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Article

Application of High-Resolution Mass Spectrometry and a Theoretical Model to the Quantification of Multifunctional Carbonyls and Organic Acids in e-Cigarette Aerosol

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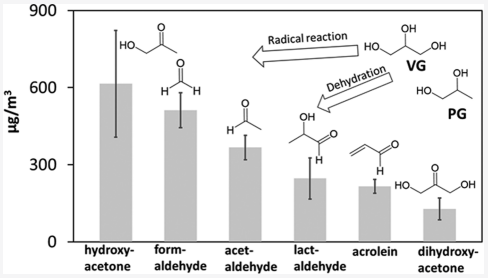
ABSTRACT: Electronic (e-) cigarette aerosol (particle and gas) is a complex mixture of chemicals, of which the profile is highly dependent on device operating parameters and e-liquid flavor formulation. The thermal degradation of the e-liquid solvents propylene glycol and glycerol often generates multifunctional carbonyls that are challenging to quantify because of unavailability of standards. We developed a theoretical method to calculate the relative electrospray ionization sensitivities of hydrazones of organic acids and carbonyls with 2,4-dinitrophenylhydrazine based on their gas-phase basicities ($\Delta G_{deprotonation}$). This method enabled quantification by high-performance liquid chromatography−high-resolution mass spectrometry HPLC-HRMS in the absence of chemical standards. Accurate mass and tandem multistage MS (MS^n) were used for structure identification of vaping products. We quantified five simple carbonyls, six hydroxycarbonyls, four dicarbonyls, three acids, and one phenolic carbonyl in the e-cigarette aerosol with Classic Tobacco flavor. Our results suggest that hydroxycarbonyls, such as hydroxyacetone, lactaldehyde, and dihydroxyacetone can be significant components in e-cigarette aerosols but have received less attention in the literature and have poorly understood health effects. The data support the radical-mediated e-liquid thermal degradation scheme that has been previously proposed and emphasize the need for more research on the chemistry and toxicology of the complex product formation in e-cigarette aerosols.



1. INTRODUCTION

Since its introduction to the United States in 2007, the electronic (e-) cigarette market has expanded significantly.[1,2] The prevalence of e-cigarette use was 3.2% for adults and 7.6% for young adults (aged 18−24) in 2018.[3] The prevalence of e-cigarette use among high school students increased from 1.5% in 2011 to 27.5% in 2019, eclipsing conventional cigarettes among youth.[4,5] With the growing population of e-cigarette users, the evidence that e-cigarette use is related to higher frequency of cigarette smoking,[6] and the lack of historical governmental regulation, there is a significant need to fill existing data gaps on chemistry, toxicology, and clinical/behavioral patterns to inform about e-cigarette consumer safety and risks.[7−9] E-cigarettes have been suggested as a reduced harm alternative to traditional tobacco-based products because of the reduced presence of well-studied toxicants formed during tobacco combustion.[10] However, the use of e-cigarette may have its own risk, such as electronic cigarette or vaping-associated lung injury,[11−13] respiratory function impairment, inhalation of carcinogenic carbonyls, and changes in gene expression.[14,15] Furthermore, as e-cigarette emissions are not completely inhaled, there is a potential for bystander or secondary exposure to nonusers from the exhaled aerosol entering the environment.[16] Recent studies[17,18] have provided insights into how e-cigarette components and emissions affect indoor air quality and exposure pathways.[19−23] However, to date, there remain major gaps in our knowledge of a complete chemical profile of the vapor generated from the vaping process, as well as detailed mechanisms producing those chemicals. Moreover, the astonishing variety of e-cigarette products and innumerable flavors available on the market, combined with the fast pace of product alterations because of the steady increase in e-cigarette popularity, present significant challenges in e-cigarette research and the estimation of user risk.[24]

The thermal degradation of propylene glycol (PG) and vegetable glycerin (VG), the primary components of e-liquid,[25−27] can generate complex chemical products through a series of reactions. Laino et al.[28] showed that the thermal degradation of VG can form formaldehyde, acetaldehyde, and acrolein by dehydration via the formation of glycidol, while PG can generate propionaldehyde and acetone via the inter-

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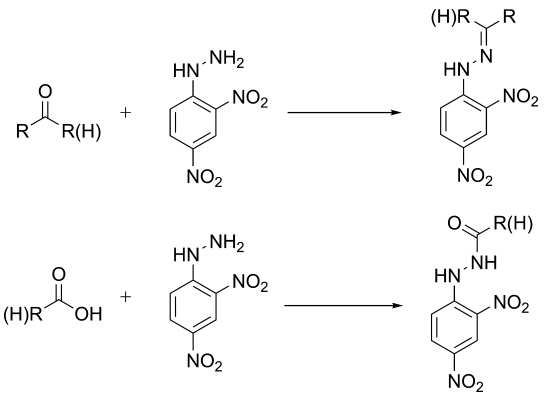
mediate formation of propylene oxide.[29,30] Díaz et al.[31] suggested PG could also participate in a heat-induced radical-mediated degradation pathway, initiated by O_2 insertion to C−H bonds to generate the OH radical that further propagate the radical chain, forming at least five degradation products. The radical-mediated pathway of VG has also been proposed by other researchers, and at least seven thermal degradation products have been observed in the process.[32−34] Some degradation products (e.g., glycolaldehyde) can react further to form simple carbonyls,[35,36] and accretion reactions between carbon-centered radicals or stable products (e.g., hemiacetal formation)[37,38] can further complicate the chemistry of e-cigarette aerosols.

