



Headspace analysis of E-cigarette fluids using comprehensive two dimensional GC×GC-TOF-MS reveals the presence of volatile and toxic compounds



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ABSTRACT

The analysis of electronic cigarette (E-cigarette) fluids by high performance liquid chromatography or gas chromatography (GC) coupled to mass spectrometry (MS), GC hyphenated to flame-ionisation detection, or nuclear magnetic resonance spectroscopy poses many challenges due to the complex matrix and extremely high number of compounds present. In order to overcome these challenges, this study focused on the detection of the multiple complex compounds classes produced by the pyrolysis of E-cigarette liquids using comprehensive two dimensional gas chromatography (GC×GC) coupled to time of flight (TOF)-MS. Gas samples were prepared by heating E-liquids inside aluminium tins for 5 min. The tins were placed in a sand bath, which was temperature controlled at 200 °C. The samples were collected using thermal desorption tubes connected to volatile organic compound (VOC) sampling pump attached and subsequently analysed using GC×GC-TOF-MS. The greater peak resolution obtained when using GC×GC-TOF-MS allowed to distinguish many toxic compounds and VOCs that could not be detected by the other methods mentioned above. As a result, a comprehensive list of volatile compounds emitted from E-cigarette fluids when heated was established, which might allow a better understanding of potential health effects of vaping. Heating E-liquids to moderate temperature results in the emission of over 1000 volatile compounds of which over 150 are toxic. These compounds are either present in the liquid or can be formed during storage or heating leading to a more complex volatile profile of E-cigarette liquids than previously assumed. The application of GC×GC-TOF-MS allows the elucidation of this profile and therefore a better understanding of possible health implications.

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1. Introduction

Currently there are several nicotine delivery systems available on the market such as cigarettes, cigars, pipes/ water pipes, the most recently introduced tobacco heating systems and electronic-cigarettes (E-cigarettes). Amongst these systems, E-cigarettes demonstrate the most rapid growth in popularity and increasing use in the last decade as result of a largely consumer-led development [1]. Since their first introduction to China's market, the popularity of E-cigarettes has rapidly increased in many different

countries around the world including USA, UK, Australia, and several countries in Europe. E-cigarettes are advertised worldwide as a safer replacement for conventional tobacco cigarettes or as an aid to smoking cessation [2].

Traditionally, an E-cigarette consists of a lithium ion battery, an atomizer chamber, a cartridge, mouthpiece and a chip attached to circuits. Recently, the third generation of E-cigarettes were introduced with bigger lithium ion batteries and the ability to change the voltage in order to increase the heat from the coil resulting in greater vapor volume and increased inhalation of nicotine [3] (Fig. 1). The vapor for inhalation is produced by heating E-cigarette liquid, also called E-juice or E-liquid, inside the device. Typical fluids contain various aerosol-forming excipients including propylene glycol and/or glycerol, different flavouring agents and optionally nicotine (by choice). The fluids are marketed according to their strength (percentage of nicotine present). In 2014, there were 466

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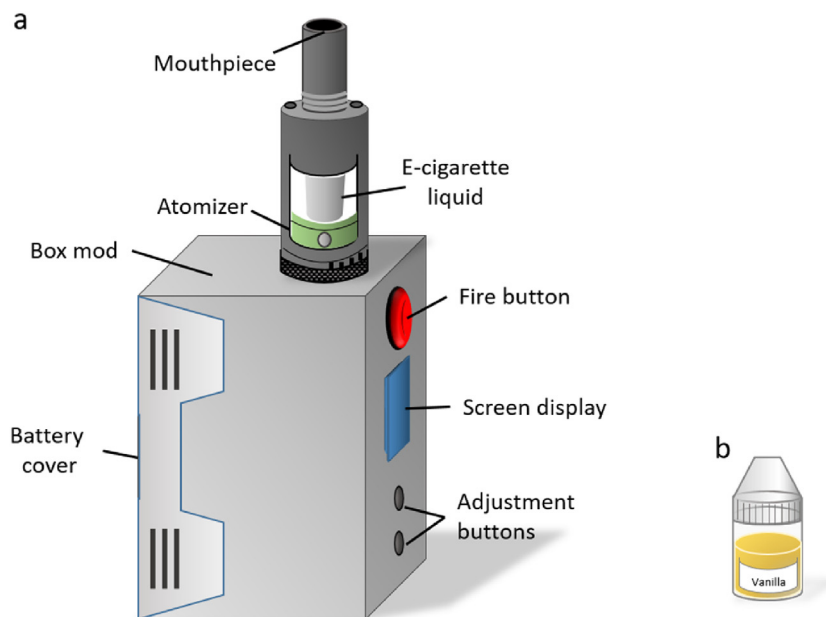


Fig. 1. Exemplary schematic of an E-cigarette device in the third generation, also called “tank-system” (a). The device comprises of a box mod including a battery chamber and cover, adjustment buttons for individually changing the voltage and thus heat, screen display and a fire button. On top of the box mod is the atomizer with the EC liquid as well as the mouthpiece or drip tip. (b) Exemplary picture of a typical E-cigarette liquid canister, in this case flavoured Vanilla.

different brands and approximately 7764 unique flavours available on the world market [4].

