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Characterisation of nonpolar compounds from Electronic cigarette aerosols by GC-MS

Karakterisering av upolare komponenter fra Elektronisk sigarett aerosoler ved GC-MS

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Abstract

Electronic cigarettes (e-cigarettes) are nicotine delivering systems without combustion. By inhaling an aerosolised liquid, e-liquid, the user can stimulate nicotine cravings. The e-liquid contains humectants, propylene glycol and glycerol, nicotine in various concentration and flavouring agents. Regulations of e-cigarettes are scarce, and health risk assessments lacking. Due to little regulations of e-cigarettes and e-liquids, the composition of the generated aerosol will vary. To accommodate the current desire for information about constituents in the aerosol from e-cigarette; this thesis identified nonpolar components from e-cigarettes using GC-MS. An ASTER e-cigarette and 11 different e-liquids were acquired at a local e-cigarette dealer. To collect aerosol generated by the e-cigarettes an aerosol-trap method was developed. A gas wash bottle with solvent coupled in series with the e-cigarette, a waste trap and a water jet for suction. Three nonpolar solvents were chosen for trapping the analytes; heptane, chloroform and ethyl acetate. The trapped aerosol was concentrated and analysed on GC-MS. The thesis contributes to mapping unknown components in e-cigarette aerosol and e-liquid. A total of 129 components were tentatively identified in the trapped aerosol, 30 were further identified with external standards, and 22 detected. 21 of the 22 detected components were quantified. Chemical classes as alcohols, aldehydes, esters and alkaloids are represented in the nonpolar layer of e-cigarette aerosol with over 60 known food additives, associated with no health risk, and at least 15 components with potential toxic character. Two components are possibly carcinogenic. Strict regulation of additives and toxicology of components alone and together should be implemented in the future and possible allergenic and toxic components should be avoided. This research point in favour of e-cigarettes potential as a healthier alternative to the regular cigarette smoke.

Sammendrag

Elektroniske sigaretter (e-sigaretter) er en alternativ måte å inhalere nikotin. Ved å trekke inn en damp bestående av flyktige stoffer, nikotin og aroma vil en e-sigarett bruker kunne få dekt nikotin behovet. En væske, e-væske, blir varmet opp av et batteridrevet varmeelement som genererer dampen. Væsken består hovedsakelig av propylenglykol, glyserol, nikotin og smakstilsetninger. Det er lite regulering av e-sigaretter og e-væsker, slik at innholdet i e-væske og damp har stor variasjon. Det er også mangel på fullstendig helserapporter om de enkelte komponenter og miksen av komponenter, både i væsken og dampen. For å imøtekomme ønsket om mer informasjon rundt dampen fra e-sigaretter ble denne avhandlingen utført for å karakterisere upolare komponenter i e-sigarett damp ved hjelp av GC-MS. En ASTER e-sigarett og 11 forskjellige e-væsker ble anskaffet fra den lokale e-sigarett forhandleren. For å fange aerosolen fra e-sigaretten ble en damp-felle metode utviklet. En gassvaskeflaske fylt med løsningsmiddel ble seriekoblet med e-sigaretten, en avfalls-fanger og en vann-jet for sug. Tre upolare løsemiddel ble brukt, heptan, kloroform og etylacetat. Etter dampen var fanget, ble prøvene konsentrert og analysert på GC-MS. Avhandlingen bidrar til kartlegging av ukjente komponenter i e-sigarett aerosol og e-væske. Med totalt 129 forskjellige komponenter midlertidig identifisert ble 30 identifisert med ekstern standard og 22 detektert. Av 22 detekterte stoffer ble 21 kvantifisert. Kjemiske klasser representert i det upolare sjiktet av aerosolen er alkoholer, aldehyder, ester og alkaloider med over 60 kjente tilsetningsstoffer med liten grad av helse risiko, og minst 15 komponenter med mulige giftige egenskaper. To av komponentene kan være kreftfremkallende. Avhandlingen bidrar til å kartlegge ukjente komponenter i e-sigarett damp og e-væske. Fremdeles vil strengere regulering og kartlegging av komponenters toksisitet alene og samlet være nødvendig, og mulige allergener og giftige stoffer bør unngås. Denne avhandlingen peker i favør e-sigarett som et sunnere alternativ til den vanlige sigaretten.

Abbreviations and Definitions

BB	**Blueberry, e-liquid**
CDC	Center for Disease Control and Prevention
CV	Creamy vanilla, e-liquid
EMP	Emperor, e-liquid
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally-recognised-as-safe
HPLC	High-performance liquid chromatography
LM	Lemon Mint, e-liquid
LOD	Limit of detection
LOQ	Limit of quantification
m/z	Mass-to-charge ratio
MT	Menthol tobacco, e-liquid
ND	Not detected
NG	Glycerol
NIST	National Institute of Standards and Technology
Peach	Peach, e-liquid
PG	Propylene Glycol
PL	Pirate's loot, e-liquid
RB	Raspberry, e-liquid
RED	Xeo vapor e-liquid
RT	Retention time

TLC	Thin-layer chromatography
TSNA	Tobacco-specific nitrosamines
UB	Unicorn Blood, e-liquid
VG	Virgina gold, e-liquid
SPME	Solid-phase microextraction
FEMA	The Flavor and Extract Manufacturers Association of the United States

Vocabulary for electronic cigarettes

Aerosol	The steam generated from an electronic cigarette
Puff	A draw from an electronic cigarette
Vape (noun)	The mixture aerosolised from an e-cigarette
Vape (verb)	To take a draw of an electronic cigarette
Vaper	The user of an electronic cigarette
E-liquid	A volatile solution is usually containing humectants propylene glycol and glycerol with flavouring ingredients and nicotine. The liquid will be heated by the e-cigarette to create an aerosol for the user to consume.
Flavouring Agents	Components added to a mixture to improve taste or odour. The agents were originally for food and medicine, but have developed to include other mixtures as e-liquids

Contents

1. Introduction

1.1 Historical background

Smoking tobacco has a long tradition all the way back to the shamanistic rituals 4000 BC in America. The famous tradesman and explorer Christopher Columbus was the first European to discover the plant which entered the European market in the 16th century and further to Norway in the early 17th century (Lund 2017).

The World Health Organization (WHO) define tobacco products as "Products made entirely or partly of leaf tobacco as raw material, which is intended to be smoked, sucked, chewed or snuffed. All contain the highly addictive psychoactive ingredient, nicotine". Several governments report that the most prominent cause for diseases and premature death in the Western world is tobacco smoking (Folkehelseinstituttet 2016) and by the year 2030, up towards 8,3 million tobacco-related deaths can occur every year (Mathers & Loncar 2006). It is therefore of absolute value to derive alternative methods to consume nicotine, without the toxic smoke.

Electronic cigarettes (e-cigarettes) were thought of already in 1963 by Herbert A. Gilbert. His idea of a smokeless non-tobacco cigarette that replaced tobacco with moist air would 40 years later start a new trend (Gilbert 1963). It was Hon Lik, a 52-year old Chinese pharmacist who first created an e-cigarette in 2003. The first-generation e-cigarette came to the European marked in 2007, an e-cigarette to mimic traditional cigarette. The evolution of e-cigarettes has continued to advance as second- and third-generation devices.

1.2 How does an e-cigarette work?

Even though e-cigarettes have different design and appearance, they operate the same way (Brown & Cheng 2014). An e-cigarette usually consists of a battery, an atomiser, a tip, heating coil and a cartridge, as shown in Figure 1.

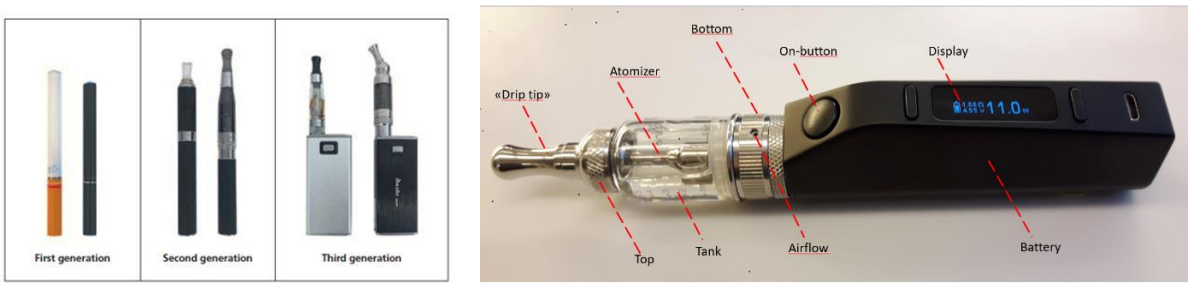


Figure 1. First to third generation e-cigarettes (Phillips 2018) and the main components to complete a third-generation e-cigarette

E-cigarettes use energy from a battery through an atomiser to evaporate a solution containing humectants, flavour and nicotine (Lund 2018). The solution is referred to as e-liquid or e-juice. Main ingredients of e-liquids are the humectants propylene glycol (PG) and glycerol (NG). Flavour ingredients and different nicotine concentration make the e-liquid complete. The humectants function as nicotine and flavouring carriers. To evaporate the e-liquid, a user takes a draw (or puff) and activate the heating element, usually by pressing a button, before inhaling the aerosolised solvent, also called vape. Adjusting the airflow and battery power can give different properties to the aerosol. Vape from e-cigarettes can mimic tobacco smoke and the burning feeling in the throat, this effect is usually referred to as "throat hit" (Herrington & Myers 2015). Too high heating power can result in "dry puffs", this comes from thermal decomposition of PG and NG and result in a "bad taste" puff (Farsalinos *et al.* 2015b). In the earlier generations (first and second) it was reported that e-cigarettes need higher suction than conventional cigarettes to smoke. The same research questioned e-cigarettes as nicotine delivery devices since the dosing were nonuniform over time (Trtchounian *et al.* 2010).

A second-generation e-cigarette has a refillable tank that contains e-liquid while the most frequent device on the market, third-generation e-cigarettes, can refill, change air flow and output on the battery, as shown in Figure 1. E-cigarettes have a lot of different replaceable parts so the output will vary (Brown & Cheng 2014). Lund (2013) and Uchiyama (2016) described e-cigarettes to mimic a traditional tobacco cigarette without combustion.

1.3 Components in e-cigarettes vapour

Zhu *et al.* (2014) counted more than 7000 unique e-liquid flavours from 466 brands, fast increasing each month. Trtchounian *et al.* (2010) questioned e-cigarettes as nicotine delivery devices while Centers for Disease Control and Prevention (CDC, 2010) found that e-cigarettes can achieve a concentration of nicotine, comparable to that from conventional cigarettes. The consequences of inhaling the aerosol and other constituents are not fully clarified. Most of the flavouring agent used in e-liquids are deemed safe in food (EU Lists of Flavourings). Food and Drug Administration (FDA) also comments that most flavouring components used in e-liquids are generally-recognised-as-safe (GRAS) as food additives. The Flavour and Extract Manufacturers Association of the United States (FEMA) who assess GRAS components do not evaluate flavouring agents in other products like e-cigarette only in human food. The human detoxification processes of all components in e-cigarette aerosols are still unknown.