The fragmentation of aliphatic alcohols tend to produce compounds that have a carbonyl (ketone or aldehyde) moeity;[39,40] however, because PG and VG are polyols, their degradation will also result in carbonyls functionalized with hydroxyl groups in addition to the simple types. Organic acid formation may also occur to an extent, possibly as a carbonyl oxidation process. Some thermal degradation products have well-documented toxicity to humans (e.g., formaldehyde, acetaldehyde, and acrolein),[41−43] while others have suspected toxicity (e.g., dihydroxyacetone, glyoxal, and formic acid). In addition to thermal degradation products,[44−46] hundreds of flavoring ingredients may be added to e-liquids and vaporized in e-cigarette aerosols, which can potentially lead to adverse health impacts.[47,48]

Jensen et al.[34] identified the largest variety of thermal degradation products to date from aerosolized e-liquid using nuclear magnetic resonance (NMR); however, the data are not quantitative in that work. Because most compounds in e-cigarettes have a carbonyl moiety, quantification is conventionally done by derivatizing with 2,4-dinitrophenylhydrazine (2,4-DNPH) to produce hydrazone adducts (Scheme 1),[49−51]

Scheme 1. Reactions Between Carbonyls or Acids and 2,4-DNPH to Form Carbonyl−DNPH Hydrazone and Acid−DNPH Adduct.[49−51]



followed by analysis with liquid-chromatography (LC) or gas chromatography (GC) using authentic carbonyl−DNPH standards for the calibration of chromatographic peak areas.[52−58] Electrostatic potential maps of representative carbonyl−DNPH adducts are shown in Figure S1. Even so, authentic carbonyl−DNPH standards are not available for many complex products. The synthesis of carbonyl−DNPH standards may be done;[59] however, the process to synthesize, purify, and purity-check is laborious, requires specialty equipment, and requires reasonable synthetic chemistry skills. The synthesis of DNPH hydrazones of multicarbonyls require additional purification steps to isolate the mono- and multihydrazones. In addition, some carbonyls (e.g., certain ketoaldehydes and others) are not commercially available as starting material, requiring their own separate synthesis. Thus, an approach to quantify without chemical standards is an attractive alternative.

Furthermore, spectroscopic chromatography methods that rely on retention time and UV−visible absorbance spectra may be limited by coelution or indistinctive spectra, even when utilizing authentic chemical standards. The coupling between chromatography and high-resolution mass spectrometry (HRMS) is a powerful tool for chemical identification,[60] as it removes the coelution limitation by enabling molecular formula assignments from exact mass. The goals of this work are twofold: (1) use high mass resolving power coupled to chromatography to better identify DNPH hydrazones of functionalized and simple carbonyls and acids, and (2) develop a method to quantify e-cigarette chemical products for which analytical standard are unavailable.

2. EXPERIMENTAL SECTION

2.1. E-Cigarette Aerosol Sample Generation and Extraction. First-generation disposable e-cigarettes from blu (Imperial Brands Inc., Bristol, United Kingdom.), a popular e-cigarette brand,[61] with "Classic Tobacco" e-liquid cartridges were used for this study. The blu e-cigarettes are comprised of a rechargeable battery with a capacity of 140 mA h, an atomizer with a coil resistance of 3.5 Ω, and a disposable, nonrefillable e-liquid cartridge with proprietary ingredients. Batteries were charged after every 20 min of usage and the e-liquid cartridge was replaced after 400 puffs. A TE-2B smoking machine (Figure S2, Teague Enterprises Inc., Woodland, CA) was used to generate e-cigarette aerosols for the analysis. The apparatus puffed two e-cigarettes, in alternating turns, at a frequency of 8 puffs/min (4 puffs for each e-cigarette) for a 2 s puff duration. The average flow rate was 2.3 L/min and the puff volume was 77 mL, quantified by a primary flow calibrator (A.P. Buck Inc., Orlando, FL). E-cigarette aerosol samples were collected through (1) 2,4-DNPH cartridges (Supelco Inc., 350 mg DNPH, Bellefonte, PA) for carbonyls/acids and (2) 47 mm polytetrafluoroethylene filters (Millipore Sigma, 0.2 μm pore, Burlington, MA) for nicotine. A total of 200 puffs were collected for each analysis, which is within the linear dynamic range of the analysis (R^2 = 0.97−0.99 from 5 to 200 puffs, Figure S3). The emission profile was stable, within the uncertainty of the analysis, for the first and second 200-puff collection of each cartridge (Figure S4). After collection, DNPH cartridges were extracted with 2 mL of acetonitrile (Fisher Scientific Inc., LC−MS grade, Hampton, NH) into 1.5 mL autosampler vials (approximately 0.5 mL remains in the cartridge). Consecutive extractions of DNPH cartridges for 40-, 80-, and 200-puff samples confirmed that >97% of both DNPH and its hydrazones were extracted after the first 2 mL volume. The samples were diluted using LC−MS acetonitrile to the desired concentrations for direct-infusion HRMS and MS^n analyses (Section 2.2). Extracts were used for high-performance liquid chromatography (HPLC)−HRMS analyses (Section 2.3) without dilution. All samples were promptly analyzed after preparation; sample collection and analyses were performed in triplicate.