Considering the well-known destructive effects of tobacco cigarettes on human health including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD) and cancer [5], concerns over possible health impacts of the various newly introduced E-fluids are increasingly raised. The concerns have increased further last year, since 68 deaths were linked to a lung illness reportedly associated to vaping in February 2020 [6]. In 2012, the U.S. Food and Drug Administration (FDA) reported approximately 100 harmful and potential harmful constituents (HPHCs) for tobacco products and tobacco smoke [7]. In 2016, the FDA released a similar list for electronic nicotine delivery systems (ENDS), whereby manufacturers were advised to screen the aerosols of their products for 29 compounds [8]. This list has now been modified resulting in an overall 33 compounds suggested to be analysed by the manufacturer [7]. However, there are still obscurities as to which analytical method is to be used and whether this list is comprehensive or not. Therefore, more research is required to define the chemical composition of aerosols from E-cigarettes and address the growing health concerns.

Several studies have analysed the volatile organic compounds (VOCs) present in the aerosol of E-cigarette fluids using hyphenated techniques such as gas-chromatography-mass spectrometry (GC-MS), thermal desorption (TD) in conjunction with GC-MS [9], liquid chromatography-MS (LC-MS) [3,10] and liquid chromatography-tandem MS (LC-MS/MS) [11]. These studies have detected several classes of harmful chemicals such as carbonyls [10], polycyclic aromatic hydrocarbons [12], glycols and nicotine [13] as well as impurities and by-products [11]. Especially for the analysis of volatile compounds, GC-MS is commonly the method of choice. However, the analysis of E-cigarette fluids can be challenging for GC-MS due to limitations such as insufficient selectivity, limited peak capacity and inability to manage dynamic range [14]. A technique with greater peak resolution and dynamic range is expected to be more suitable for complex mixtures as potentially present in E-cigarette fluids.

In this study, comprehensive two-dimensional GC hyphenated to time of flight mass spectrometry (GC×GC-TOF-MS) was applied

for the first time to the analysis of E-liquid pyrolysis products collected using TD tubes. The peak capacity of GC×GC-TOF-MS is superior to conventional one-dimensional GC due to the addition of a secondary column. Analytes eluting from the first column can be focused onto the second, which provides a higher number of satisfactorily resolved peaks per unit length of the column. Furthermore, the presence of two columns with different stationary phases provides better selectivity for complex mixtures than when only a single column with one stationary phase is used. Hence, GC×GC-TOF-MS is often employed to overcome the limitations of GC-MS for the analysis of complex samples containing a broad dynamic range of VOCs. TOF-MS has a higher acquisition rate than the more traditional quadrupole MS, which is important considering the narrow peaks obtained in GC×GC analysis.

Current studies have mainly focused on few set of compounds in EC fluids [10,15]. In the presented manuscript we demonstrate for the first time the application of a GC×GC-TOF-MS method to the separation and identification of VOCs released from six E-cigarette fluids. Since GC×GC-TOF-MS allows for better peak separation and resolution, we provide the most comprehensive list of VOCs in E-cigarette liquids so far, which allows a better understanding of the pyrolysis of E-liquids and potentially which compounds might be produced during vaping.

2. Materials and method

2.1. E-cigarette fluids

E-liquids of six common flavours (Vanilla, Butterscotch, Tobacco, Cinnamon, Apple and Menthol) were acquired from a commercial online source (<https://www.vaperempire.com.au/>). For each flavour, two strengths of nicotine, i.e. 0 mg/ mL (N0) and 18 mg/ mL (N18), were purchased, whereby 18 mg/ mL of nicotine in an E-cigarette fluids defines high nicotine strength [13]. Samples were collected in two replicates for each flavour and strength. A punctured empty tin sealed with plastic tape, however containing no sample of nicotine nor flavour additives was used as blank along with experimental samples.

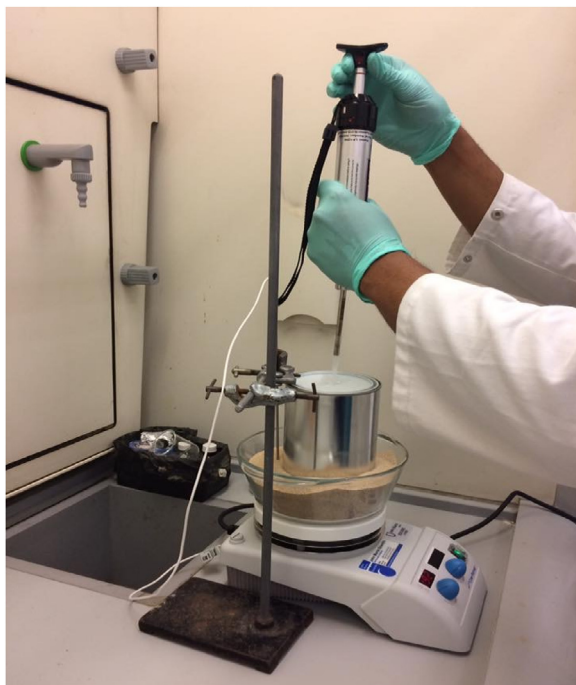


Fig. 2. Example setup of the sample collection method. 5 μL of EC fluid was deposited inside a 1 L tin. The tin was heated at 200 $^{\circ}\text{C}$ in a sand bath for 5 min. 100 mL of the produced VOCs were collected using a TD tube, which had a VOC sampling pump attached and was connected to the tin via a pipette tip.