Herrington and Myers (2015) found 60-70 different components in the e-liquid and 80-90 in the aerosol. The difference may indicate that during aerosolisation components react to form new components. Oxidation of the humectants may occur when the liquid touches the heating element and result in carbonyl generation, like the aldehydes formaldehyde, acetaldehyde and acrolein (Uchiyama *et al.* 2013; Uchiyama *et al.* 2016). Figure 2 shows possible oxidation steps of PG and NG. Hutzler *et al.* (2014) found a total of 141 different components in their study on vape from 28 different e-liquids, most were flavouring additives, but some had other characteristics like ethylene glycol added to substitute other humectants like PG and NG. Ethylene glycol is a known toxic for humans if digested (Hess *et al.* 2004). Sassano *et al.* (2018) screened 148 e-liquids to identify constituents and relative toxicity. They also revealed that e-liquids are very heterogeneous.

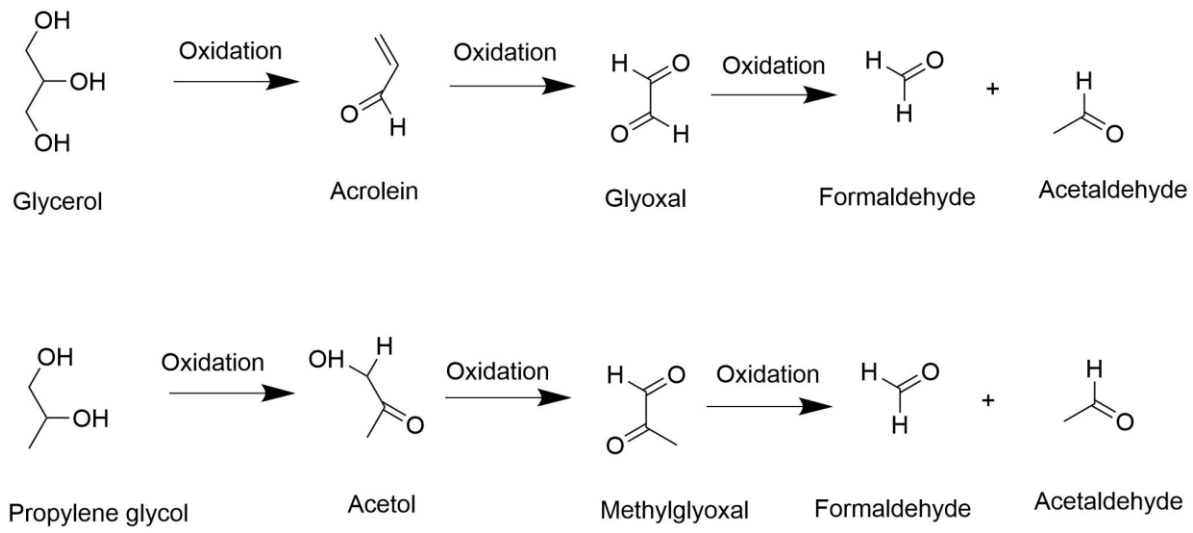


Figure 2. Oxidation steps of glycerol and propylene glycol to the aldehydes formaldehyde and acetaldehyde (Uchiyama et al. 2016)

Some flavouring components that are deemed safe to consume do not have the same metabolism when inhaled. Diacetyl and acetyl propionyl have been identified in several e-cigarette aerosols and are associated with respiratory diseases when inhaled (Allen *et al.* 2016). According to Farsalinos *et al.* (2015a), this represents an avoidable risk since similar sweet flavour is achievable without diacetyl and acetyl propionyl.

A test to determine acrylamide and acrolein in tobacco smoke and e-cigarette aerosol showed acrylamide in tobacco smoke and acrolein in both tobacco smoke and e-cigarette aerosol (Papousek *et al.* 2014). Under dry puff conditions Farsalinos *et al.* (2015b) discovered 30-250 times higher amounts of formaldehyde, acetaldehyde and acrolein than normal. As for tobacco-specific nitrosamines (TSNAs) and volatile organic components (VOCs), there are known hazardous components in e-cigarettes reported consistently by Herrington *et al.* (2015), Goniewicz *et al.* (2014) and Kosmider *et al.* (2014). Goniewicz *et al.* (2014) reported significantly lower (9-450 times) concentrations of some hazardous components in e-cigarettes aerosol compared to conventional cigarette smoke. The components compared were formaldehyde, acetaldehyde, acrolein, toluene, N'–nitrosonornicotine and 4-(methylonitrosoamino)-1-(3-pirydyl)-l-butanone.

1.4 Regulation of e-cigarettes

As e-cigarettes do not combust, users will not be exposed to some of the potentially harmful components related to smoking. The aerosol has been reported to be less complex than the smoke from combusting cigarettes since it mostly consists of humectants, PG or NG (Margham *et al.* 2016). The complex smoke from tobacco cigarettes contains about 5000 different components where at least 70 are carcinogenic (Talhout *et al.* 2011). It is commonly accepted that vaping e-cigarettes exposes the user to less hazardous components than tobacco cigarettes. Due to the uncertainty of long-term health risks, e-cigarettes have been held back from some markets, like in India (Naskar & Jakati 2017). Since e-cigarettes came on the market in 2007 many countries, have had challenges regulating the products without sufficient scientific background. Some countries decided to ban e-cigarettes while others allowed them without any regulations (Etter *et al.* 2011).

Brown and Cheng (2014) requested a standardised e-cigarette testing regime to allow production comparisons. WHO reported recently (2014) on the regulation of e-cigarettes and similar products, where they stated that e-cigarettes were an "evolving frontier filled with promise and threat for tobacco control", and that regulations are needed. Already in 2011, Trtchounian and Talbot urged regulators to remove all electronic nicotine delivery systems (ENDS), including electronic cigarettes, due to design flaws, inadequate labelling and lacking quality control. Regulations of e-cigarettes should prevent promotion towards young people and non-smokers, minimise potential health risk to the user and whoever is exposed. Since there are no standardised method or evaluation of components in e-liquids, the content can vary in concentration in each brand. A Polish study (Kucharska *et al.* 2016) concluded that there was a poor agreement between manufacturers listed flavour substances and identified aroma components. These studies indicate the complexity of both the e-liquid and aerosol profile and encourage regulation.

The Tobacco Products Directive (*Directive 2014/40/EU* 2014) were available in 2016 and started to regulate the e-cigarette industry to some extent. The directive gave clear limits of nicotine content (20 mg/mL), the volume of e-liquids and some other quality and safety requirements like child-proof corks. E-cigarettes also got a quality control were each puff with the same strength and duration should result in the same concentration of nicotine. Information of other constituents in the e-liquids should be presented with the package. Some components were prohibited in e-liquids. EU members were considered regulators of advertisement of e-electronic cigarettes in their own country.

1.5 Objective

The objective of this thesis was to identify nonpolar components in e-cigarette aerosol with GC-MS. The objective was further developed to also quantify some of the identified components. A sub-objective of this thesis was to develop a method to trap the aerosol. Nonpolar components were targeted due to little research in the field. Polar components were excluded since many studies already have been performed.

2. Theory

2.1 Gas-chromatography

To separate unknown constituents in a complex solution, like trapped vape from an e-cigarette, gas-chromatography (GC) with a mass spectrometer (MS) detector is preferred. Chromatographic principles are based on a component's affinity to the stationary phase and mobile phase. In GC a solution is vaporised after injection and eluted by the mobile phase (carrier gas) through a column and the stationary phase. Due to the components different boiling point and affinity to the stationary phase, they will be separated and have different retention time (RT). The most used carrier gases are nitrogen, helium and hydrogen, which are chosen for their inert characteristics. Different types of columns can achieve different separation of a complex mix. Capillary columns and packed columns are the common columns, where capillary columns made of fused silica coated with the stationary phase are the most used. Capillary columns are open and long compared to packed columns. Open and long columns give better separation due to less pressure drop and more theoretical plates (Rahman *et al.* 2015). The stationary phase is chosen accordingly to the analyte of interest, for nonpolar analytes dimethylpolysiloxane is preferred. Their applications are broad, and span from environment analysis and quality control in pharmaceutical products to food testing, even used to separate aerosol from e-cigarettes (McAuley *et al.* 2012).

2.2 Mass spectrometry

Electron ionisation (EI) is the most common technique to ionize components for analysis in mass spectrometry. Electrons are accelerated to 70 eV and collide with gaseous molecules in the source; this will ionize the molecules and accelerated them through the mas filter and towards the mass detector. EI is a hard ionisation technique, which results in fragmentation of the analyte in the ion source.

Along with mass accuracy and resolution, mass range limit, analysis speed and transmission are essential characteristics for measuring the performance of a mass analyser (de Hoffmann 2007). Resolution is the degree the mass analyser can separate two masses with small mass to charge (*m/*z) difference. Mass accuracy is the difference between a measured mass and the actual mass. With a mass spectrometer, both qualitative and quantitative information can be achieved. Previous studies of e-cigarette liquids and aerosol have used GC-FID and GC-MS in their study. Mass filters like ion trap and single quadrupoles have previously been used in studies to identify components in both the aerosol and e-liquid. No previous studies for quantification and qualification of aerosols in e-cigarettes have been performed using a sector instrument.

A sector instrument can have both magnetic (B) and electromagnetic (E) sectors, as shown in Figure 3. Both sectors apply a force to an ion, with mass *m* and charge *q*, perpendicular to its path. The vertical force will give the ion a circular path with radius r, without a change in velocity v. The force applied from the magnetic sector on an ion is denoted F_M.

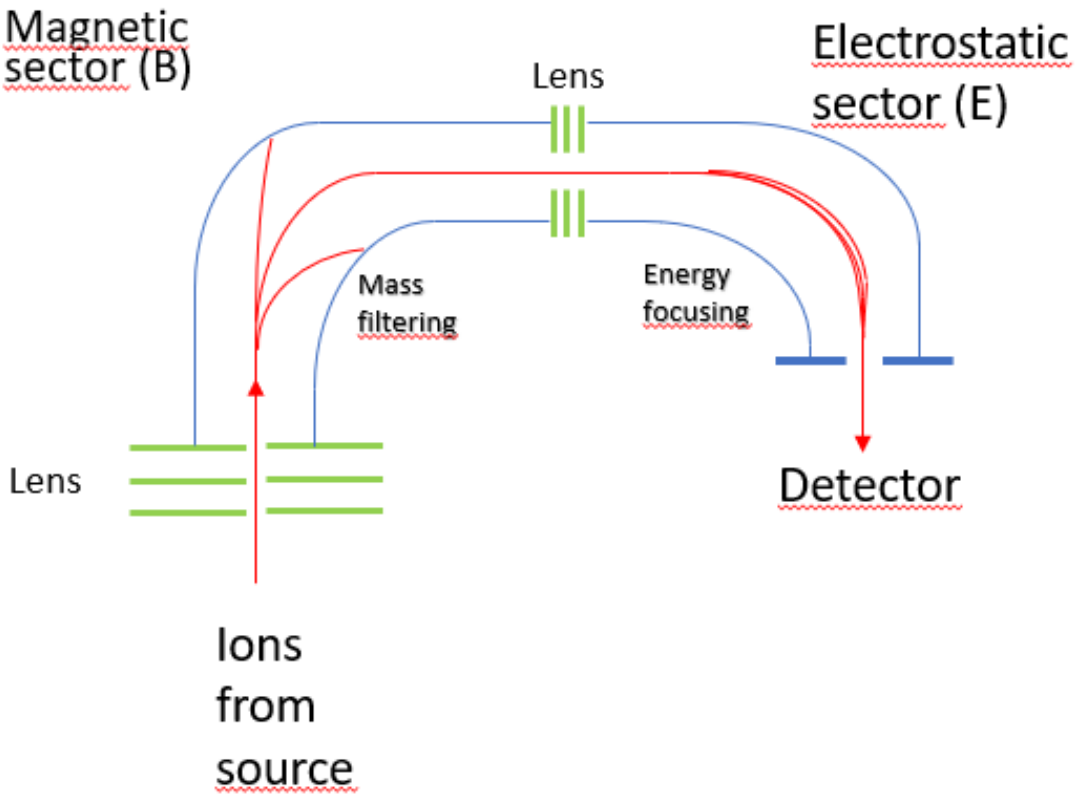


Figure 3. Sketch of a sector instrument with reverse geometry (BE)

Ions entering the magnetic field get different trajectories, based on their momentum, called angular dispersion, shown in Equation 1. While in the electrostatic field (E), energy dispersion occurs. Ions are separated based on the ions kinetic energy (E_k) entering the field, shown in Equation 2.