2.2. HRMS and Tandem MS. Diluted carbonyl−DNPH extracts were analyzed for molecular composition using a

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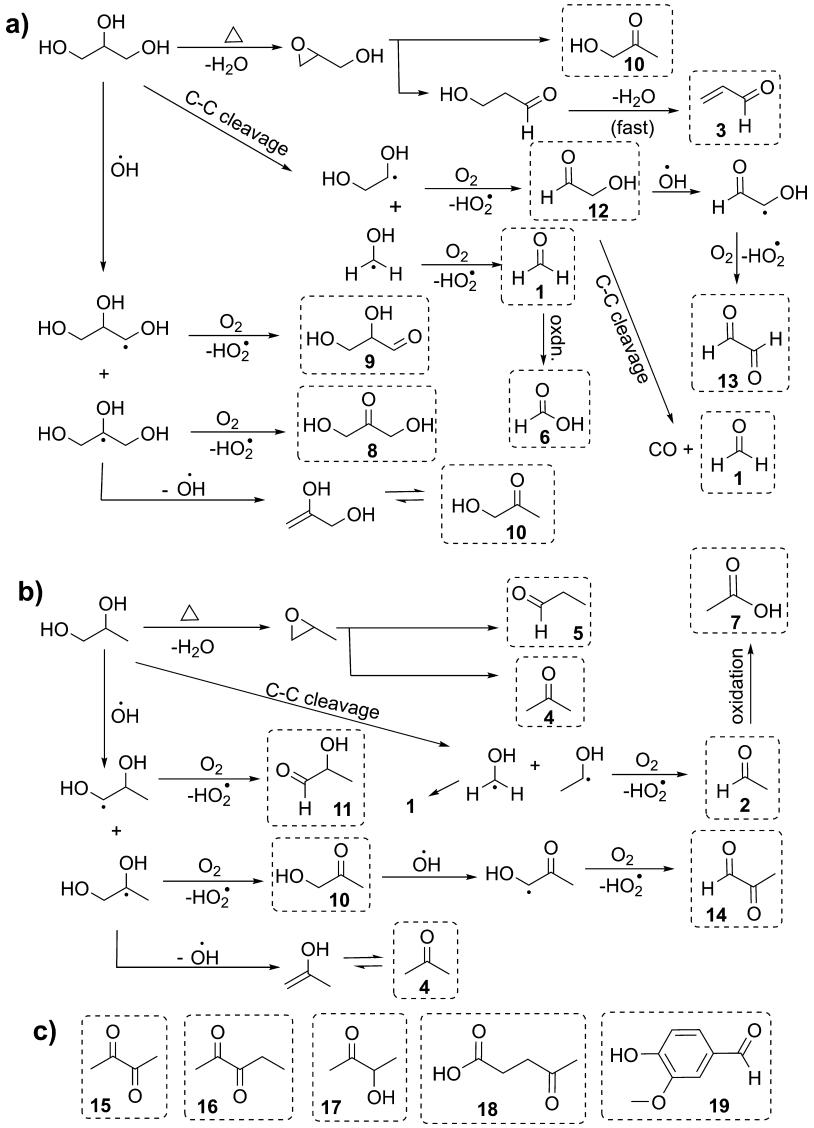
Scheme 2. Representative Reaction Pathways for the Formation of Carbonyls and Acids in E-Cigarette Aerosol by Thermal Degradation of (a) VG and (b) PG.[29−36] Select Flavoring Chemicals are Shown in (c)[a]



[a]Compounds in boxes have been quantified in this work. Legend key: (1) formaldehyde, (2) acetaldehyde, (3) acrolein, (4) acetone, (5) propionaldehyde, (6) formic acid, (7) acetic acid, (8) dihydroxyacetone, (9) glyceraldehyde, (10) hydroxyacetone, (11) lactaldehyde, (12) glycolaldehyde, (13) glyoxal, (14) methylglyoxal, (15) diacetyl, (16) 2,3-pentanedione, (17) acetoin, (18) levulinic acid, and (19) vanillin.

linear-trap-quadrupole Orbitrap (LTQ-Orbitrap) mass spectrometer (Thermo Corp., Waltham, MA) at a mass-resolving power of ∼60,000 $m/\Delta m$ at m/z 400. The extracts were directly infused into a capillary nano-electrospray ion source (50 μm fused-silica capillary tip, 4 kV spray voltage, 275 °C capillary temperature, 5 μL min^{-1} flow) and the spectra taken in the negative ion mode. An external mass calibration was performed using the ESI-L tuning mix (Agilent Inc., Santa Clara, CA) immediately prior to the MS analysis, such that the mass accuracy was adjusted to be approximately 1 ppm for standard compounds. Molecular assignments were performed using the MIDAS v.3.21 molecular formula calculator (Florida State Univ.). Insights into the molecular structure were obtained using collision-induced dissociation (CID) multistage tandem MS (MS^n, stages 2−4) in the LTQ-Orbitrap. The CID energy was tuned for each mass, such that the precursor ion has 10−20% normalized abundance. Thermo Xcalibur software was used for data processing.

2.3. HPLC Coupled with HRMS (HPLC−HRMS). DNPH hydrazones were quantified by HPLC coupled to the same LTQ-Orbitrap in the Section 2.2 with an electrospray ionization (ESI) source, operating in the negative ion mode at a mass range of m/z 150−500 to cover the mass range of carbonyl−DNPH and dicarbonyl-$(DNPH)_2$ adducts observed in this work. Separation by HPLC was performed using a C_{18} column end-capped with dimethyl-*n*-octadecyl silane (Poroshell EC-C_{18}, 2.1 × 100 mm, 2.7 μm, 120 Å pores, Agilent Technologies, Inc., Santa Clara, CA) and a mobile phase of LC−MS grade water with 0.1% formic acid (A) and acetonitrile (B). The analytes were eluted over the course of 37 min at 0.27 mL/min with the following gradient program: 40% B (3.33 min), 50% B (14.6 min), 60% B (20 min), 100% B (32 min), and 40% B (37 min). After the separation by chromatography, single ion chromatography (SIC) for the accurate m/z of DNPH adducts that were identified by the methods in the Section 2.2 was used for quantification. A