2.2. Sample collection method

One liter aluminium tins were used to accumulate headspace VOCs released from the EC fluids. The tins were punctured in the lid to allow VOC collection using a pipette. The holes were sealed with plastic tape until collection. Conditioning of the tins was achieved by baking them at 110 $^{\circ}\text{C}$ for 24 h in an oven. Thereby all background VOCs within the tins were removed, which was confirmed by GCxGC-TOF-MS analysis. After conditioning the tins, 5 μL of E-fluid were deposited on the base of the tin. The tin was placed in a sand bath in order to release the vapor from the E-fluids. The temperature of the sand bath, i.e. 200 $^{\circ}\text{C}$, was controlled using a temperature probe which was kept under the base of the tin. Thus, the base of the tin was kept at 200 $^{\circ}\text{C}$. This temperature represents actual vaping conditions, whereby temperatures in “temperature control” E-cigarettes commonly range between 100 $^{\circ}\text{C}$ –315 $^{\circ}\text{C}$ [16]. The VOCs accumulation time was 5 min. For VOC collection a pipette tip was attached to a stainless Tenax TA sorbent tube (Markes International Ltd., UK). A Markes VOC sampling pump (Markes International Ltd., UK) was connected to the other side of the TD tubes and used to collect 100 mL of headspace accumulated within the tin on to the sorbent tube (Fig. 2).

2.3. VOC sample analysis

Prior to sample analysis by GCxGC-TOF-MS, 2 μL of the internal standard bromobenzene (150 ppm; GC grade, Sigma-Aldrich, Castle Hill, NSW, Australia) in methanol (HPLC Grade, Sigma-Aldrich) were pipetted onto each sorbent tube using an eVol[®] XR handheld automated analytical syringe (SGE Analytical Science, Weatherill Park BC, NSW, Australia). This allows the normalisation of analytes versus the internal standard for data analysis purposes. Initially, the Tenax tubes were thermally desorbed using a Markes Unity 2 Thermal Desorber and Series 2 ULTRA multi-tube autosampler (Markes International Ltd., UK). Each tube was thermally desorbed for 5 min at 300 $^{\circ}\text{C}$ under a helium flow of 40 mL/min and concentrated onto

a general purpose cold trap (TenaxTA/ Carboxgraph1TD) maintained at -10 $^{\circ}\text{C}$. The cold trap was heated for 5 min at 300 $^{\circ}\text{C}$ with a split desorption flow of 6 mL/min (i.e. split ratio of 4:1). After thermal desorption, each tube was reconditioned for 30 min at 330 $^{\circ}\text{C}$ under a split flow of 50 mL/min. The total run time, including tube desorption, of GCxGC-TOF-MS analysis and tube reconditioning was 1.5 h. Finally, tubes were wrapped in aluminium foil and stored in a glass jar ready for sample collection.

The thermal desorption unit was connected to a Pegasus[®] 4D GCxGC-TOF-MS (LECO, Castle Hill, NSW, Australia) system via a 1 m uncoated fused silica transfer line using an Ultimate Union Kit (Agilent Technology, Mulgrave, NSW, Australia). The transfer line between the TD unit and GCxGC-TOFMS was maintained at 120 $^{\circ}\text{C}$ in order to avoid condensation of the compounds. In the GCxGC part, a 30 m \times 0.250 mm inner diameter (ID), 1.40 μm film thickness (df) Rxi[®]-624Sil MS column (Restek Corporation, Bellefonte, PA, USA) was used in the first dimension (1D). The second dimension (2D) column was a 2 m \times 0.250 mm ID, 0.50 μm df Stabilwax[®] column (Restek Corporation). A SilTite μ -Union (SGE Analytical Science) was used to connect the 1D and 2D columns. High purity helium (BOC, Sydney, NSW, Australia) was used as the carrier gas at a constant flow of 2.00 mL/min. The temperature program was initially optimised using a direct injection of a nicotine and propylene glycol standard. The 1D oven temperature was maintained at 40 $^{\circ}\text{C}$ for 2 min and then increased to 230 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{min}$. The modulator temperature offset was 5 $^{\circ}\text{C}$ and the 2 D oven temperature offset was 25 $^{\circ}\text{C}$. The modulation period was 4 s with a 4 s hot pulse. The temperature of the MS transfer line was maintained at 250 $^{\circ}\text{C}$. Spectra were acquired at a rate of 200 spectra/s with a mass acquisition range of 25–500 amu. The filament was switched off between 390 s and 510 s. The ion source temperature was 200 $^{\circ}\text{C}$, the electron ionisation energy was 70 eV and the detector voltage was programmed with an offset of +200 V above the optimised detector voltage.

