 mesh), acetonitrile (HPLC grade, >99.9%), methanol (anhydrous, 99.8%), ethanol (>99.5%), phosphoric acid (85% solution in water), (+)-nicotine (≥99%), and ammonium acetate (99.99%) were purchased from Sigma-Aldrich, Inc. (MO, USA). DNPH hydrochloride (≥98%) was purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan).

Preparation of the sorbent cartridge, Cambridge filter pad, and concentrated DNPH solution

Gaseous compounds generated from E-cigarettes were collected with a sorbent cartridge (CX-572) packed with Carboxen-572 particles. Three hundred milligrams of Carboxen-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, the carbon adsorbents were packed into a polyethylene cartridge (Rezorian tube, 1 mL, Supelco Inc., Bellefonte, PA) placed with end frits.

Particulate matter generated from E-cigarettes was collected with a Cambridge filter pad (CFP). CFPs were washed with acetonitrile and dried in a vacuum to 5-8 MPa at room temperature. Washed CFPs were used after conditioning for 48 h in a constant temperature and humidity chamber set to 25°C and 55%, respectively.

Carbonyl compounds generated from E-cigarettes were analyzed by derivatization with DNPH. Phosphoric acid (10 mL) and DNPH hydrochloride (1 g) were combined in a 50-mL volumetric flask, and then the solution was diluted to 50 mL with acetonitrile. This mixture was continuously stirred with a magnetic stirrer until becoming clear, and then stored in a refrigerator at 4°C.

Collection of E-cigarette smoke using CX-572 and CFP

An E-cigarette was attached to the CFP at a 30° angle (Fig. 1) on the smoking machine. The CX-572 was installed between the CFP and the pump of the smoking machine. Main-stream E-cigarette smoke collection was performed according to the Health Canada Intense (HCI) regime. Smoke constituents were collected under conditions that involved a 55-mL puff volume, 2 s puff duration, and 30 s puff interval. CX-572 cartridge method has adequate adsorption efficiencies for carbonyl compounds and VOCs to mainstream cigarette smoke generated from traditional cigarette. In this experiments, the substances collected on CFP and CX-572 are defined as particulate and gaseous matters, respectively.

Analysis of carbonyl compounds and VOCs collected in CX-572 and CFP

The analysis of carbonyl compounds and VOCs in CX-572 was performed by the two-step elution method, followed by GC/MS and HPLC. A flow chart of the analytical procedure for simultaneous determinations of VOCs and carbonyls in mainstream E-cigarette smoke is shown in Fig. 2.

CX-572: An 8 mL vial was filled with 1 mL of carbon disulfide, to which 4 mL of methanol was slowly added. The solution in the vial was separated into two phases (carbon disulfide was the lower phase). After cigarette smoke collection, the CX-572 particles with frits were removed from the cartridge and deposited into a vial. The CX-572 particles settled into the lower carbon disulfide phase, where the nonpolar compounds
were eluted. After the sample had sat for 3 min, the solution was slowly stirred. The two-phase solution of carbon disulfide and methanol was then solved into a single phase, and the polar compounds were eluted. At this point, the desorbed nonpolar compounds remained in solution. For VOC analysis, 1 mL of the eluate solution was transferred to a 1.5-mL autosampler vial. This solution was analyzed by GC/MS under the previously described conditions. A 0.5-mL aliquot of the eluate solution was transferred to a 5-mL volumetric flask for carbonyl analysis. Then, 0.2 mL of the enriched DNPH solution was added. After 10 min, this solution was diluted to 5 mL with ethanol and analyzed by HPLC.

CFP: After collecting E-cigarette smoke, the CFP was placed in a 50-mL Erlenmeyer flask. Then, 10 mL of methanol was added to an Erlenmeyer flask and stirred on a rotary shaker at 120 cycles per min for 20 min. VOCs and carbonyl compounds were analyzed according to the previously described procedure.

E-cigarettes tested

Ten brands of E-cigarettes commercially available in Japan were used in this study. When the products were packaged with the specific E-liquids, the packed E-liquids were used as is. When the product did not contain any additional E-liquid, a comparable E-liquid (Apple, Premium smoke juice, Liqua, RITCHY Group Ltd.) was used as the test sample. Table 1 provides an outline of the E-cigarettes tested in this study. The voltage and resistance were the measured values obtained with a multimeter (U1271A, Agilent Technologies, Inc., CA, USA).