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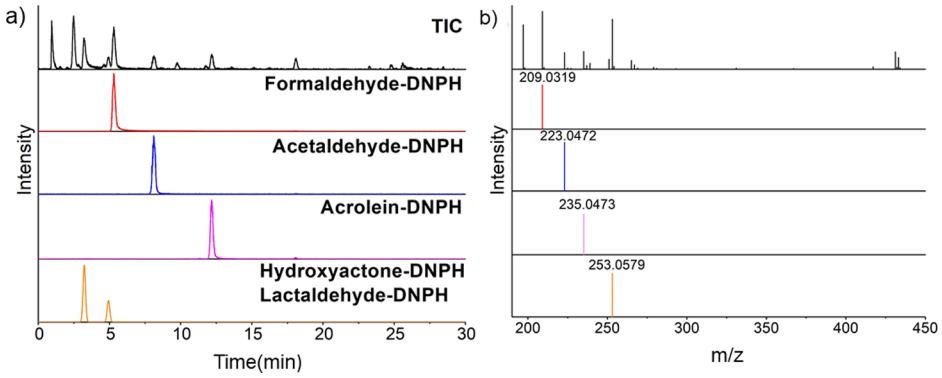

Figure 1. (a) Total ion chromatogram of the e-cigarette aerosol sample extracts and single ion chromatograms for four select deprotonation ions of carbonyl−DNPH hydrazones (b) corresponding integrated mass spectrum of the total ion chromatogram and individual mass spectra of four single-ion chromatograms.

carbonyl−DNPH standard solution (M-1004-10X, AccuStandard, Inc., New Haven, CT) that comprised 13 carbonyl−DNPH analytes was used to obtain the concentration standard curves for calculating the concentration of formaldehyde, acetaldehyde, acetone, acrolein, and propionaldehyde in e-cigarette aerosols (Figure S5). From the application of the standard curves and propagating the remaining errors of the analysis (e.g., uncertainties in peak area determination, standard concentration uncertainties, and syringe volume uncertainties), the $\pm 1\sigma$ uncertainty for calibrated compounds is 10−20%. The concentration of the remaining carbonyls and organic acids are calculated by their SIC peak areas and the calculated sensitivities to the ESI negative ion mode in the Section 2.4. The concentration of nicotine was also measured by the same method using the positive ion mode.

2.4. Theoretical Calculations. The chemical structures of the DNPH hydrazones affect their deprotonation efficiency in the ESI negative ion mode and thus, their calibration sensitivity in HPLC−HRMS. The Gibbs free energy change of the deprotonation reaction (ΔG_d) of carbonyl−DNPH and acid−DNPH compounds that occur in the ESI negative ion mode (Figure S1) was calculated by Gaussian 09 (Gaussian Inc., USA) in both the gas-phase and solution phase. The structural geometry optimization and frequency calculation was performed by density functional theory (DFT) using the M06-2X functional and 6-31g+(d,p) basis set, which has been recommended for the study of main-group thermochemistry in recent years.[62−64] First, the ΔG_d values for the 13 carbonyl−DNPH compounds in the analytical standard mixture were calculated to obtain a relationship to their measured ESI sensitivities. The relationship between the ΔG_d and ESI sensitivities was then extended to calculate the relative theoretical ESI sensitivities for the DNPH hydrazones for which commercial standards are not available. Calculated sensitivities were then used to estimate the concentrations of carbonyl−DNPH hydrazones in e-cigarette aerosol extractions with the method described in the Section 2.3. The mass concentrations of different carbonyls/acids in air ($\mu g/m^3$) were calculated by the total mass of the specific carbonyls/acids in the HPLC−HRMS analysis divided by the total volume of air that flowed through the DNPH cartridge during the vaping collection process.

3. RESULTS AND DISCUSSION

The method reported in this work offers unambiguous identification and a large quantification range for functionalized carbonyl compounds and organic acids. This is useful for studying e-cigarette thermal degradation chemistry, as well as other environmental chemistry topics (e.g., gas-phase chemistry, aqueous oxidation reactions, etc.). A total of nineteen DNPH hydrazones in the e-cigarette aerosol sample were observed (Scheme 2): five simple carbonyls, six hydroxycarbonyls, four dicarbonyls, three acids, and one phenolic carbonyl. Hydroxycarbonyls comprised 3 of the top 6 most abundant compounds. Uchiyama et al.,[82] recently found that some compounds are emitted purely as gas-phase species (e.g., acetaldehyde and acrolein), some as purely particulates (e.g., glyoxal and nicotine), and some as both (e.g., formaldehyde). Both the concentration and phase information are useful for the estimation of exposure risk.

Much of the chemical identification for DNPH hydrazones ($C_cH_hO_oN_n$) can be directly derived from the exact mass of the detected $[M - H]^-$ ions alone. As the formation of DNPH hydrazones replaces only one atom (O with N, Scheme 1), it is straightforward to deduce the original molecular formula of the carbonyl or acid from the hydrazone formula. The chemical structures were confirmed as in the Section 3.1. All analytes are baseline separated in the chromatographic spectrum using accurate mass SIC. Figure 1a shows the total ion chromatography (TIC) and SIC of select carbonyl−DNPH compounds, and Figure 1b shows the corresponding integrated mass spectrum of TIC and each SIC. From the TIC, it is clear that e-cigarette aerosol is a complex system, which contains a large number of carbonyls/acids. Coelution is common in the TIC (e.g., lactaldehyde−DNPH coeluted with formaldehyde−DNPH); however, SIC isolates the chromatographic peaks of the desired m/z, avoiding coelution and misidentification. We also found that acetone−DNPH coeluted with vanillin−DNPH in the chromatography. This will have led to an overestimation of the abundance of acetone using a chromatography method without HRMS, as vanillin−DNPH is not commercially available.