Sector instruments are used for both qualitative and quantitative analysis. With sector instruments, one gets large dynamic range and classic mass spectra. With high reproducibility, sensitivity and resolution sector instruments are well suited as detectors.

Equation 1. The action of the magnetic field in sector instrument.

$$F_M = qvB$$

$$qvb = \frac{mv^2}{r} \text{ and } mv^2 = 2qV_s \text{ give}$$

$$\frac{m}{q} = \frac{r^2B^2}{2V_s}$$

$$r = \frac{\sqrt{2mE_k}}{qB}$$

Equation 2. The action of the electrostatic field in sector instrument

$$qE = \frac{mv^2}{r}$$

$$\frac{m}{q} = \frac{rE}{v^2}$$

$$r = \frac{2E_k}{qE}$$

2.3 Scan modes

Total ion chromatogram (TIC) gives a signal for every ion detected for all compounds detected in the analysed sample. For TIC to work as intended, it is common not to scan for *m/z* less than 40, to avoid interfering molecules from the air. From a TIC, one can extract specific ions to make a reconstructed ion chromatogram (RIC). The RIC will then reveal peaks with the specific ion/ions of interest. RIC can be a useful tool to improve sensitivity, to check and resolve suspected co-eluting substances, give clean chromatograms of interest or to detect suspected but not found components (Miller 2009). In selected ion monitoring (SIM) mode, the mass filter only passes one *m/z* ratio through the instrument at a given time segment. This technique has a high sensitivity and is mostly used in quantitative studies.

To identify components from their mass spectra after analysis, libraries of mass spectra are available. The library will give a mass spectrum a match score, based on how similar the analysed spectrum was compared to the one in the library. NIST is well known for their different MS libraries.

2.4 Determination of components in vape from electronic cigarettes

There are still no standard guidelines for testing e-cigarettes, nor a detailed description of constituents in the aerosol produced when vaping. It makes a comparison, between the few studies available, difficult. Various aerosol generating methods have been tested; Werley *et al.* (2016) and Utchiyama *et al.* (2016) mimic traditional tobacco smoke with a smoking machine, which generated and trapped the aerosol. Werley *et al.* (2016) used GC-MS to give a chemical fingerprint of the aerosol and analysed nicotine with GC-FID. Utchiyama *et al.* (2016) identified both nicotine and volatile organic compound (VOC) with GC-MS.

For aldehyde detection in e-cigarette vape, Ogunwale *et al.* (2017) trapped the aerosol in Tedlar bags with a "10 puff method", that mimic puffing done by e-cigarette users. After a microreactor oximation of the aldehydes or ketone, GC-MS analysis was performed with an ion trap as a detector.

Another study screened constituents in a single puff and used a simple syringe to draw vape through a thermal desorption tube. Analysed with a single quad detector in a GC-MS system (Herrington *et al.* 2015).

A more comprehensive study, screening components in both liquids and aerosol from e-cigarettes were performed by Hutlzer *et al.* (2014). Identification and quantification were done with headspace GC-MS to determine aldehydes in the aerosol. Single quadrupole GC-MS was used to determine the nicotine content in the aerosol and GC-FID was used to analyse e-liquid solutions.

In a multicomponent analysis of e-liquid done by Kavvalakis *et al. (2015)*, a single quadrupole GC-MS system in SIM mode was used to determine polycyclic aromatic hydrocarbons (PAHs) and main humectants.

3. Experimental

3.1 Electronic cigarettes and e-liquids

The e-cigarette and 11 e-liquids, Table 1, were commercially bought at TV-Boden Svinesund, Sweden. “The most common e-cigarette used” ASTER Eleaf (Shenzhen, China) e-cigarette was chosen, a third-generation e-cigarette. The e-liquids were selected to achieve different flavouring, nicotine concentration, PG and NG ratio and manufacturers. After purchase, the e-liquids were stored in a freezer at -20 ºC.

Table 1. 11 e-liquids analysed in this thesis, listed with PG and NG ratio and nicotine concentration in each liquid.

Name	Abbreviation	Manufacturer	Flavour	PG (%)	NG (%)	Nicotine (mg/mL)
Blueberry	BB	LIQUA (Shenzhen, Guangdong, China)	Blueberry	70	30	6
Peach	Peach	LIQUA (Shenzhen, Guangdong, China)	Peach	70	30	18
Lemon Mint	LM	VIVO (Warszawa, Poland)	Mint	50	50	6
Xeo vapor E-liquid	RED	XEO (Hannover, Germany)	American Blend Red	50	50	3
Unicorn blood	UB	FUZION VAPOR (Saint Johns, Florida, USA)	Fruity and sweet	50	50	6
Pirate's loot	PL	VAPING PIRATES (Strömstad, Sweeden)	Key Lime Cheesecake	40	60	0
Emperor	EMP	EJUICE (Höör, Sweden)	Tobacco	50	50	6
Virgina gold	VG	EJUICE (Höör, Sweden)	Mild Tobacco	50	50	24
Creamy vanilla	CV	EJUICE (Höör, Sweden)	Vanilla	50	50	6
Raspberry	RB	EJUICE (Höör, Sweden)	Raspberry	50	50	6
Menthol Tobacco	MT	EJUICE (Höör, Sweden)	Menthol	50	50	6

3.2 E-cigarette settings

An ASTER e-cigarette with Nautilus Aspire tank system (Shenzhen, China) was used throughout the study, Figure 4. E-cigarette settings was as shown in Table 2, if not otherwise specified. ASTER was used up to 23 W. Power setting tested was 11 W, 18 W and 23 W with atomisers 7-11 or 10-14, 14-18 W and 20-23 W. The airflow settings tested were 0, 1, 2 and 3 where the number corresponds to air flow; 0 equal closed hatchet and 3 max flow. To prevent carryover from earlier e-liquids, the e-cigarette system was thoroughly cleaned, and a new atomiser was used if necessary.

Figure 4. ASTER Eleaf battery with Nautilus Aspire tank system at 11 W, 1 in air flow and an empty tank.

Table 2. Most used settings on ASTER e-cigarette with Nautilus Aspire tank system.

Electronic cigarette	Possible settings	Used settings
Battery		ASTER Eleaf
Tank system		Nautilus Aspire
Air flow	(Closed) 0, 1, 2, 3 (Max)	3 (Max)
Atomizer	Aspire BVC	7-11 W
Power	0-75 W	11 W (1.88 Ohm, 4.55 V)
Volume e-liquid	0-5 mL	4 mL
Temperature	-15 up to room-temperature	Room-temperature

3.3 Collection of vape

Collection of vape and sample preparation were performed in a fume hood at room-temperature if not otherwise specified.

To trap vape, a gas wash bottle (250 mL, Lenz, Wertheim, Germany) filled with 100 ml solvent, Table 3, and a waste-trap were coupled in series and connected to an e-cigarette with a water jet for suction, as outlined in Figure 5. The waste-trap was used to trap possible residue from the hose or e-liquid not aerosolised. The trap was set up between the e-cigarette and gas wash bottle and connected with a rubber hose (diameter 1 cm; length 5cm) to the e-cigarette. A similar hose coupled the trap and bottle together and eventually to a water jet pump.

Table 3. Overview of solvent used with grade, CAS and manufacturer.

Solvent	Grade	CAS	Manufacturer
Heptane	LCMS	142-82-5	Rathburn (Walkerburn Scotland)
Chloroform	HiPerSolv	67-66-3	VWR Chemicals (Radnor, PA, USA)
Ethyl acetate	AnalaR Normapur	141-78-6	VWR Chemicals (Radnor, PA, USA)

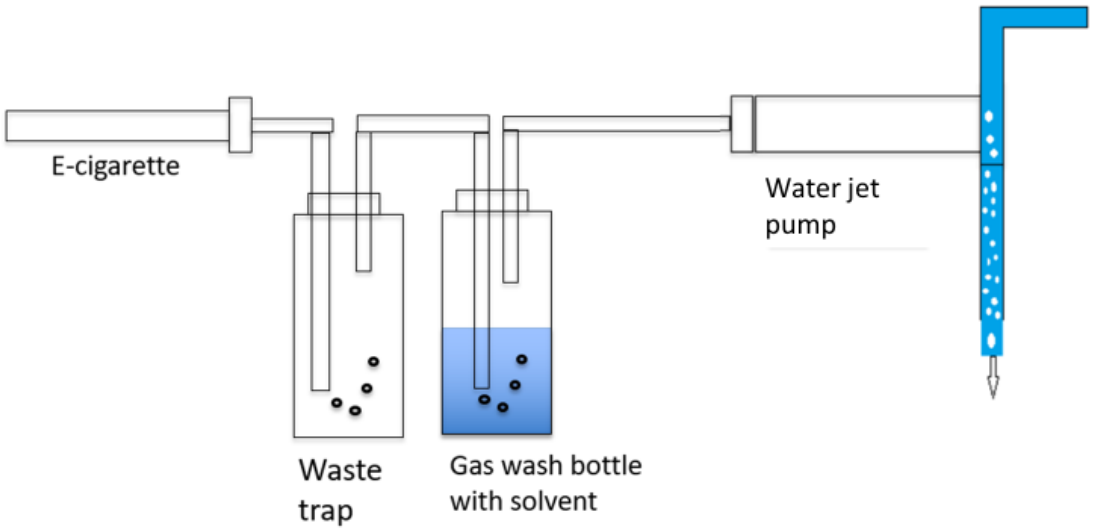


Figure 5. Sketch of used system. An aerosol trap with a waste chamber and a gas wash bottle coupled in series connected to a water jet pump

3.4 Sample preparation

After extraction, two samples of 30 mL solution were taken from the gas wash bottle. One sample was concentrated using Nitrogen gas, the other using an Syn-core evaporator (Büchi Syncore, vacuum pump V-700, vacuum controller V-855), to end volume 5 mL. The vacuum pump was set to 47 mBar (heptane), 207 mBar (chloroform), 95 mBar (ethyl acetate) and was used for 120 minutes, with 105 rpm stirring. Evaporation by nitrogen gas took approximately 90 minutes. The samples were then stored in a freezer at -20 ºC.

3.5 Blank solutions

Two solutions of each solvent; heptane, chloroform and ethyl acetate were analysed. From each solvent, one blank was made with ASTER filled with RB attached to the system, but not turned on. The other blank was made without ASTER attached. The solutions were then concentrated with vacuum and nitrogen gas with the same parameters as the other samples. Then the blanks were analysed on the same GC-MS under the same settings, Table 4, as the rest of the samples.