2.4. Data processing

ChromaTOF[®] (version 4.51.6.0; LECO) was used to carry out data processing with a signal-to-noise ratio (S/N) of 150 and a baseline offset (data processing feature which tracks baseline through the middle of noise) of 0.8. The 1D and 2D expected peak widths were set at 30 s and 0.15 s, respectively. A peak table containing a list of compounds was created using mass spectral matching at a match threshold of 80 % with the National Institute of Standards and Technology (NIST) Mass Spectral Library. No individual standards were used for identification purposes considering the large number of compounds detected. The compounds were categorized into the following compound groups: Carboxylic acids, ester, aldehyde, ketone, ether, aromatic, alkane/ alkene/ cycloalkane/ -alkene, halogenated, miscellaneous.

3. Results

Headspace samples of six different E-cigarette fluids and two different nicotine strengths (N0 and N18) were collected and analysed via GCxGC-TOF-MS. After removing peaks produced from solvents (methanol), the internal standard, i.e. bromobenzene, and column bleed, over 1000 different compounds were identified with S/N over 150. These compounds were classified into compounds classes and are listed in the Supplementary Information A. The number of compounds detected for each flavour and the two nicotine strengths are displayed in Table 1.

Initially, each compound was assigned to only one compound class based on the functional or characteristic group with the highest priority (Supplementary Information A; classification sys-

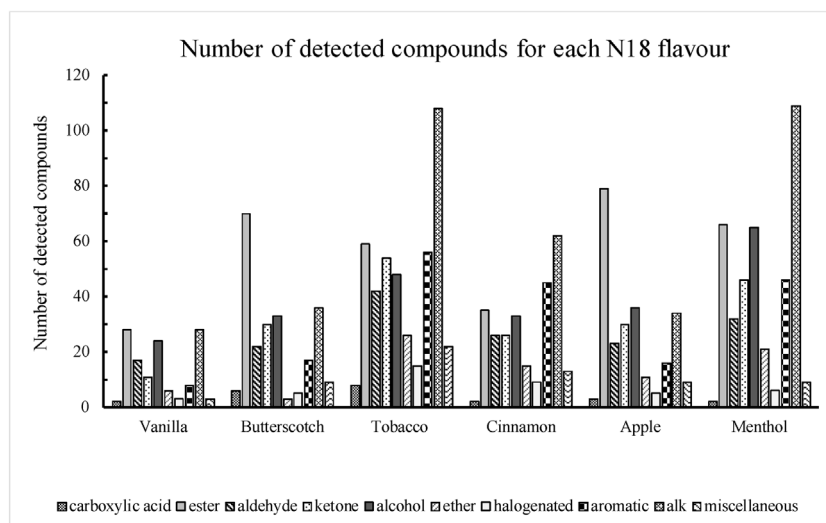


Fig. 3. Number of compounds detected in the headspace of EC fluids with 18 mg/ mL of nicotine detected and displayed for the various flavours. The compounds are categorized into carboxylic acid, ester, aldehyde, ketone, alcohol, ether, halogenated, aromatic, alk (alkanes/ alkenes/ alkynes) and miscellaneous (classification system 1). For each E-cigarette liquid 5 μ L were deposited on the bottom of a 1 L conditioned aluminium tin. After heating the tin in a sand bath at 200 °C for 5 min, VOC collection was achieved using a Markes VOC sampling pump connected to an opening at the top of the tin lid. 100 mL of headspace gas were collected and analysed via GCxGC-TOF-MS.

Table 1

Overall number of compounds detected for each flavour and nicotine strength.

E-cigarette fluids	Number of compounds detected	
	N0	N18
Tobacco	242	438
Menthol	429	402
Cinnamon	272	266
Butterscotch	279	231
Vanilla	142	130
Apple	239	246

tem 1). Hereby the sequence of priority followed carboxylic acid, ester, aldehyde, ketone, alcohol, ether, halogenated, aromatic, alkane/alkene/ alkyne and finally miscellaneous such as nitro-group, amines etc. Following this classification systems, the 1064 were subdivided into 18 carboxylic acid, 204 esters, 73 aldehydes, 118 ketones, 121 alcohols, 60 ethers, 33 halogenated, 132 aromatics, 267 alk (alkane/ alkene/ alkyne), and 39 miscellaneous.

Considering all functional groups in a molecule, 1355 compounds were listed in the different compound classes (Supplementary Information B; classification system 2). This number differs to the above mentioned 1064 since some compounds were assigned to multiple compound classes. For instance vanillin was assigned to aldehyde, ketone, alcohol and aromatic according to the various functional groups present in the molecule. Following this classification system, the 1355 compounds were subdivided into 18 carboxylic acids, 204 esters, 73 aldehydes, 131 ketones, 163 alcohols, 112 ethers, 44 halogenated compounds, 287 aromatics, 267 alk (alkanes/ alkenes/ alkynes) and 56 miscellaneous. A detailed presentation of the number of compounds in the various compound classes for each flavour is shown in Fig. 3 (N18) and 5 (N0). Overviews of the number of compounds detected in each compound class for the different flavours are provided for N18 in Fig. 4 and N0 in Fig. 6.