3.1. Chemical Structures of Multifunctional Carbonyls and Acids. Beyond molecular formulas, it is advantageous to confirm the exact bonding sites of carbonyls and other moieties to give insight into chemical mechanisms and aid in theoretical calculations of reaction energies, as these calculations are sensitive to structures. The chemical structure

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Table 1. Carbonyls/Acids Characterized by HPLC−HRMS[a]

number in Scheme 2	carbonyls/acids	observed m/z of DNPH hydrazones	specific fragmentation pathway of DNPH hydrazones/adducts	mass per 10 puff (μg)	concentration ($\mu g/m^3$)
1	formaldehyde	209.032	209.03 → 167.01($-CH_2N_2$) 209.03 → 179.02($-CH_2O$)	1.5 ± 0.2	512 ± 68
2	acetaldehyde	223.047	223.05 → 179.02($-CH_3CHO$) 223.05 → 178.03($-CH_3NO$)	1.1 ± 0.13	367 ± 47
3	acetone	237.063	237.06 → 179.02($-C_3H_6O$) 237.06 → 163.03($-C_3H_6O_2$)	0.24 ± 0.05	88 ± 18
4	acrolein	235.047	235.05 → 163.03($-C_3H_4O_2$) 235.05 → 167.01($-C_3H_4N_2$)	0.61 ± 0.08	216 ± 27
5	propionaldehyde	237.063	237.06 → 179.02($-C_3H_6O$) 237.06 → 209.03($-C_2H_4$)	0.14 ± 0.03	48 ± 11
6	formic acid	225.027	225.03 → 182.02($-HOCN$)	0.04 ± 0.02	13 ± 7
7	acetic acid	239.042	239.04 → 209.04($-NO$) 239.04 → 179.02($-C_2H_4O_2$)	0.07 ± 0.03	23 ± 9
8	dihydroxyacetone	269.053	269.05 → 179.02($-C_3H_6O_3$) 269.05 → 182.02($-C_3H_5O_2N$)	0.37 ± 0.12	128 ± 43
9	glyceraldehyde	269.053	269.05 → 179.02($-C_3H_6O_3$) 269.05 → 182.02($-C_3H_5O_2N$) 269.05 → 251.04($-H_2O$)	0.05 ± 0.02	19 ± 8
10	hydroxyacetone	253.058 433.085	253.06 → 182.02($-C_3H_5ON$) 253.06 → 177.03($-N_2$,-CH_4O_2)	1.8 ± 0.6	615 ± 208
11	lactaldehyde	253.058 433.085	253.06 → 182.02($-C_3H_5ON$)	0.71 ± 0.23	247 ± 80
12	glycoaldehyde	239.042	239.04 → 179.02($-C_2H_4O_2$) 239.04 → 167.03($-NO$, $-C_2H_2O$)	0.04 ± 0.02	14 ± 6
13	glyoxal	417.055	417.05 → 182.02($-C_8H_5N_5O_4$) 417.05 → 348.02($-C_2H_3N_3$)	0.02 ± 0.01	6 ± 3
15	diacetyl	265.058 445.086	265.06 → 218.06 ($-HNO_2$) 265.06 → 177.03($-N_2$, $-C_2H_4O_2$)	0.16 ± 0.04	56 ± 15
16	2,3-pentanedione	279.073 459.100	279.07 → 176.05($-HNO_2$, $-C_3H_4O$) 279.07 → 182.02($-C_5H_7NO$)	0.07 ± 0.02	24 ± 7
14	methylglyoxal	431.071		0.13 ± 0.04	46 ± 14
17	acetoin	267.073		0.11 ± 0.03	55 ± 12
18	levulinic acid	295.069		0.07 ± 0.02	23 ± 8
19	vanillin	331.069		0.14 ± 0.05	46 ± 19

[a]Each experiment was performed in triplicate, and the data are expressed as the average (±SD), errors are 1σ including delivering aerosol, DNPH derivatization, and ESI sensitivity calculation. The concentrations of five simple carbonyls were calculated by concentration calibrations using authentic standards, while the concentrations of the rest of the compounds were calculated using calculated ESI sensitivities. Concentrations in $\mu g/m^3$ were calculated by the total mass of the compounds divided by the total volume of air flowing through the smoking machine during sampling. Because of potential sampling losses, reported concentration values represent a lower limit.

of DNPH adducts was identified by their neutral and radical losses in tandem multistage MS^n using CID,[65,66] which often helps to elucidate the exact carbon location of the moiety-of-interest for small molecules. For example, alcohols adjacent to a beta carbon with an abstractable hydrogen (e.g., glucose) can lose H_2O by H-shift rearrangement,[67] while those bonded to aromatic (e.g., phenol) or other nonabstractable sites do not show this loss in the negative ion mode. For nitroaromatics such as DNPH, the electron-withdrawing groups of NO_2 exerts a strong stabilizing effect on anion radicals, and facilitates NO_2-mediated rearrangements (often leading to loss of NO or an O-migration).[68,69]