3.6 Degree of sensitivity

Four degrees of sensitivity was used referring to components. In increasing order of complexity

1. Tentatively identified: Identification with NIST library with match factor above 700, without an external standard.
2. Identified: The component was verified with an external standard with corresponding retention time and over 700 as match factor from NIST. The component has concentration under the limit of detection (<LOD).
3. Detected: A component has concentration between the limit of detection and the limit of quantification (<LOQ) with over 700 in match factor from NIST and corresponding retention time.
4. Quantified: A component has a concentration above the limit of quantification, inside the linear area, with over 700 in match factor from NIST and corresponding retention time.

3.6 GC-MS analysis

An Agilent 6890N GC coupled with a micromass AutoSpec Ultima MS system was used to qualitatively and quantitatively determine components in the trapped aerosol from electronic cigarettes. The MS was a sector instrument with EBE geometry and electron ionisation (EI). The sector instrument had full-scan mode enabled and was tuned to a resolution of 1000 with mass range 40-600 *m/z*. The temperatures in the ion source and transfer line were 250 $^{\circ}$C. The GC-MS system parameters are given in Table 4 with temperature gradients plotted in Figure 6.

The software used for GC-MS analysis was Masslynx 4.0 (Waters, Milford, MA, USA) and NIST 08 Mass Spectral Library (Gaithersburg, MD, USA) was used to identify components in the aerosol.

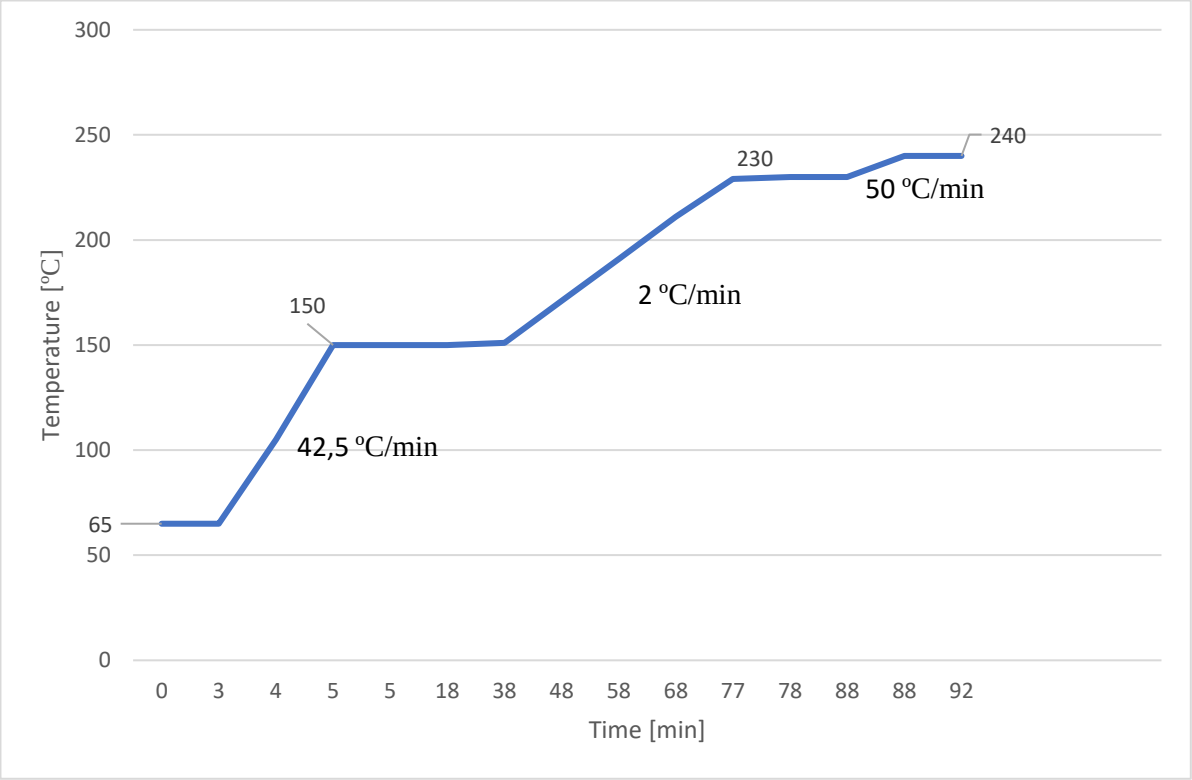


Figure 6. Temperature gradient in Agilent 6890N GC used for separation of components in e-cigarette aerosol

Table 4. GC-MS system parameters

GC-MS	**Manufacturer**	
GC	Agilent Technology, Wilmington, DE, USA	Agilent 6890N
Column	Restek Corporation, 255 Bellefonte, PA, USA	60 m Restek column (Rtx®-2330) with 0.25 mm I.D and 0.2 µm film thickness of fused silica 254 biscyanopropyl cyanopropylphenyl polysiloxane stationary phase
Injection	CTC 256 Analytics AG, Zwinger, Switzerland),	CTC PAL Autosampler
	Inj. Volume	1.0 µL at a split ratio of 1:10
	Inj. Temp	250 °C
Carrier gas	Yara, Rjukan, Norway; 99,9999%, Helium at a constant pressure of 95 kPa	
	Temperature gradients	Figure 6
MS detector	Miromass, AutoSpec – Ultima, Model: M629	
	Mode	Scan positive mode
	Transfer line temp.	250 °C
	Analyzer type	Sector instrument, EBE geometry
	Electron energy	70 eV
	Detector	Selectron multiplier
	Tune type	EBE
	Ionization mode	EI
	Range	40-600 *m/z*

3.7 Other settings and conditions for trapping vape

Three e-liquids, VG, EMP and UB, were tested with the same conditions, listed in Table 2, except for effect 23 W. VG, EMP and UB aerosols were vaped with 23 W. UB was additionally tested under two other temperature conditions -10 ºC and 0 ºC with 23 W. To trap aerosol below zero, an ethanol bath was used with a Heftofrig cooling bath (Heto Birkerød Danmark with 8 L ethanol). To use higher effect, the atomiser had to be switched, and settings on the battery were changed to 23 W.

One e-liquid, CV, was only tested under special conditions; 15 ºC and 18 W.

3.8 Standards

To identify, detect and quantify 34 of the tentatively identified components external standards were purchased from different suppliers listed in Table 5. To identify a component, 5 mg external standard was mixed with 5 mL heptane. 28 components were then mixed in one sample and 7 components in another, then evaluated with GC-MS. Hamilton syringes (700 series, Sigma-Aldrich, St. Louis, MO, USA) 50 µL and 100 µL and VWR disposable antistatic microspatulas were used with an analytical weight, Sartorius CP 2P (0-2,1 g, d = 0,005 mg). Four degrees of sensitivity were used, tentatively identified, identified, detected and quantified.

3.8.1 Calibration curves

The solution used to identify components were further diluted with heptane to give the concentrations found in Appendix II Table 15. Calibration curves were calculated after least square method. Outliers were identified and removed based on Dixon's Q-test Appendix III.

Table 5. Analytical standards used for identification, detection and quantification. Peak number (no) denotes components in descending order from most frequently identified in an aerosol to least frequent identified.

Peak no	Trivial name	IUPAC name	CAS no	Analytical grade	Manufacturer
1	Nicotine	3-[(2S)-1-methylpyrrolidin-2-yl]pyridine	23950-04-1	Analytical Standard	Sigma Aldrich (Munich, Germany)
2	Benzene, 1,3-bis(1,1-dimethylethyl)-	1,3-ditert-butylbenzene	1014-60-4	>97%	Sigma Aldrich (Munich, Germany)
4	Butylated hydroxytoluene	2,6-ditert-butyl-4-methylphenol	128-37-0		Sigma Aldrich (St. Louis, MO, USA)
6	Dodecyl acrylate	dodecyl prop-2-enoate	2156-97-0	Technical grade, 90%	Sigma Aldrich (Dorset, United Kingdom)
8	Linalool	3,7-dimethylocta-1,6-dien-3-ol	78-70-6	Pure	Koch-Light Laboratories LTD (Haverhill, United Kingdom)
9	gamma-Nonalactone	5-pentyloxolan-2-one	104-61-0	analytical reference material	Sigma Aldrich (St. Louis, MO, USA)
10	Acetic acid, phenylmethyl ester	benzyl acetate	140-11-4	Analytical Standard	Sigma Aldrich (Munich, Germany)
11	d-Limonene	(4R)-1-methyl-4-prop-1-en-2-ylcyclohexene	5989-27-5		Chemi-Teknik AS (Oslo, Norway)
15	Decane	Decane	124-18-5	Analytical Standard	Sigma Aldrich (Shanghai, China)
16	Heptacosane	Heptacosane	593-49-7	Analytical Standard	Sigma Aldrich (Buchs, Switzerland)

Table 5. Analytical standards used for identification, detection and quantification. Peak number (no) denotes components in descending order from most frequently identified in an aerosol to least frequent identified.

19	Triacetin	2,3-diacetyloxypropyl acetate	102-76-1	Analytical Standard	Sigma Aldrich (St. Louis, MO, USA)
20	gamma-Undecalactone	5-heptyloxolan-2-one	104-67-6	Analytical Standard	Sigma Aldrich (St. Louis, MO, USA)
22	beta-Damascone	(E)-1-(2,6,6-trimethylcyclohexen-1-yl)but-2-en-1-one	35044-68-9	technical >90%	Sigma Aldrich (Buchs, Switzerland)
23	2-Heptanone	Heptan-2-one	110-43-0	Analytical Standard	Sigma Aldrich (Shanghai, China)
24	delta-Decalactone	6-pentyloxan-2-one	705-86-2	Analytical Standard	Sigma Aldrich (Munich, Germany)
27	*cis*-3-hexenyl acetate	[(Z)-hex-3-enyl] acetate	3681-71-8	Analytical Standard	Sigma Aldrich (Tokyo, Japan)
28	Acetic acid, hexyl ester	hexyl acetate	142-92-7	Analytical Standard	Sigma Aldrich (Buchs, Switzerland)
29	*p*-Anisaldehyde	4-methoxybenzaldehyde	123-11-5	>98 %	Fluka AG (Oslo, Norway)
30	beta-Ionone	(E)-4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one	79-77-6	Analytical Standard	Sigma Aldrich (St. Louis, MO, USA)
34	Eucalyptol	2,2,4-trimethyl-3-oxabicyclo[2.2.2]octane	470-82-6	>98 %	Carl Roth KG (Karlsruhe, Germany)
42	Pulegone	(5R)-5-methyl-2-propan-2-ylidenecyclohexan-1-one	89-82-7	92 %	Acros Organics (Geel, Belgium)
64	3,4-Dimethoxybenzaldehyde	3,4-dimethoxybenzaldehyde	120-14-9	99 %	Aldrich (St. Louis, MO, USA)
66	3-Heptanone	Heptan-3-one	106-35-4	Analytical Standard	Sigma Aldrich (St. Louis, MO, USA)
73	alpha-Ionone	(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	127-41-3	Analytical Standard	Sigma Aldrich (St. Louis, MO, USA)

Table 5. Analytical standards used for identification, detection and quantification. Peak number (no) denotes components in descending order from most frequently identified in an aerosol to least frequent identified.