3.1. E-cigarette fluids N18

Looking at the different N18 flavours (Fig. 3), the highest number of compounds were detected in the vapor of Tobacco N18 (438), followed by Menthol N18 (402), Cinnamon N18 (266), Apple N18 (246) and Butterscotch N18 (231). The least amount of compounds

(130) was detected in the vapor of the Vanilla flavoured N18 E-liquid (Supplementary Information A). Furthermore, Tobacco N18 E-liquid produced the highest number of carboxylic acids, aldehydes, ketones, ethers, halogenated substances, aromatics and miscellaneous. The highest number of esters were detected in Apple N18 vapor, while the highest number of alcohols and alks were found in the headspace of Menthol N18 (Supplementary Information B; Fig. 4). The compound classes varied highly between the different flavours. However, aromatics and alks represented most of the time approximately one third of the detected compounds. The least amount of compounds were assigned to carboxylic acids and halogenated compounds.

3.2. E-cigarette fluids N0

In the headspace of E-cigarette fluids with 0 mg/ mL nicotine strength, Menthol N0 produced the highest number of VOCs (429, Fig. 5, Supplementary Information A). 279 compounds were detected in the headspace of Butterscotch N0. E-cigarette fluids of Cinnamon N0, Tobacco N0 and Apple N0 released 272, 242 and 239 VOCs respectively (Fig. 5, Supplementary Information A). As with the higher nicotine strength sample, the vapors from Vanilla N0 showed the lowest detected number of VOCs with 142 (Figs. 3 and 5).

The compound class abundance between the flavours was highly variable at both nicotine concentrations (Figs. 3 and 5). Similar to the E-liquids containing 18 mg/ mL nicotine, approximately one third of the compounds was assigned to aromatics and alks for the N0 flavours. In accordance to the N18 vapors, esters and alcohols represented the third and fourth most represented compound classes.

The core ingredients of E-cigarette fluids, namely propylene glycol and glycerol [17,18], were detected in all vapors. Main detected flavouring agents were pyridine, 3-(1-methyl-2-pyrrolidinyl)-, also called nicotine, associated with Tobacco flavoured fluid, d-menthol for Menthol fluid (also found in Vanilla, Butterscotch and Apple), cinnamaldehyde for Cinnamon fluid, diacetyl and acetoin for a buttery or caramel flavour, and ethylvanillin and vanillin for a vanilla note. Other detected flavouring additives included linalool, maltol or ethyl maltol, menthol, the ketones diacetyl, acetoin, and pulegone, and phellandren. There were 164 toxic VOCs detected in the

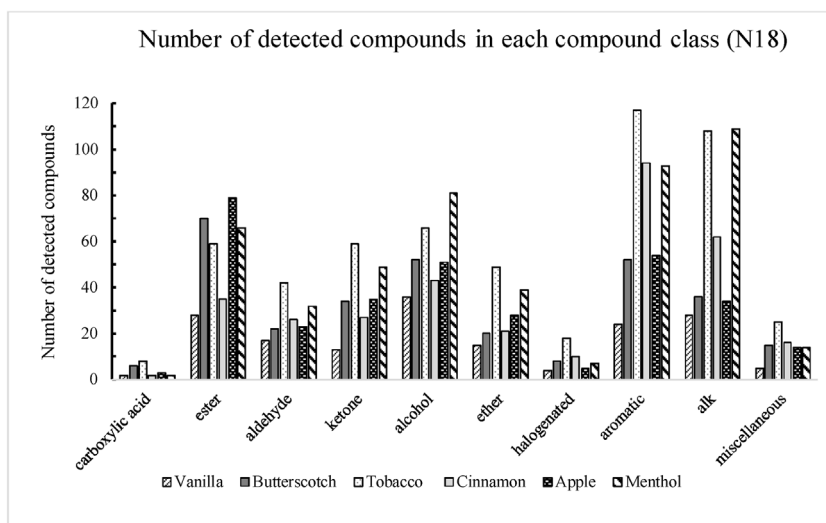


Fig. 4. Number of compounds detected in the headspace of EC fluids with 18 mg/ mL of nicotine detected and categorized into different compound categories: carboxylic acid, ester, aldehyde, ketone, alcohol, ether, halogenated, aromatic, alk (alkanes/ alkenes/ alkynes) and miscellaneous (classification system 2). For each E-cigarette liquid 5 μ L were deposited on the bottom of a 1 L conditioned aluminium tin. After heating the tin in a sand bath at 200 °C for 5 min, VOC collection was achieved using a Markes VOC sampling pump connected to an opening at the top of the tin lid. 100 mL of headspace gas were collected and analysed via GCxGC-TOF-MS.