For small ions such as acetaldehyde−DNPH, there is no other reasonable carbonyl structure that exists for the molecular formula, and MS^n confirms this structure with expected fragmentation of CH_3NO and CH_3CHO (Scheme S2). However, there are some ambiguous formulas such as $C_3H_6O_3$, which may belong to structural isomers dihydroxyacetone and glyceraldehyde. Both of these hydroxycarbonyls are proposed to exist in the e-cigarette aerosol after the NMR analysis but are impossible to distinguish with chromatography, as they have the same UV-absorption and m/z.[34] With MS^n fragmentation, we found that dihydroxyacetone (Scheme S7) is the main product. Even though several fragmentation pathways for these isomers are similar [e.g., 269.05 → 179.02 (C_3H_6O loss) and 269.05 → 239.04 (NO loss)], the H_2O loss and $C_2H_4O_2$ loss that is expected for glyceraldehyde−DNPH were observed to be negligible in the mass spectrum (Scheme S8). The preferred formation of dihydroxyacetone over glyceraldehyde supports the radical-mediated oxidation pathways suggested by Díaz et al.,[31] as radical abstraction of the H in VG should lead preferentially to a secondary alkyl radical compared to the primary radical (Scheme 2, leading to **8** and **9**, respectively). The initiating radicals are suggested to be reactive oxygen species such as hydroxyl radical,[32] and as such, the degradation products can be described by processes that occur in atmospheric chemistry.[70] Some of the products identified here can be expected from the thermal degradation of PG and VG (e.g., formaldehyde, acetaldehyde, acetone, hydroxyacetone, glycolaldehyde, dihydroxyacetone, etc.),[34]

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which is in agreement with the proposed mechanism, while others are likely to be flavoring additives (e.g., 2,3-pentanedione, vanillin, acetoin, etc.).[47]

A shared product ion after fragmentation of the DNPH hydrazones is $C_6H_3N_4O_3^-$ (m/z 179.02), which is the modified DNPH after the O-rearrangement loss of the original carbonyl/acid. Other similar loss pathways are those of the DNPH itself, including loss of HONO, NO_2, and NO (after NO_2 rearrangement to ONO). There are also distinctive fragmentation pathways for each ion, which are summarized in Table 1. MS^n fragmentation schemes for all DNPH hydrazones are shown in Schemes S1−S13.

3.2. Quantification of DNPH Hydrazones of Multifunctional Carbonyls and Acids. While the process of ionization in ESI is complex, it has been demonstrated that there are key factors influencing the ionization efficiency (IE) of different compounds.[62,71−74] For example, for the same family of compounds, there is a relationship between negative ion ESI response and pKa[75] of the dissociation equilibrium HA ⇆ A^- + H^+, which is directly related to basicity. We calculate the basicity (as defined for A^-) in terms of $\Delta G_{deprotonation}$ (ΔG_d)[76,77] because the deprotonated $[M - H]^-$ ion is usually detected in the ESI negative mode. Our calculations of the electrostatic potential (ESP) maps of carbonyl−DNPH hydrazones show that they have a primary acidic proton (Figure S1); thus, they are excellent candidates for which gas-phase basicity (ΔG_d) can be used to parameterize IE in the ESI negative mode. We emphasize that the theoretical chemistry results in this work only provide a relative indication of sensitivity, not absolute calibration factors, and only for the same family of compounds that are protonated or deprotonated. The relative theoretical sensitivities are then anchored by absolute ESI calibrations for the carbonyl−DNPH compounds where standards are commercially available.

Figure 2 shows the relationship between the measured negative ion mode ESI sensitivities of 13 carbonyl−DNPH standards and their calculated ΔG_d (R^2 = 0.63). Valeraldehyde was excluded because it was considered as an outlier by Cook's distance (D_i = 0.84).[78] The deviation from the linear trend line is ±31%, which, when propagated with the peak integration uncertainty in the HPLC−HRMS analysis, result in an average 1σ uncertainty of 31−50% for all compounds lacking in commercial standards. ΔG_d were also calculated in the acetonitrile solution phase, which also gave reasonable correlations to ESI sensitivity (Figure S6). Although we opted to use gas-phase ΔG_d because of the stronger correlation, both theoretical models yielded results that are different by 1−24% (Table S1). Wheeler and Houk[79] found that integration grid or molecular orientation may influence the DFT-based energy calculation at a certain theory level for specific molecular systems. In this work, three random orientations for formaldehyde−DNPH, acetaldehyde−DNPH, and acetone−DNPH hydrazones were chosen to test the sensitivity of the calculation to the DFT initiation factors; we found that the values of ΔG_d are identical after geometry optimization and free energy calculation. Table S1 also shows that concentrations calculated using authentic standards are different than those of theoretical models by 10−53% (a combination of uncertainties in both methods). The uncertainty in concentrations calculated by the theoretical model for compounds lacking in commercial standards (31−50%) remains considerable, and may be targeted for improvement in future studies. While this uncertainty range is larger than the uncertainty when using analytical standards (~10−20%), it is an improvement to the alternatives of (a) not having quantitative data for complex carbonyls and acids, or (b) using "proxy" chemical standards to estimate concentration, which may have highly different analytical sensitivities for similar molecular formulas (e.g., the DNPH derivative of C_3H_4O is 350% more sensitive than that of C_3H_6O in ESI) and, thus, introduce uncertainties of over 100%.

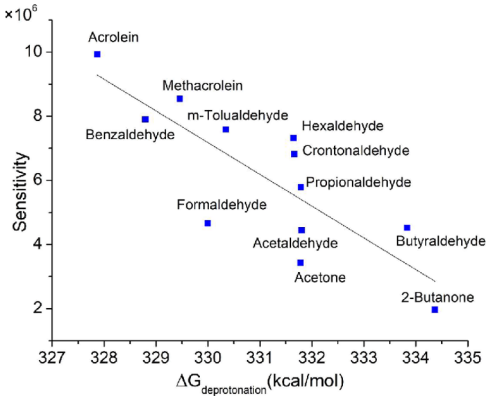

Figure 2. Correlation between the observed ESI sensitivities of standard carbonyl−DNPH hydrazones and calculated ΔG_d (gas phase, R^2 = 0.63). The data point of valeraldehyde was excluded because it was considered as an outlier by Cook's distance (D_i = 0.84).[78]