75	Benzaldehyde	Benzaldehyde	100-52-7	Analytical Standard	Sigma Aldrich (St. Louis, MO, USA)
77	Isovanillin	3-hydroxy-4-methoxybenzaldehyde	621-59-0	99 %	Aldrich (St. Louis, MO, USA)
82	Ethyl butyrate	Ethyl butanoate	105-54-4	Analytical Standard	Sigma Aldrich (Munich, Germany)
83	Butanoic acid, hexyl ester	Hexyl butanoate	2639-63-6	Analytical Standard	Sigma Aldrich (Toluca, Mexico)
84	Carvone	(5R)-2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one	6485-40-1	99+%	Acros Organics (Geel, Belgium)
101	Guaiacol	2-methoxyphenol	90-05-1	>98 %	Fluka AG (Oslo, Norway)
104	Hexanoic acid	hexanoic acid	142-62-1	Analytical Standard	Sigma Aldrich (St. Louis, MO, USA)
107	Hydrocoumarin	3,4-dihydrochromen-2-one	119-84-6	Analytical Standard	Sigma Aldrich (Shanghai, China)
111	Linalyl acetate	3,7-dimethylocta-1,6-dien-3-yl acetate	115-95-7	Analytical Standard	Sigma Aldrich (Shanghai, China)
119	Pentadecane	Pentadecane	629-62-9	Analytical Standard	Sigma Aldrich (Buchs, Switzerland)

3.8.2 Quantitative analyses

Peak areas were integrated with MassLynx™ 4.0 SCN530 software; these areas were used to calculate the concentration based on the calibration curves in Appendix I. Outliers were calculated with Dixon's Q-test (Appendix III).

3.8.3 LOD and LOQ

The signal to noise (S/N) was measured for each analytical standard and limit of detection (LOD), and limit of quantification (LOQ) was calculated using Equation 3 and Equation 4, respectively.

Equation 3.

$$LOD = 3\frac{[Analytical\ standard]}{S/N}$$

Equation 4.

$$LOQ = 10\frac{[Analytical\ standard]}{S/N}$$

3.9 E-liquid extraction

Two e-liquids were extracted using different solvents. 1 mL of RB and PL were extracted 1:10 with heptane and chloroform. PL was also tested with ethyl acetate. The solution was mixed using yellowline TTS 2 vortex mixer at 1800 rpm for 2 minutes. It was further centrifuged with Eppendorf Centrifuge 6430 R for 2 minutes at 4500 RCF. The solvent phase was transferred to GC-vials and analysed by GC-MS.

4. Results

A total of 129 different components (Table 6) were found in the aerosol samples from 11 different e-liquids (Table 1) vaped by the same e-cigarette (Aster Eleaf with Nubilant tank system). Of those 129 components, 126 were identified using NIST library with match factor over 700 and 30 were additionally identified with external standards. Three components were suggestivly identified with less than 700 in match factor, methyl dec-2-enoate, ethyl oleate and methyl hexanoate. 21 components were quantitatively determined.

4.1 Blanks

Blank samples without e-cigarette attached had no components tentatively identified.

The heptane trap with e-cigarette attached had four tentatively identified components, benzyl acetate, dodecyl acrylate, butylated hydroxytoluene and nicotine. In the chloroform trap with e-cigarette attached benzyl acetate, butylated hydroxytoluene and nicotine were tentatively identified. Ethyl acetate only revealed nicotine in the trap with e-cigarette attached.

Benzyl acetate, dodecyl acrylate and butylated hydroxytoluene were detected in blank samples. Neither had concentrations high enough for quantification (<LOQ) in any solvent trapped aerosol. The only significant peak was nicotine with a concentration of $5{,}5 \pm 4{,}6$ µg/mL in heptane and chloroform trapped aerosol.

4.2 Components

126 components had a NIST score over 700 and 113 had a score over 800. 56 of the identified components had a score over 900 in at least one sample. Nicotine was the only component found in all samples.

18 peaks were found in the aerosol sample of UB, 3 peaks were only tentatively identified in UB; alpha-terpineol, methyl cinnamate and methyleugenol. In Figure 7 a chromatogram of trapped aerosol from UB 11 W is shown, with identified peaks at corresponding RT. The chromatogram is scaled and includes only RT of interest, from 5-55 min. RT before 5 min includes only the solvent and retention time after 55 min had no peaks. There was no significant difference when increasing the effect from 11 W to 23 W.

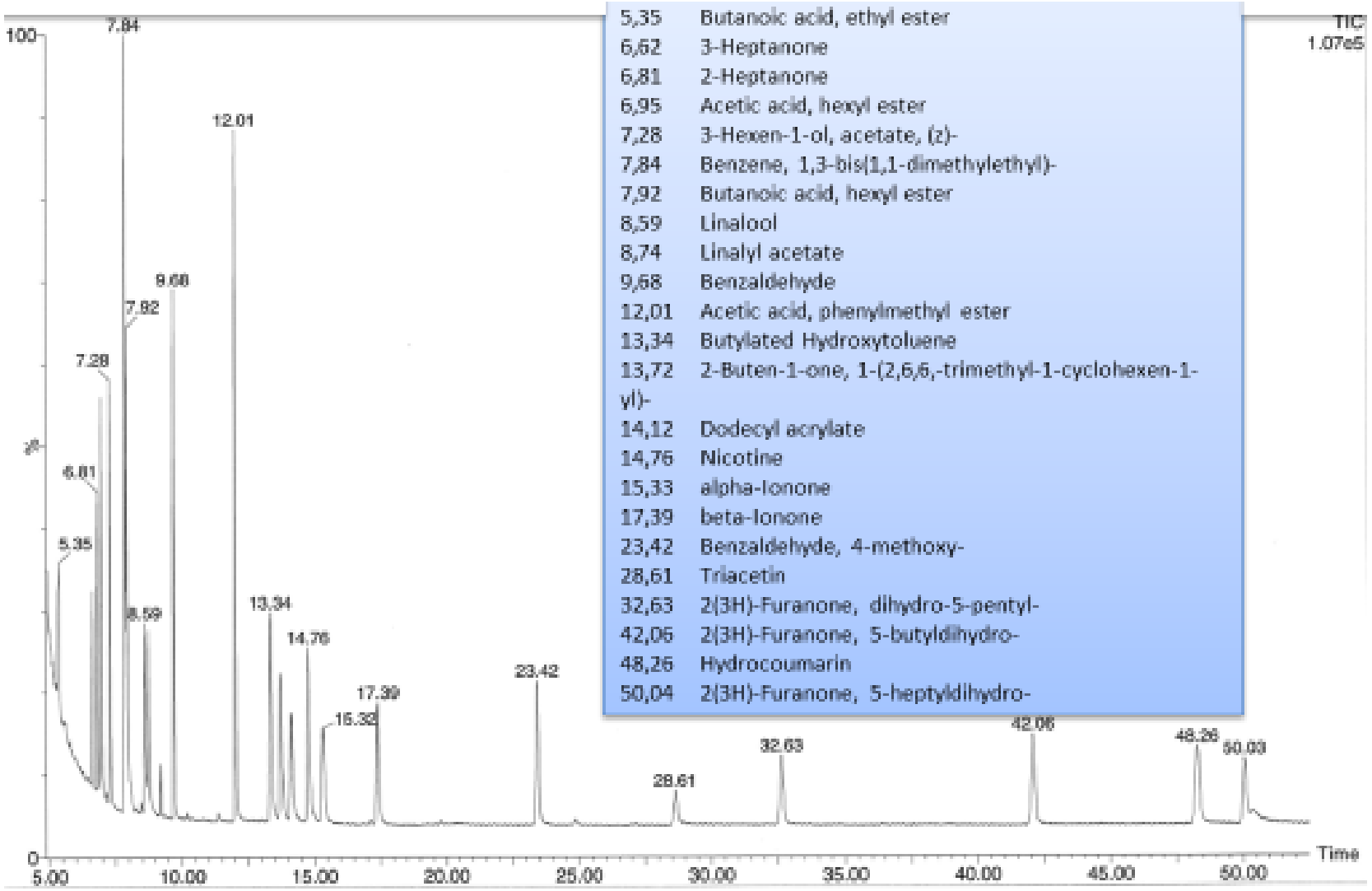


Figure 7. Chromatogram of 28 analytical standards with concentrations found in C1 Appendix II Table 15. 23 components identified with TIC and 5 components identified with RIC.

In EMP, only nicotine was found with 11 W. By changing the atomiser and output to 23 W, 3 additional peaks were detected: d-limonene, 2-heptanone and 1,3-ditert-butylbenzene.

From aerosol trap of BB 15 components were tentatively identified. 12 of the components were alkanes with chain lengths from C10 to C27.

From a total of 39 components in Peach 17 unique components were tentatively identified in the aerosol. 13 of these components were known food additives.

From aerosol samples of LM, 4 constituents were uniquely discovered, methyl dec-2-enoate, beta-citral, isomenthol and *O*-ccetylcitric acid triethyl ester

In trapped aerosol from RED, 6 unique components were tentatively identified from a total of 15 components found in the sample.

In PL aerosol 25 components were unique for this aerosol, all solvents were tested. Additionally, 5 components were tentatively identified with the liquid-liquid extraction of PL from all three solvents.

In VG aerosol 22 components were tentatively identified and 8 components were unique to the trapped aerosol.

One component was found only in CV, hydrocoumarin (107), and verified with an external standard.

In RB aerosol, 8 components were unique from a total of 24 components found.

In trapped aerosol from MT, 8 components were tentatively identified only in this sample, from a total of 27 components.

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed. Components are listed in decreasing order of times identified and given an appropriate number (NO) accordingly.

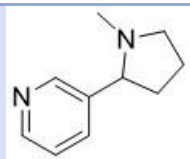
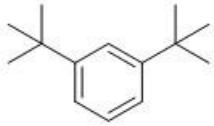
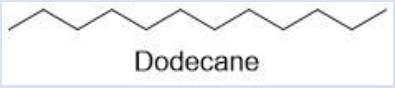
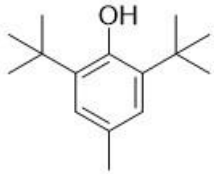
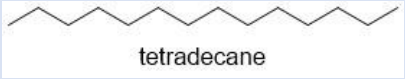
Peak no	Component	Synonym	IUPAC	CAS	Match	Probability	E-liquid	2D Structure
1	3-(1-Methyl-2-pyrrolidinyl)pyridine	Nicotine	3-[(2S)-1-Methylpyrrolidin-2-yl]pyridine	23950-04-1	958	80,40 %	All	 Nicotine
2	Benzene, 1,3-bis(1,1-dimethylethyl)-	1,3-Di-tert-butylbenzene	1,3-Ditert-butylbenzene	1014-60-4	936	80,70 %	UB, RB, MT, RED, EMP, CV, LM, BB and VG	 1,3-Di-tert-butylbenzene
3	Dodecane	N-Dodecane	Dodecane	112-40-3	948	29,00 %	RB, Peach, MT, RED, LM, VG, EMP, BB and UB	 Dodecane
4	Butylated hydroxytoluene	2,6-Di-tert-butyl-4-methylphenol	2,6-Ditert-butyl-4-methylphenol	128-37-0	944	76,10 %	RB, Peach, BB, MT, PL and VG	 2,6-ditert-butyl-4-methylphenol
5	Tetradecane	N-Tetradecane	Tetradecane	629-59-4	946	30,40 %	Peach, MT, VG, LM, EMP, BB and UB	 tetradecane