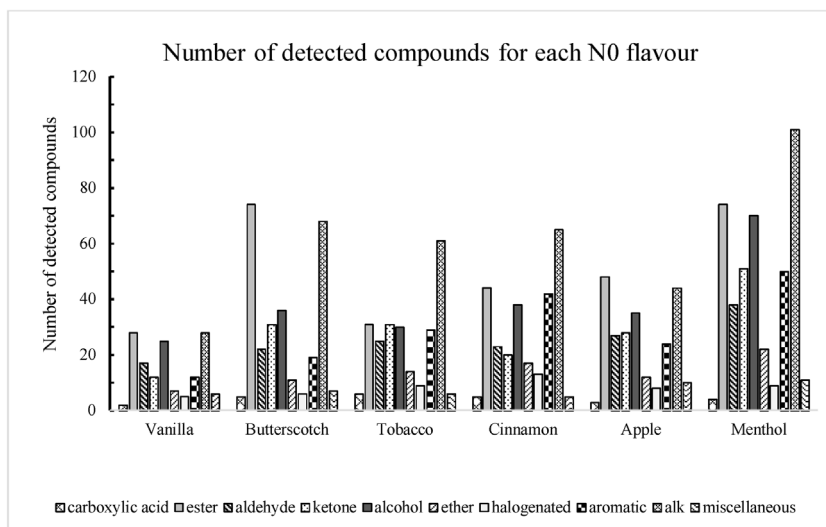


Fig. 5. Number of compounds detected in the headspace of EC fluids with 0 mg/ mL of nicotine detected and displayed for the various flavours. The compounds are categorized into carboxylic acid, ester, aldehyde, ketone, alcohol, ether, halogenated, aromatic, alk (alkanes/ alkenes/ alkynes) and miscellaneous (classification system 1). For each E-cigarette liquid 5 μ L were deposited on the bottom of a 1 L conditioned aluminium tin. After heating the tin in a sand bath at 200 °C for 5 min, VOC collection was achieved using a Markes VOC sampling pump connected to an opening at the top of the tin lid. 100 mL of headspace gas were collected and analysed via GCxGC-TOF-MS.

e-cigarette vapors (Supplementary Information C). Carbonyl VOCs were detected in all flavours of both nicotine strengths, i.e. N18 and N0. The other class of potentially harmful VOCs detected were long chain alkanes, cyclic alkanes/ alkenes and poly aromatic hydrocarbons. A detailed list of the various VOCs detected in the headspace of the E-cigarette liquids with indicated toxicity (highlighted in green) can be found in the Supplementary Information B.

4. Discussion

Using the technical advantages of GCxGC-TOF, an overall 1064 compounds were identified in the headspace of six differently flavoured E-liquids and two different nicotine strengths. This presents a very comprehensive list revealing various toxic and non-toxic compounds, which were not previously detected in the headspace of E-liquids. Although this number is still lower as the compounds found in traditional cigarette smoke [9], it is shown,

that heating E-cigarette vapor in the presented experimental setup is complex and contains a larger number of compounds that suggested by other previously reported experiments.

4.1. E-cigarette fluids N18

In between the N18 E-cigarette vapors, Tobacco (438) and Menthol (402) showed the highest numbers of compounds detected. Interestingly, these are flavours similar to the traditional cigarettes and might be consumed more often by smokers, who would like to experience the traditional taste while transferring from traditional smoking to E-cigarettes.

Within Tobacco, Cinnamon and Menthol, the compound class with the highest number of compounds detected was alks (108, 62 and 109 respectively), while for Butterscotch and Apple esters were the strongest represented compound class (70 and 79 respectively). In Vanilla vapor, 28 esters and 28 alks were identified representing