The trend of ΔG_d and ESI sensitivity (Figure 2) arises from the intrinsic relationship between the deprotonation efficiency and the ability of the aromatic product ion to stabilize the negative charge initially formed on the N atom (Figure S1).[62,75] Acrolein is the most sensitive compound in the ESI negative mode because it has conjugated double bonds, that is, additional π orbitals for the negative charge to be delocalized.[80] Also, ketones have lower sensitivities than aldehydes because the electron donating group (methyl group and ethyl group) on both sides of the C═N bond slightly destabilizes the negative ions.[81] A limitation of this model occurs for compounds that have similar ΔG_d. In this situation, other factors such as molecular volume and polarity (log P) may also play an important role for these compounds.[74] Despite the limitations, this method is applicable to the compounds found in the e-cigarette aerosol and enables the first estimation of concentrations for complex carbonyls that have not yet been quantified with acceptable uncertainty. Furthermore, this computational technique offers an advantage compared to the time expenditure, costs, and chemical usage of synthesizing standards.

The calculated concentrations of e-cigarette constituents characterized in this work are given in Table 1 as mass per volume or mass per ten puffs analyzed. The most abundant compounds in the blu e-cigarette aerosol for our study conditions are hydroxyacetone, formaldehyde, acetaldehyde, lactaldehyde, acrolein, and dihydroxyacetone. While, within uncertainty, the exact order of abundance is not definitive, it is clear that hydroxycarbonyls are just as important as simple carbonyls to the composition of the e-cigarette aerosol. Hydroxyacetone (acetol) has been found to be a major, sometimes dominant, emission in other e-cigarette brands and e-liquids, as quantified by GC.[58,82] The agreement of the high abundance of hydroxyacetone lends support to the theoretical

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approach in this work, which enables all carbonyls and acids to be quantified by the same method. The high abundance of hydroxyacetone may be due to its multiple formation pathways, as given in Scheme 2, and its possible role as an impurity in the e-liquid, for example, Sleiman et al.,[58] found hydroxyacetone in concentrations of <1% of the sum of PG and VG in the e-liquids they used. We were not able to test the e-liquid in this work because of the cartridge design; thus, we are unable to comment on the extent of hydroxyacetone impurity in the e-liquid, if present.

Dihydroxyacetone and lactaldehyde, in contrast, have not been regarded as major e-cigarette emissions until their unambiguous identification in this work. Their formation pathways from PG and VG (Scheme 2) are highly feasible, so their higher abundance is not unexpected. It's not clear why these compounds have not been reported earlier; we suspect analytical challenges may be a reason. As we discussed previously, lactaldehyde−DNPH coeluted with formaldehyde−DNPH in the TIC (Figure 1). Thus, HPLC−UV, one of the most frequently used instruments for studying carbonyl compounds in e-cigarette aerosols, will not be able to identify and quantify lactaldehyde. However, the HPLC−HRMS method overcomes coelution challenges by distinguishing compounds based on their exact mass from the SIC and mass fragmentation patterns. Dihydroxyacetone−DNPH appeared to be baseline-separated in HPLC−UV, with a retention time slightly shorter than DNPH itself; however, its unambiguous identification is not possible without HRMS and/or authentic standards. Furthermore, both of these compounds are quite polar, and thus, not conventionally compatible with GC.

A comparison of the absolute emission concentrations of thermal degradation products between studies is not straightforward, even for the same brand of e-cigarettes,[53,83,84] as the puffing regimens and apparatus of reported studies are all different and individual puffing parameters have nonlinear effects on the thermal degradation chemistry.[82,83] Klager et al.,[53] also reported high variability of carbonyl concentrations (e.g., acetaldehyde 229−1870 $\mu g/m^3$) for the same brand, puffing regimen, and flavor, suggesting that the factors driving the thermal degradation chemistry are not yet fully understood. Our work should be primarily viewed as a demonstration of a new method to the chemical characterization of our specific e-cigarette model at the stated puffing conditions, with noted insights into the thermal degradation mechanism.

Formaldehyde, acetaldehyde, and acrolein are known to produce pathological and physiological effects on the respiratory tract. They are known to cause sensory irritation, inflammation, and changes in the pulmonary function; formaldehyde is also carcinogenic.[85−87] The average daily dose of aldehydes can be calculated by the amount of aldehydes per puff multiplied by the average number of puffs a user inhales per day. For example, the median puffs per day for e-cigarette users can be assumed to be 250,[88] so the average daily exposure dose of formaldehyde is 37.5 μg/day for this e-cigarette device, e-liquid, and operating conditions. The California Office of Health Hazard Assessment (OEHHA) Chronic Reference Exposure Levels (chREL) for formaldehyde is 9 $\mu g/m^3$, which could be translated to an acceptable daily dose of 180 μg/day (assuming the inhalation volume as 20 m^3 per day) and is higher than the e-cigarette aerosol exposure for formaldehyde in this work. In addition, OEHHA has a no significant risk level (NSRL) recommendation of 40 μg/day, which is intended to protect against cancer; this NSRL level is close to the exposure dose of formaldehyde in this work. The average exposure dose of acrolein for blu e-cigarettes is 15.2 μg/day according to Table 1, which is higher than the OEHHA chREL value (7 μg/day). Logue et al.[16] used a similar approach to estimate health impacts and found that both formaldehyde and acrolein can exceed maximum daily doses derived from occupational health guidelines. Differences in results are likely due to the different devices, e-liquids, and puffing regimens used.