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

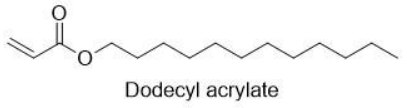
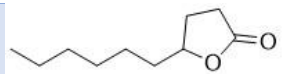
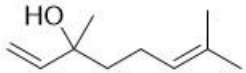
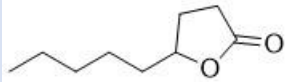
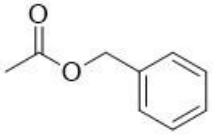
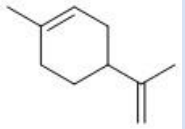
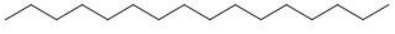
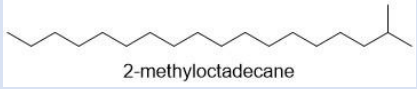
6	Dodecyl acrylate	-	Dodecyl prop-2-enoate	2156-97-0	942	62,40 %	CV, MT, RED, UB, RB and Peach	 Dodecyl acrylate
7	2(3H)-Furanone, 5-hexyldihydro-	gamma-Decalactone	5-Hexyloxolan-2-one	706-14-9	950	86,70 %	MT, PL, Peach, UB and LM	 5-hexyloxolan-2-one
8	Linalool	3,7-Dimethyl-1,6-octadien-3-ol	3,7-Dimethylocta-1,6-dien-3-ol	78-70-6	918	66,70 %	Peach, MT, PL, LM and UB	 Linalool
9	2(3H)-Furanone, dihydro-5-pentyl-	gamma-nonalactone	5-Pentyloxolan-2-one	104-61-0	913	61,90 %	RB, LM, CV and PL	 5-pentyloxolan-2-one
10	Acetic acid, phenylmethyl ester	-	Benzyl acetate	140-11-4	966	92,40 %	RB, Peach, MT and RED	 Benzyl Acetate
11	d-Limonene	Carvene	(4R)-1-Methyl-4-prop-1-en-2-ylcyclohexene	5989-27-5	934	25,80 %	MT, LM, EMP and UB	 D-Limonene
12	Hexadecane	N-Hexadecane	Hexadecane	544-76-3	953	36,50 %	Peach, LM, UB, VG	 hexadecane
13	Octadecane, 2-methyl-	-	2-Methyloctadecane	1560-88-9	861	7,42 %	Peach, RB, BB and RED	 2-methyloctadecane

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

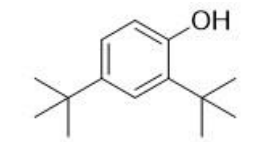
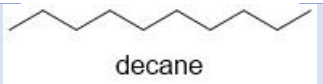
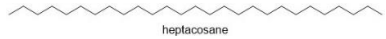
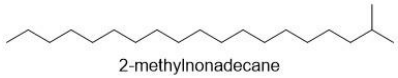
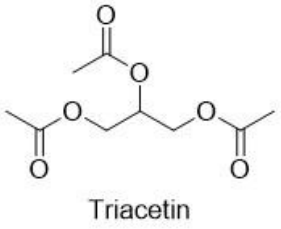
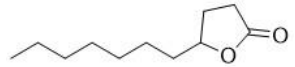
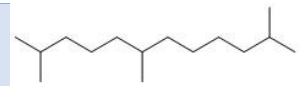
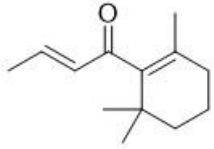
14	2,4-Di-tert-butylphenol	Phenol, 2,4-bis(1,1-dimethylethyl)-	2,4-Ditert-butylphenol	96-76-4	866	43,00 %	RED, LM and VG	 2,4-Di-tert-butylphenol
15	Decane	N-Decane	Decane	124-18-5	929	46,40 %	LM, BB and Peach	 decane
16	Heptacosane	N-Heptacosane	Heptacosane	593-49-7	860	6,57 %	VG, BB and Peach	 heptacosane
17	Nonadecane	Nonadekan	Nonadecane	629-92-5	850	15,70 %	MT. BB and UB	 nonadecane
18	Nonadecane, 2-methyl-	2-Methylnonadecane	2-methylnonadecane	1560-86-7	869	6,69 %	RB, BB and UB	 2-methylnonadecane
19	Triacetin	Glycerol triacetal	2,3-diacetyloxypropyl acetate	102-76-1	942	53,50 %	CV, Peach and RB	 Triacetin
20	2(3H)-Furanone, 5-heptyldihydro-	gamma-undecalactone	5-heptyloxolan-2-one	104-67-6	950	77,90 %	Peach and UB	 5-heptyloxolan-2-one
21	2,6,11-Trimethyldodecane	Dodecane, 2,6,11-trimethyl-	2,6,11-Trimethyldodecane	31295-56-4	875	7,59 %	LM and VG	 2,6,11-Trimethyldodecane
22	2-Buten-1-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	beta-Damascone	(E)-1-(2,6,6-Trimethylcyclohexen-1-yl)but-2-en-1-one	35044-68-9	914	78,50 %	RB and Peach	 beta-Damascone

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

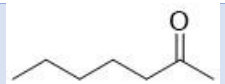
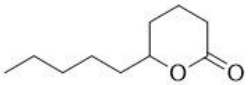
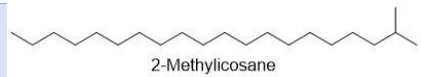
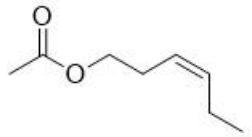
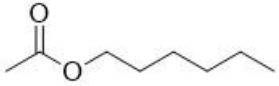
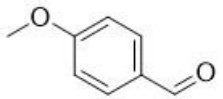
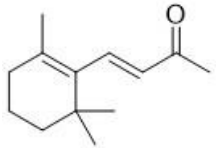
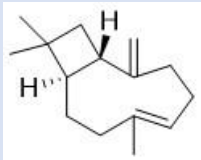
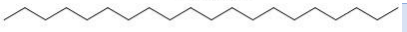
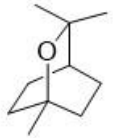
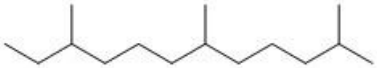
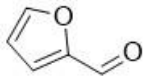
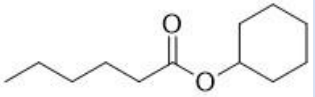
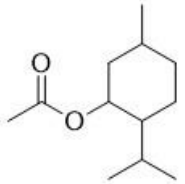
23	2-Heptanone	-	Heptan-2-one	110-43-0	936	69,80 %	Peach and EMP	 heptan-2-one
24	2H-Pyran-2-one, tetrahydro-6-pentyl-	delta-Decalactone	6-Pentyloxan-2-one	705-86-2	924	72,20 %	Peach and MT	 d-Decalactone
25	2-Methylicosane	Eicosane, 2-methyl-	2-Methylicosane	52845-08-6	868	6,68 %	LM and VG	 2-Methylicosane
26	2-Methylundecane	Undecane, 2-Methyl-	2-Methylundecane	7045-71-8	806	12,70 %	BB and Peach	 2-methylundecane
27	3-Hexen-1-ol, acetate, (z)-	*cis*-3-Hexenyl acetate	[(Z)-Hex-3-enyl] acetate	3681-71-8	866	27,00 %	RB and Peach	 [(Z)-hex-3-enyl] acetate
28	Acetic acid, hexyl ester	-	Hexyl acetate	142-92-7	962	93,40 %	RB and Peach	 hexyl acetate
29	Benzaldehyde, 4-methoxy-	*p*-Anisaldehyde	4-Methoxybenzaldehyde	123-11-5	906	65,50 %	PL and RB	 4-methoxybenzaldehyde
30	beta-Ionone	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	(E)-4-(2,6,6-Trimethylcyclohexen-1-yl)but-3-en-2-one	79-77-6	930	50,40 %	RB and Peach	 beta-Ionone

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

31	Caryophyllene	beta-Caryophyllene	(1R,4Z,9S)-4,11,11-Trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	87-44-5	871	12,40 %	MT and RED	 Caryophyllene
32	Docosane	Heneicosane	Docosane	629-97-0	918	20,50 %	BB and VG	 Docosane
33	Eicosane	-	Icosane	112-95-8	918	25,40 %	BB and VG	 icosane
34	Eucalyptol	Cineole	2,2,4-Trimethyl-3-oxabicyclo[2.2.2]octane	470-82-6	930	85,00 %	MT and PL	 Eucalyptol
35	Farnesane	Dodecane, 2,6,10-trimethyl-	2,6,10-Trimethyldodecane	3891-98-3	851	6,44 %	LM and RED	 2,6,10-trimethyldodecane
36	Furfural	2-Furancarboxaldehyde	Furan-2-carbaldehyde	98-01-1	868	59,00 %	Peach and PL	 Furfural
37	Hexanoic acid, cyclohexyl ester	-	Cyclohexyl hexanoate	6243-10-3	805	43,50 %	RB and MT	 cyclohexyl hexanoate
38	Isomenthol acetate	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate, (1.alpha.,2.beta.,5.beta.)-	[(1S,2R,5R)-5-Methyl-2-propan-2-ylcyclohexyl] acetate	20777-45-1	958	24,00 %	MT and LM	 Isomenthol acetate

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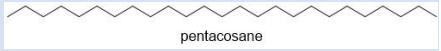
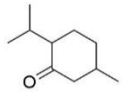
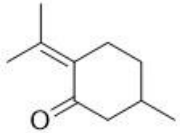
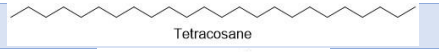
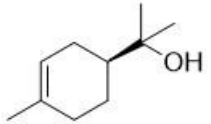
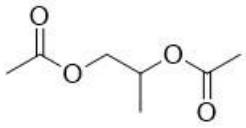
39	Pentacosane	Tetracosane, methyl-	Pentacosane	629-99-2	720	6,51 %	BB and VG	 pentacosane
40	*p*-Menthan-3-one	2-Isopropyl-5-methylcyclohexanone	5-Methyl-2-propan-2-ylcyclohexan-1-one	10458-14-7	954	28,10 %	MT and LM	 5-methyl-2-propan-2-ylcyclohexan-1-one
41	Propylene glycol	1,2-Propanediol	Propane-1,2-diol	57-55-6	750	55,40 %	MT and PL	Propylene glycol
42	Pulegone	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	(5R)-5-Methyl-2-propan-2-ylidenecyclohexan-1-one	89-82-7	909	32,00 %	MT and LM	 Pulegone
43	Tetracosane	Lignocerane	Tetracosane	646-31-1	800	4,42 %	BB and VG	 Tetracosane
44	(L)-alpha-Terpineol	(-)-alpha-Terpineol	2-[(1S)-4-Methylcyclohex-3-en-1-yl]propan-2-ol	98-55-5	700	29,00 %	PL	 (-)-alpha-Terpineol
45	1,2-Propanediol, 1-acetate	Propylene glycol 1-acetate	2-Hydroxypropyl acetate	627-69-0	914	93,20 %	Peach	1,2-Propanediol, 1-acetate
46	1,2-Propanediol, 2-acetate	Propylene glycol 2-acetate	1-Hydroxypropan-2-yl acetate	6214-01-3	858	84,50 %	Peach	 1,2-Propanediol, 2-acetate
47	1,2-Propanediol, diacetate	1,2-Diacetoxypropane	2-Acetyloxypropyl acetate	623-84-7	945	96,30 %	Peach	 2-acetyloxypropyl acetate