Results and Discussion

Analysis of chemical substances generated from E-cigarettes

Chemical compounds were detected in some of the E-cigarettes whose smoke was analyzed by the two-step elution method described. Figure 3 shows representative HPLC chromatograms of carbonyl compounds obtained from the CFP (particulate matter) and CX-572 (gaseous compounds). Figure 4 shows representative GC/MS chromatograms of chemical compounds obtained from the CFP (particulate matter) and CX-572 (gaseous compounds). Nicotine-free E-liquid was used in the E-cigarettes because nicotine products are forbidden by the Pharmaceutical Affairs Law in Japan. When an E-cigarette with E-liquid containing nicotine was analyzed, the chromatogram indicated its presence with a dark line, as can be seen in Fig. 4. The nicotine peak was detected at a retention time of 41 min.

All carbonyl compounds detected in E-cigarette smoke are comprised of carbon numbers 1 to 3. Glyoxal (GO) and methylglyoxal (MGO) exist mainly as particulate matter. Acetaldehyde (AA), acetone (AC), acrolein (ACR), propanal (PA), and acetol (AT) exist mainly as gaseous compounds. AT is detected by GC/MS, but not by HPLC. Formaldehyde (FA) exists as both particulate and gaseous compounds. The main components of E-liquid in E-cigarettes are PG and GLY. A possible cause for carbonyl generation is the oxidation of liquids, such as PG and GLY, in the E-cigarette upon incidental contact with the heated Nichrome wire in the atomizer. The liquids are oxidized to formaldehyde (C1), acetaldehyde (C2), ...
acrolein (C3), glyoxal (C3), and methylglyoxal (C3) (Scheme 1).

Bis-DNPH derivatives, such as GO and MGO, have different UV-visible absorption spectra from monocarbonyl compounds. Figure 5 shows UV-visible absorption spectra of DNPH derivatives at concentrations of 20 \( \mu \text{mol/L} \). The maximum wavelengths and molar absorption coefficients of UV-visible absorption spectra are as follows: FA, 354 nm, \( 2.0 \times 10^4 \) L/mol/cm; AA, 363 nm, \( 2.1 \times 10^4 \) L/mol/cm; ACR, 372 nm, \( 2.6 \times 10^4 \) L/mol/cm; PA, 363 nm, \( 2.1 \times 10^4 \) L/mol/cm; GO, 437 nm, \( 4.9 \times 10^4 \) L/mol/cm; MGO, 413 nm, \( 4.5 \times 10^4 \) L/mol/cm. The spectra are divided into two groups based on a maximum wavelength of 360 or 425 nm. As a result, HPLC analysis was performed using the maximum wavelength range of 350 - 450 nm for detection. PG and GLY are both detected in the CFP (particulate matter), but not in CX-572 (gaseous compound). AT is detected in both CFP and CX-572. As previously described, E-cigarette smoke is generated from E-liquids, such as PG and GLY, by heating with a Nichrome coil. These results confirm that CFPs can effectively collect E-cigarette smoke. Many esters, such as ethyl acetate, ethyl butyrate, butyl acetate, ethyl isovalerate, isoamyl isovalerate (banana oil), and isoamyl acetate (apple oil), are detected as gaseous compounds in the smoke. This suggests that these esters are contained in E-liquid as a perfume.

**Carbonyl generation with puff number**

Smoking a traditional cigarette is “static” and has a puff number of between 8 and 12 per cigarette. However, smoking an E-cigarette is “flexible” and does not have a puff number per E-cigarette, because smoking continues until no E-liquid remains. Therefore, we measured the changes in E-cigarette carbonyl generation according to the puff number in order to identify the generation profile. Figure 6 shows changes in the carbonyl compound generation amount from E-cigarettes with the puff number. The puff intervals were set to 15, 30, and 60 s. The upper panels indicate individual puff data, and lower panels indicate cumulative puff data.

The profile of carbonyl generation with the puff number by an

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**Fig. 4** Representative chromatographic profiles of VOCs in gaseous compounds generated from E-cigarettes. AT, Acetol; PG, propylene glycol; GLY, glycerol; NT, nicotine; EA, ethyl acetate; EB, ethyl butyrate; BA, butyl acetate; EV, ethyl isovalerate; BO, isoamyl isovalerate (banana oil); HA, \( n \)-hexyl acetate; AO, isoamyl acetate (apple oil).

**Fig. 5** UV-visible absorption spectra of monoaldehyde-DNPhydrazones (solid lines) and dicarbonyl-DNPhydrazones (broken lines).