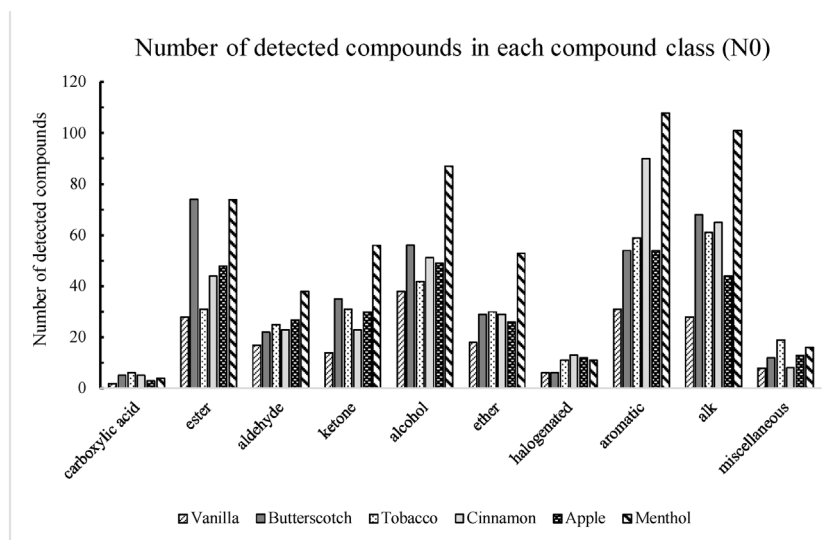


Fig. 6. Number of compounds detected in the headspace of EC fluids with 0 mg/mL of nicotine detected and categorized into different compound categories: carboxylic acid, ester, aldehyde, ketone, alcohol, ether, halogenated, aromatic, alk (alkanes/ alkenes/ alkynes) and miscellaneous (classification system 2). For each E-cigarette liquid 5 μ L were deposited on the bottom of a 1 L conditioned aluminium tin. After heating the tin in a sand bath at 200 °C for 5 min, VOC collection was achieved using a Markes VOC sampling pump connected to an opening at the top of the tin lid. 100 mL of headspace gas were collected and analysed via GCxGC-TOF-MS.

the classes with the highest number of VOCs. For all flavours, the fewest number of compounds that were found are carboxylic acids.

Overall, the N18 EC fluids produced a higher number of volatile compounds than the N0 EC fluids (Supplementary Information A). When looking closer at the data, it can be seen however that except for Tobacco and Apple, the number of detected compounds in N0 is higher than in N18 vapor. The biggest differences are observed for Tobacco. For Tobacco, 196 compounds less are detected in N0 than in N18. It is therefore not possible to state that nicotine containing E-liquids generally release more compounds than nicotine-free E-liquids.

4.2. E-cigarette fluids N0

The highest number of compounds in N0 vapor was found for Menthol (430). This were 28 compounds more than identified in N18 Menthol vapor. In Butterscotch, Tobacco, Cinnamon and Apple 279, 242, 272, and 239 VOCs respectively, were identified. Equally as for N18 cigarette fluids, the highest number of compounds detected in Tobacco, Cinnamon and Menthol belonged to alks (61, 65, and 101 respectively), while for Butterscotch and Apple esters were found the most. In Vanilla vapor, esters (28) and alks (28) represented the two most prevalent compound groups. Carboxylic acids were the lowest number of compounds detected.

4.3. General considerations

Overall, 164 toxic compounds were detected in the vapor of the six types of E-liquids with both nicotine strengths (Supplementary Information C). The highest number of toxic compounds were found in the headspace of N18 Tobacco (102). N0 Tobacco vapor contained fewer toxic compounds (63). Considering that traditional smokers using E-cigarettes as an aid to quit smoking or looking for a healthier alternative might prefer the classic tobacco flavour, we confirm, that thermal degradation compounds form already at medium vaping temperatures and can therefore be present even when applying lower voltages.

For all other flavours, only small variation in the number of toxic compounds between N18 and N0 were found. Menthol vapor contained the second highest number of toxic compounds with 83 compounds in N0 and 74 in N18, followed by Cinnamon (66 in N18

and 62 in N0 vapor), Apple (51 in N18 and 55 in N0 vapor), Butterscotch (42 in N18 and 59 in N0 vapor) and Vanilla (33 in N18 and 38 in N0 vapor). For Tobacco and Cinnamon, the E-liquid containing nicotine released a higher number of toxic compounds into the vapors, while for Menthol, Apple, Butterscotch and Vanilla, a higher number of toxic compounds was found in N0 vapor. Thus, no prediction on the number of toxic compounds can be made based on the nicotine levels in E-liquid. Although many identical toxic compounds were discovered in the N18 and N0 vapor of the same flavour, none of the toxic profiles was exactly the same. Between flavours, the number and composition of toxic compounds varied greatly. Toxic compounds detected in all flavours and nicotine strengths are 2,3-butanedione, 2-propenal, acetaldehyde, α -pinene, benzene, propyl benzene, methyl cyclohexane, dodecane, 2,6,10-trimethyl dodecane, formaldehyde, heptane, hexadecane, methacrolein, phenylethyl alcohol, tetradecane, toluene, tridecane and undecane. Overall, toxic compounds associated to all compounds classes, i.e. carboxylic acids, esters, aldehydes, ketones, alcohols, ethers, halogenated, aromatics, alks and miscellaneous, could be identified (Supplementary Information C).

Many characterizing flavouring aldehydes were detected in the headspace of the different E-liquids including benzaldehyde, cinnamaldehyde, citral, piperonal, methacrolein, vanillin, and ethylvanillin. Since no regulations are yet in place in order to limit their use in E-liquids, health concerns considering their potential respiratory and cardiovascular toxicological effects have risen [19]. The effects are attributed to the activation of the transient receptor potential (TRP) ion channels, TRPA1 and TRPV1, leading to inflammatory consequences and cardiovascular reflexes [20,21]. Moreover, not only the aldehydes themselves can influence the toxicological effects of E-liquids, but also their reaction products. In the presented study here, PG acetals for benzaldehyde and piperonal (heliotropine) were found in vapors of Butterscotch, N18 and N0, and in Apple N18. These results support a previous study claiming that flavour aldehydes are chemically unstable and can react with other ingredients in E-liquid such as propylene glycol and vegetable glycerol and potentially result in different physiological and toxicological effects. Other detected flavouring compounds are for instance the alcohols linalool (flowery smell), maltol or ethyl maltol (caramelized sugar flavour), menthol, the ketones diacetyl (buttery flavour), acetoin, pulegone, and phellandrene. Similar as

for the aldehydes, some of these compounds have toxicological effects to human health. While maltol, ethyl maltol and menthol have only low toxicity levels according to the FDA, diacetyl used for artificial butter flavouring, can result in injuries replicating features of human obliterative bronchiolitis [22]. Monoterpenoids such as phellandrene, linalool, pulegone can be used as natural pesticides due to their neurotoxic effects [23]. While these compounds have shown various toxic effects on insects, roaches, worm and rodents, health effects on humans and effective doses should be further investigated.