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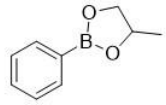
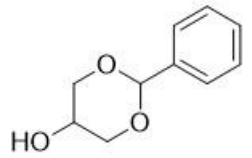
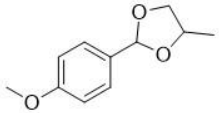
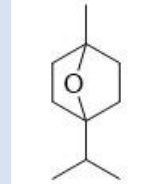
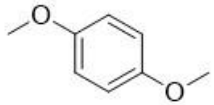
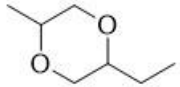
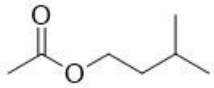
48	1,3,2-Dioxaborolane, 4-methyl-2-phenyl-	AC1LBWNZ	4-Methyl-2-phenyl-1,3,2-dioxaborolane	4406-75-1	905	92,20 %	Peach	 4-Methyl-2-phenyl-1,3,2-dioxaborolane
49	1,3-Dioxan-5-ol, 2-phenyl-	2-Phenyl-1,3-dioxan-5-ol	2-Phenyl-1,3-dioxan-5-ol	1708-40-3	800	77,80 %	PL	 2-Phenyl-1,3-dioxan-5-ol
50	1,3-Dioxolane, 2-(4-methoxyphenyl)-4-methyl-	Anisaldehyde propylene glycol acetal	2-(4-Methoxyphenyl)-4-methyl-1,3-dioxolane	6414-32-0	870	94,40 %	RB	 2-(4-methoxyphenyl)-4-methyl-1,3-dioxolane
51	1,4-Cineole	Isocineole	1-Methyl-4-propan-2-yl-7-oxabicyclo[2.2.1]heptane	470-67-7	927	77,60 %	PL	 1,4-Cineole
52	1,4-Dimethoxybenzene	*P*-Methoxyanisole	1,4-Dimethoxybenzene	150-78-7	900	80,30 %	PL	 1,4-dimethoxybenzene
53	1,4-Dioxane, 2-ethyl-5-methyl-	-	2-Ethyl-5-methyl-1,4-dioxane	53907-91-8	718	17,50 %	RB	 1,4-Dioxane, 2-ethyl-5-methyl-
54	1-Butanol, 3-methyl-, acetate	Isoamyl acetate	3-Methylbutyl acetate	123-92-2	885	79,30 %	Peach	 3-methylbutyl acetate

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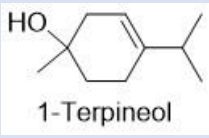
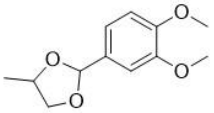
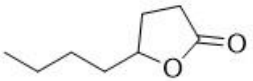
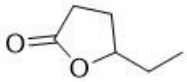
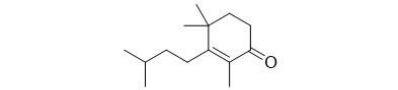
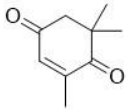
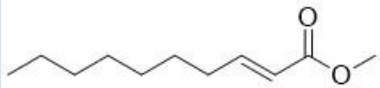
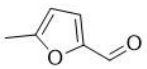
55	1-Terpineol	*P*-Menth-3-en-1-ol	1-Methyl-4-propan-2-ylcyclohex-3-en-1-ol	586-82-3	906	38,30 %	PL	 1-Terpineol
56	2-(3,4-Dimethoxyphenyl)-4-methyl-1,3-dioxolane	Veratraldehyde propylene glycol acetal	2-(3,4-Dimethoxyphenyl)-4-methyl-1,3-dioxolane	-	838	91,80 %	PL	 Veratraldehyde propylene glycol acetal
57	2(3H)-Furanone, 5-butyldihydro-	gamma-Octalactone	5-Butyloxolan-2-one	104-50-7	939	59,70 %	Peach	 5-butyloxolan-2-one
58	2(3H)-Furanone, 5-ethyldihydro-	gamma-Caprolactone	5-Ethyloxolan-2-one	695-06-7	932	69,00 %	PL	 gamma-Caprolactone
59	2,4,4-Trimethyl-3-(3-methylbutyl)cyclohex-2-enone	3-Isopentyl-2,4,4-trimethyl-2-cyclohexen-1-one	2,4,4-Trimethyl-3-(3-methylbutyl)cyclohex-2-en-1-one		734	34,70 %	VG	 2,4,4-trimethyl-3-(3-methylbutyl)cyclohex-2-en-1-one
60	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	4-Oxoisophorone	2,6,6-Trimethylcyclohex-2-ene-1,4-dione	1125-21-9	931	92,10 %	RED	 2,6,6-Trimethyl-2-cyclohexene-1,4-dione
61	2-Decanoic acid, methyl ester	Methyl dec-2-enoate	Methyl (E)-dec-2-enoate	2482-39-5	651	11,40 %	LM	 Methyl dec-2-enoate
62	2-Furancarboxaldehyde, 5-methyl-	5-Methylfurfural	5-Methylfuran-2-carbaldehyde	620-02-0	896	64,20 %	Peach	 5-methylfuran-2-carbaldehyde

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

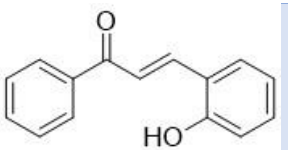
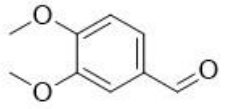
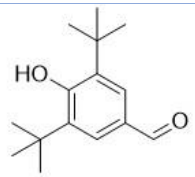
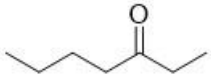
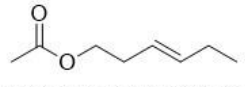
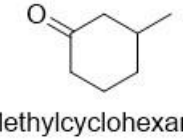
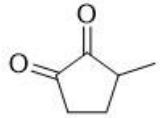
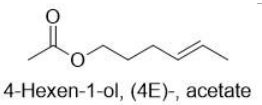
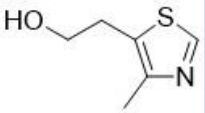
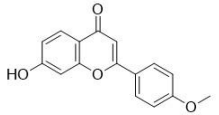
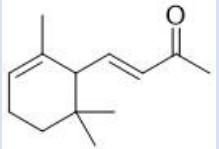
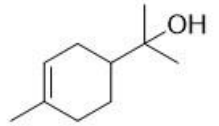
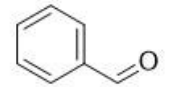
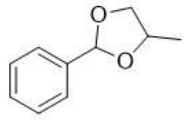
63	2-Hydroxychalcone	-	(E)-3-(2-Hydroxyphenyl)-1-phenylprop-2-en-1-one	644-78-0	918	36,90 %	PL	 2-Hydroxychalcone
64	Vanillin methyl ether	-	3,4-Dimethoxybenzaldehyde	120-14-9	936	91,70 %	PL	 Vanillin methyl ether
65	Benzaldehyde, 3,5-di-tert-butyl-4-hydroxy-	-	3,5-Ditert-butyl-4-hydroxybenzaldehyde	1620-98-0	862	67,40 %	VG	 3,5-ditert-butyl-4-hydroxybenzaldehyde
66	3-Heptanone	-	Heptan-3-one	106-35-4	835	44,90 %	Peach	 heptan-3-one
67	3-Hexen-1-ol, acetate, (E)-	*trans*-3-Hexenyl acetate	[(E)-hex-3-enyl] acetate	3681-82-1	788	14,30 %	MT	 3-Hexen-1-ol, acetate, (E)-
68	3-Methylcyclohexanone	Cyclohexanone, 3-methyl-	3-Methylcyclohexan-1-one	591-24-2	755	32,80 %	MT	 3-Methylcyclohexanone
69	3-Methylcyclopentane-1,2-dione	1,2-CYCLOPENTANEDIONE, 3-METHYL-	3-Methylcyclopentane-1,2-dione	765-70-8	918	42,70 %	PL	 3-Methylcyclopentane-1,2-dione

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

70	4-Hexen-1-ol, acetate	4-Hexen-1-ol, (4E)-, acetate	[(E)-Hex-4-enyl] acetate	72237-36-6	912	32,20 %	Peach	 4-Hexen-1-ol, (4E)-, acetate
71	5-Thiazoleethanol, 4-methyl-	Sulfurol	2-(4-Methyl-1,3-thiazol-5-yl)ethanol	137-00-8	839	93,80 %	PL	 Sulfurol
72	7-Hydroxy-4'-methoxyflavone	Pratol	7-Hydroxy-2-(4-methoxyphenyl)chromen-4-one	487-24-1	840	83,40 %	Peach	 7-hydroxy-2-(4-methoxyphenyl)chromen-4-one
73	alpha-Ionone	3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	127-41-3	923	70,80 %	RB	 alpha-Ionone
74	alpha-Terpineol	P-Menth-1-en-8-ol	2-(4-Methylcyclohex-3-en-1-yl)propan-2-ol	98-55-5	866	48,30 %	UB	 P-Menth-1-en-8-ol
75	Benzaldehyde	Benzoic aldehyde	Benzaldehyde	100-52-7	939	72,50 %	Peach	 Benzaldehyde
76	Benzaldehyde propylene glycol acetal	4-Methyl-2-phenyl-1,3-dioxolane	4-Methyl-2-phenyl-1,3-dioxolane	2568-25-4	903	91,10 %	PL	 Benzaldehyde propylene glycol acetal

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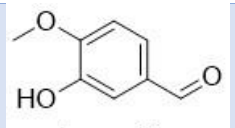
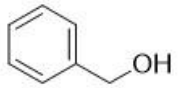
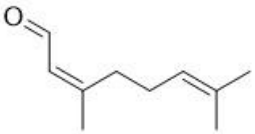
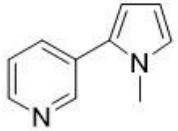
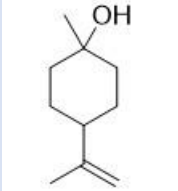
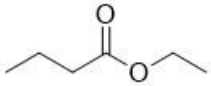
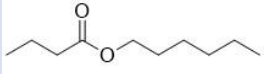
77	Benzaldehyde, 3-hydroxy-4-methoxy-	Isovanillin	3-Hydroxy-4-methoxybenzaldehyde	621-59-0	800	18,30 %	PL	 Isovanillin
78	Benzyl alcohol	-	Phenylmethanol	100-51-6	799	33,90 %	RED	 Benzyl Alcohol
79	beta-Citral	2,6-Octadienal, 3,7-dimethyl-(z)	(2Z)-3,7-Dimethylocta-2,6-dienal	5392-40-5	913	35,00 %	LM	 (2Z)-3,7-dimethylocta-2,6-dienal
80	beta-Nicotyrine	Nicotyrine	3-(1-Methylpyrrol-2-yl)pyridine	487-19-4	708	31,20 %	VG	 Nicotyrine
81	beta-terpineol	P-Menth-8-en-1-ol	1-Methyl-4-prop-1-en-2-ylcyclohexan-1-ol	138-87-4	818	40,00 %	PL	 Beta-terpineol
82	Butanoic acid, ethyl ester	Ethyl butyrate	Ethyl butanoate	105-54-4	891	89,20 %	Peach	 ethyl butanoate
83	Butanoic acid, hexyl ester	Hexyl butyrate	Hexyl butanoate	2639-63-6	956	79,80 %	Peach	 hexyl butanoate