While the reported emissions in this work may not be generalized to all e-cigarettes and use scenarios, it is informative to compare the aldehyde emissions normalized by nicotine, as e-cigarette users transitioning from traditional tobacco products (e.g., combustible cigarettes) will self-titrate nicotine intake when using e-cigarette products.[89,90] Figure 3 shows the comparison between carbonyl emissions per mg nicotine with the work of Farsalinos et al.[91] for tobacco products. In this work, the nicotine yield is 10.4 ± 1.9 μg/10 puffs. We did not observe evidence of nicotine oxidation[92] under the puffing conditions of this work, which will impact the ratio. The formaldehyde/nicotine ratio is 144 ± 32 μg/mg nicotine, which is 4 times higher than the formaldehyde/nicotine ratio in combustible cigarettes (37 ± 7 μg/mg).[91] The acrolein/nicotine ratio measured in this work is close to that of tobacco products (59 ± 13 μg/mg compared to 62 ± 8 μg/mg), while the acetaldehyde/nicotine ratio (106 ± 23 μg/mg compared to 663 ± 92 μg/mg) and propionaldehyde/nicotine ratio (13 ± 4 μg/mg compared to 60 ± 10 μg/mg) are lower than that in combustible cigarettes. Logue et al.[16] observed similar trends using different e-cigarette products; however, the results were not normalized for nicotine, so a direct comparison is not possible. Thus, we find e-cigarettes do not necessarily emit lower carbonyl compounds than tobacco products, but the comparisons may change depending on the specific e-cigarettes or tobacco products, or different puffing/smoking regimens.[93]

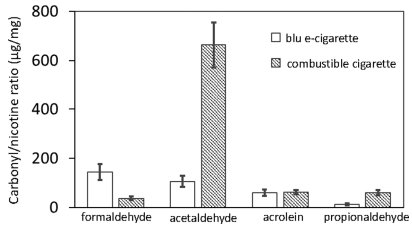

Figure 3. Comparison of carbonyl/nicotine ratios for the e-cigarette device, e-liquid, and puffing regimen used in this work and those of combustible cigarettes as reported by Farsalinos et al.[91] (2018).

Although hydroxycarbonyls are abundant in e-cigarette aerosols, a general lack of toxicological data precludes health risk assessment. Smith et al.[94] found that exogenous exposure to dihydroxyacetone is cytotoxic and will cause cell death by apoptosis. Glycolaldehyde is also suspected to have biological toxicity.[95] For hydroxyacetone and lactaldehyde, toxicology data are currently unavailable on many toxicology databases such as the Hazardous Substances Data Bank (HSDB), the European Chemicals Agency (ECHA), and the Research Institute of Fragrance Materials (RIFM).

In addition to thermal degradation products, flavoring chemicals are also found to be significant components in e-cigarette aerosol. Allen et al.[47] measured the concentration of diacetyl (<LOD—238.9 μg/e-cigarette), 2,3-pentanedione

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(<LOD—64.4 μg/e-cigarette), and acetoin (<LOD—529.2 μg/e-cigarette) in 51 e-cigarettes from different brands and flavors, with the highest concentrations found in e-liquid flavors such as "Peach Schnapps." In this work, the estimated concentrations of the flavoring chemicals diacetyl (~6.4 μg/e-cigarette), 2,3-pentanedione (~2.8 μg/e-cigarette), and acetoin (~4.4 μg/e-cigarette) are fairly consistent with some measurements for the Classic Tobacco flavor[53,96] but higher than others.[47,53] Of note is that Klager et al.[53] found that diacetyl concentration in 16 different e-cigarettes varies from 0.028 to 3.69 $\mu g/m^3$, while our results show a concentration of 56 ± 15 $\mu g/m^3$. It is clear that the amount of flavoring chemicals largely depends on the individual e-liquids, puffing regimen, and collection methods. In addition, vanillin and levulinic acid have been quantified here for the first time in e-cigarette aerosols.[48,97] Acid additives (e.g., levulinic acid) may be introduced as a part of nicotine salts and may be used to control the acidity of e-liquids.[98] Inhaling either diacetyl, or the related flavoring 2,3-pentanedione, has been associated with bronchiolitis obliterans ("popcorn lung").[99,100] As the composition of e-cigarette aerosol is complex and the range of products is vast, a more systematic understanding of the fundamental chemistry (e.g., the molar yield of thermal degradation products from PG and VG, the dependence on vaping temperature, the phase characteristics, the impacts of nicotine and flavorings) is needed.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.9b07387.

Information regarding ESP map and ionization reaction of carbonyl−DNPH hydrazones in ESI negative mode, experiment setup, linear dynamic range of different puffs, standard curves of 13 carbonyl−DNPH hydrazones, MSMS of different DNPH adducts, linear relationship between calculated ΔG_d and sensitivities of 13 carbonyl−DNPH hydrazones in ESI negative mode in the solution phase, and comparison of five simple carbonyl compounds between the concentrations calculated by authentic standard and by calculated sensitivities by models both in the gas phase and solution phase (PDF)

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Author Contributions

T.B.N. and Y.L. designed the experiments, Y.L. and A.E.B. carried out the experiments. All authors contributed original data and data analyses. T.B.N. and Y.L. prepared the draft manuscript. All co-authors have reviewed and edited the manuscript.

Notes

The authors declare the following competing financial interest(s): One of the authors (AKM) is also employed by a scientific consulting firm, Cardno ChemRisk, which provides scientific advice to the government, corporations, law firms, and various scientific/professional organizations. Cardno ChemRisk has been engaged by various electronic nicotine delivery system (ENDS) and e-liquid manufacturers to provide general consulting and expert advice on scientific matters in litigation and in the context of regulatory requirements. This article was prepared and written exclusively by the authors without review or comment by any outside organization.

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