Carbonyl degradation products identified in the headspaces of all E-liquids were formaldehyde, methacrolein and acetaldehyde, which is in accordance with previous studies [24,25]. Pyrolysis of glycerol has been shown to result in dehydration and fragmentation of the molecule resulting in the formation of these compounds [26]. Additionally, we confirm, that thermal degradation compounds form already at medium temperatures. Translating this information to the vaping process, it could be possible that these compounds can already be present even when applying lower voltages [10]. Acute exposure to these compounds may lead to vascular injury, endothelial dysfunction and platelet activation whereas chronic exposure can accelerate cardiovascular disease [27].

Interestingly, nicotine was detected in all vapors originating from E-liquids with N18 and N0 except for N0 vanilla and N0 cinnamon. This is in line with reports from three previous studies, which also documented nicotine levels for E-liquids labelled as nicotine-free [13,28,29]. No remarks about possible differences in nicotine levels between N18 and N0 vapor can be made here, as the experimental set up did not allow for the quantification of the measured compounds. Furthermore, the vapor was produced by heating the E-liquid in a tin located in a heated sand bath and does not represent the exact vaporization process inside an E-cigarette.

Although the FDA published a list for initial HPHC testing in E-cigarette liquids, HPHC detection and concentrations in the aerosols remains poorly understood and requires further investigation. Using GCxGC-TOF-MS, we were able to broaden the knowledge about the presence of HPHC in E-cigarette vapor. A HPHC list including the current recommendation is provided in Supplementary Information D. Eight of the listed compounds were identified in all vapors, i.e. acetone, methacrolein, benzene, n-butanol, formaldehyde, glycerol, propylene glycol and toluene. Since no information about the concentration levels of these compounds can be provided using the untargeted semi-quantitative approach presented here, further studies should quantitatively detect those compounds to supply information about their doses and related health effects and compare the result to previously published studies [15,24,25,30]. The quantitative information would be required to obtain further knowledge about the toxicity of the pyrolysis products of E-liquids. For this purpose, analytical techniques such as GC-MS, GC-MS/MS or LC-MS/MS would be suitable. GCxGC-TOF-MS is advantageous for untargeted and semi-quantitative analysis due to the superior peak-resolution as shown here. Using GC-MS, data acquisition and data analysis can be more straight forward. Thus, the time required for the quantitative analysis of a set of known compounds shorter. Tandem MS techniques such as GC-MS/MS or LC-MS/MS offer the advantage of selective quantification of target compounds in samples with high background due to the removal of matrix interferences.

Additionally, changes related to different temperatures/ voltages and common puffing volumes should be further investigated.

5. Conclusion

Using GCxGC-TOF-MS, a comprehensive list of over 1064 VOCs produced by heating E-liquids with different flavours was estab-

lished. Out of these 1064 compounds, 164 were toxic. To our best knowledge, this is the most comprehensive list of possible E-cigarette vapor compounds to-date. The number of VOCs was not dependent on the nicotine strength of the flavour, i.e. no generic statement on whether nicotine containing E-liquids lead to a higher or lower number of VOCs than nicotine-free E-liquids can be made. Interestingly, the highest number of VOCs as well as toxic VOCs were detected in Tobacco vapor, which could be of interest to smokers with preference for traditional cigarette flavour. Nicotine was found in the vapors of all tested E-liquids, nicotine-containing and nicotine-free, except for N0 vanilla and N0 cinnamon. Overall, various different flavouring agents as well as their reaction products with E-liquid solvents and carbonyl degradation products were identified. These results demonstrate the complexity of the toxic profile of E-cigarette vapors which is aggravated by production of hundreds of potentially toxic carcinogenic compounds by the vaping process and broadens the understanding of the potential toxicity of the aerosols produced by vaping and their potential harmful effects on humans health.

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CRedit authorship contribution statement

Darshil Patel: Methodology, Investigation, Data curation, Writing - original draft. **R. Verena Taudte:** Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Katie Nizio:** Methodology, Writing - original draft. **George Herok:** Conceptualization, Writing - original draft. **Charles Cranfield:** Conceptualization, Writing - original draft. **Ronald Shimmon:** Conceptualization, Methodology, Investigation, Data curation, Writing - original draft.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jpba.2021.113930>.

Declaration of Competing Interest

The authors report no declarations of interest.

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