**Scheme 1** The oxidation reaction of propylene glycol and glycerol to form various aldehydes.
E-cigarette is very different from the profile by a traditional cigarette. Carbonyl compound generation is very low until the critical points: 15 puffs at the 15 s interval, 13 puffs at the 30 s interval, and 11 puffs at the 60 s interval. However, when the puff number exceeds these critical points, carbonyl generation increases rapidly, and remains constant. An adequate amount of E-liquid remains in the E-cigarette vial for at least 30 puffs.

It has been reported that compounds in an E-cigarette liquid, such as GLY and glycols, incidentally come into contact with the heated Nichrome wire and undergo pyrolysis and oxidation to form FA, AA, ACR, GO, and MGO. The theoretical vaporization temperature of the heating element may reach up to 350°C. This temperature is sufficiently high to induce physical changes in the E-liquids and chemical reactions between the constituents of E-liquids. Solvents at this temperature may undergo thermal decomposition, leading to the formation of potentially toxic compounds. These results suggest that a hot-spot may appear in the atomizer as result of elevated temperature due to repeated puffs. When the temperature exceeds a critical point, carbonyl compound generation increases rapidly. E-cigarettes can be smoked until the E-liquid vial is empty. If the puff number is set to ten puffs per E-cigarette, similar to a traditional cigarette, the measurement value is too low for correct evaluation. Therefore, these results indicate that the standard protocol used to measure chemical substances in E-cigarette smoke, as determined by the HCl regimen, should be 30 puff numbers.

Relationship between carbonyl generation and applied voltage

It is possible to increase the generation of carbonyl compounds by the applied voltage. Figure 7 shows changes in the amount of carbonyl compound generation from E-cigarettes with the applied voltage to the heater coil in the atomizer. The atomizer of brand D and a battery (brand H) with a variable output range were used in the test.

E-cigarette smoke mist increases with increased applied voltage from 3.2 to 4.4 V, but decreases slightly at up to 4.8 V. Carbonyl compounds are generated at voltages exceeding 4 V and increase rapidly until 4.8 V. It was reported that the battery output voltage affects the concentration of carbonyl compounds in the emission. Certain newer types of E-cigarettes allow users to increase the vapor production and nicotine delivery by changing the battery output voltage. Kosmider et al. showed that increasing the voltage from 3.2 to 4.8 V resulted in a 4 to >200 times increase in the FA, AA, and AC levels. Ohta et al. also reported that increased levels of carbonyl compounds, such as FA and AA, were observed at a voltage of over 3 V. Consequently, commercial E-cigarettes with 4 – 5 V batteries are sufficient to generate carbonyl compounds. The battery output voltage significantly affects the concentration of carbonyl compounds in the E-cigarette aerosol. As a result, high-voltage E-cigarettes may expose users to high levels of carbonyl compounds.

Carbonyl generation from various E-cigarettes

The carbonyl compound concentrations in smoke mist from nine brands of E-cigarettes sold in Japan were determined by the CX-572/two-step elution method. Table 2 gives the amounts...
(μg/10 puff) of major carbonyl compounds generated by them. The smoking machine was set at 30 puffs, and the amounts were converted into 10 puff values for comparisons with traditional cigarettes. Analysis of these E-cigarette brands reveals very large variations in carbonyl concentrations among different brands as well as different samples of the same brand. Typical distributions of carbonyl concentrations were not observed for any of the E-cigarettes tested. The mean values differ greatly from the median values. Data from the reference cigarette 3R4F\(^5\) are listed in the table for comparison. The overall maximum values are very high, 790 μg/10 puff from the median values. Data from the reference cigarette in traditional cigarettes have been reported: GO, 0.1 – 0.5 μg/10 cigarette. The measured GO and MGO levels are substantially lower than those in the E-cigarette data. Figure 8 shows the amounts of FA, AA, ACR, GO, MGO, and smoke mist generated from 9 brands of E-cigarettes. These results represent triplicate measurements for five samples. E-cigarettes of brands B, C, D, and E generate high amounts of carbonyl compounds. Five (F, G, H, I, and J) of the nine E-cigarette brands show little carbonyl compound generation. The measurement values of carbonyl compounds vary among the five products, and also among three measurements of each.

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**Supporting Information**

The relationship between carbonyl generation and E-cigarette smoke (Fig. S1) and the relationship between gaseous formaldehyde and particulate formaldehyde (Fig. S2) are provided in supporting information. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

**References**

Fig. 8 Amounts of various aldehydes and smoke mist generated from nine brands of E-cigarettes.

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