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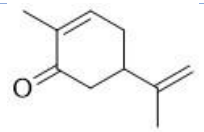
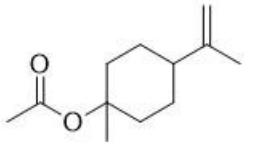
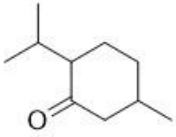
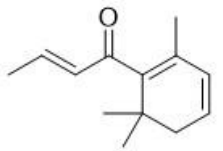
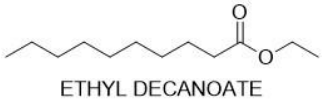
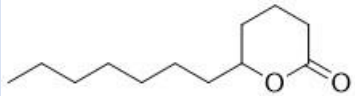
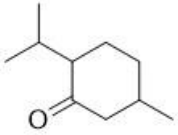
84	Carvone	-	(5R)-2-Methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one	6485-40-1	932	39,40 %	MT	 Carvone
85	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate	beta-Terpinyl acetate	(1-Methyl-4-prop-1-en-2-ylcyclohexyl) acetate	10198-23-9	923	20,70 %	Peach	 (1-methyl-4-prop-1-en-2-ylcyclohexy
86	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-*cis*)-	D-Isomenthone	(2R,5R)-5-Methyl-2-propan-2-ylcyclohexan-1-one	1196-31-2	954	27,50 %	MT	 D-isomenthone
87	Damascenone	beta-Damascenone	(E)-1-(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one	23696-85-7	834	74,90 %	RED	 Beta-Damascenone
88	Decanoic acid, ethyl ester	-	Ethyl decanoate	110-38-3	828	52,30 %	PL	 ETHYL DECANOATE
89	delta-Dodecalatone	Dodecan-5-olide	6-Heptyloxan-2-one	713-95-1	909	64,20 %	PL	 Delta-dodecalactone
90	d-Menthone	(+)-Menthone	(2R,5S)-5-Methyl-2-propan-2-ylcyclohexan-1-one	89-80-5	831	22,80 %	RED	 D-menthone

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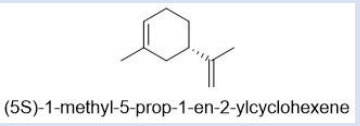
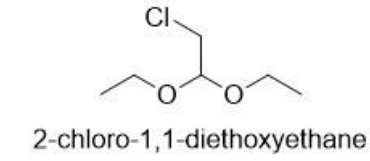
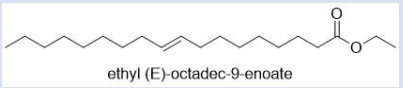
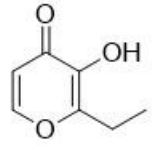
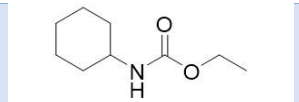
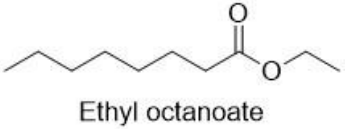
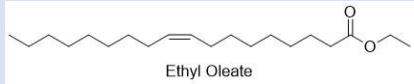
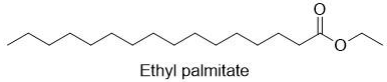
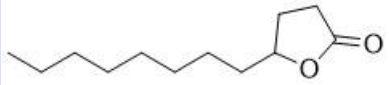
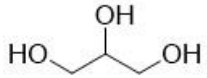
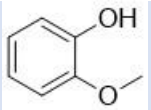
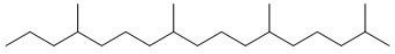
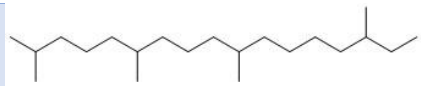
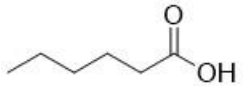
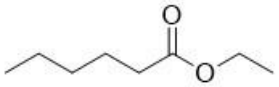
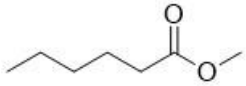
91	d-Sylvestrene	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, ®-	(5S)-1-Methyl-5-prop-1-en-2-ylcyclohexene	1461-27-4	820	14,70 %	MT	 (5S)-1-methyl-5-prop-1-en-2-ylcyclohexene
92	Ethane, 2-chloro-1,1-dietoxy-	Chloroacetaldehyde diethyl acetal	2-Chloro-1,1-diethoxyethane	621-62-5	847	97,10 %	PL	 2-chloro-1,1-diethoxyethane
93	Ethyl Elaidate	(E)-9-Octadecenoic acid ethyl ester	Ethyl (E)-octadec-9-enoate	6114-18-7	823	28,10 %	PL	 ethyl (E)-octadec-9-enoate
94	Ethyl maltol	2-Ethyl-3-hydroxy-4H-pyran-4-one	2-Ethyl-3-hydroxypyran-4-one	4940-11-8	904	92,60 %	PL	 2-Ethyl-3-hydroxy-4H-pyran-4-one
95	Ethyl N-cyclohexylcarbamate	-	Ethyl N-cyclohexylcarbamate	1541-19-1	762	78,70 %	RB	 ethyl N-cyclohexylcarbamate
96	Ethyl octanoate	Octanoic acid, ethyl ester	Ethyl octanoate	106-32-1	863	86,60 %	PL	 Ethyl octanoate
97	Ethyl oleate	Oleic acid ethyl ester	Ethyl (Z)-octadec-9-enoate	111-62-6	699	22,10 %	VG	 Ethyl Oleate
98	Ethyl palmitate	Hexadecanoic acid, ethyl ester	Ethyl hexadecanoate	628-97-7	889	83,40 %	VG	 Ethyl palmitate

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

99	gamma-Dodecalactone	4-Dodecanolide	5-Octyloxolan-2-one	2305-05-7	885	50,30 %	PL	 Gamma-dodecalactone
100	Glycerin	Glycerol	Propane-1,2,3-triol	56-81-5	842	89,10 %	PL	 Glycerol
101	Guaiacol	-	2-Methoxyphenol	90-05-1	872	57,50 %	RED	 Guaiacol
102	Heptadecane, 2,6,10,14-tetramethyl-	-	2,6,10,14-Tetramethylheptadecane	18344-37-1	890	4,85 %	VG	 2,6,10,14-tetramethylheptadecane
103	Heptadecane, 2,6,10,15-tetramethyl-	-	2,6,10,15-Tetramethylheptadecane	54833-48-6	879	7,58 %	RB	 2,6,10,15-tetramethylheptadecane
104	Caproic acid	-	Hexanoic acid	142-62-1	805	NA	RB	 hexanoic acid
105	Hexanoic acid, ethyl ester	Ethyl caproate	Ethyl hexanoate	123-66-0	900	78,00 %	PL	 Hexanoic acid, ethyl ester
106	Hexanoic acid, methyl ester	-	Methyl hexanoate	106-70-7	699	36,10 %	PL	 methyl hexanoate

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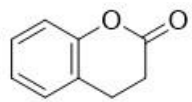
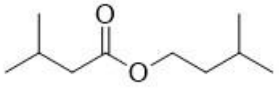
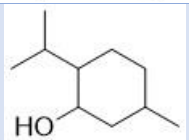
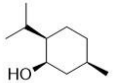
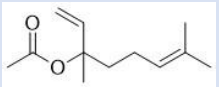
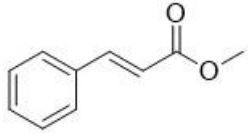
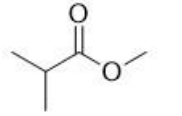
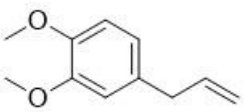
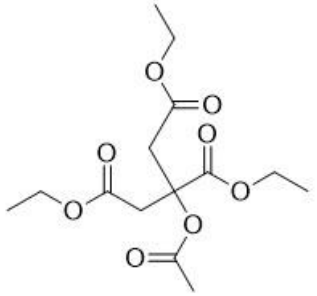
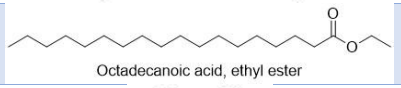
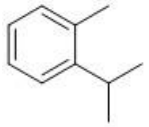
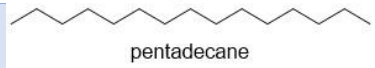
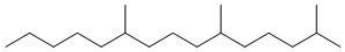
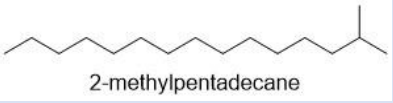
107	Hydrocoumarin	-	3,4-Dihydrochromen-2-one	119-84-6	876	51,60 %	CV	 3,4-dihydrochromen-2-one
108	Isoamyl Isovalerate	-	3-Methylbutyl 3-methylbutanoate	659-70-1	942	61,10 %	PL	 3-methylbutyl 3-methylbutanoate
109	Isomenthol	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5β)-	(1S,2R,5R)-5-Methyl-2-propan-2-ylcyclohexan-1-ol	23283-97-8	882	25,90 %	LM	 Isomenthol
110	Isoneomenthol	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	(1R,2R,5R)-5-Methyl-2-propan-2-ylcyclohexan-1-ol	491-02-1	772	18,70 %	RED	 (1R,2R,5R)-5-methyl-2-propan-2-ylcyclohexan-1-ol
111	Linalyl acetate	Linalol acetate	3,7-Dimethylocta-1,6-dien-3-yl acetate	115-95-7	842	28,60 %	Peach	 Linalyl acetate
112	Methyl cinnamate	2-Propenoic acid, 3-phenyl-, methyl ester	Methyl (E)-3-phenylprop-2-enoate	103-26-4	912	68,80 %	UB	 Methyl cinnamate
113	Methyl isobutyrate	-	Methyl 2-methylpropanoate	547-63-7	820	78,20 %	PL	 Methyl isobutyrate

Table 6. 129 different components tentatively identified with GC-MS and NIST library. Their IUPAC name, CAS registration number, match factor from NIST, probability from NIST and in which e-cigarette aerosol the components were identified is listed.

114	Methyleugenol	Eugenol methyl ether	1,2-Dimethoxy-4-prop-2-enylbenzene	93-15-2	864	41,20 %	UB	 Methyleugenol
115	Neopentyl glycol	-	2,2-Dimethylpropane-1,3-diol	126-30-7	845	17,40 %	PL	 Neopentyl Glycol
116	O-Acetylcitric acid triethyl ester	ATEC	Triethyl 2-acetyloxypropane-1,2,3-tricarboxylate	77-89-4	709	70,30 %	LM	 O-Acetylcitric acid triethyl ester
117	Octadecanoic acid, ethyl ester	Ethyl stearate	Ethyl octadecanoate	111-61-5	862	76,40 %	PL	 Octadecanoic acid, ethyl ester
118	*O*-Cymene	2-Isopropyltoluene	1-Methyl-2-propan-2-ylbenzene	527-84-4	906	25,90 %	PL	 O-Cymene
119	Pentadecane	N-Pentadecane	Pentadecane	629-62-9	853	7,07 %	Peach	 pentadecane
120	Pentadecane, 2,6,10-trimethyl-	-	2,6,10-Trimethylpentadecane	3892-00-0	878	5,23 %	VG	 2,6,10-Trimethylpentadecane
121	Pentadecane, 2-methyl-	-	2-Methylpentadecane	1560-93-6	858	4,94 %	VG	 2-methylpentadecane

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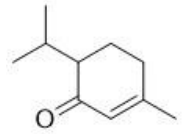
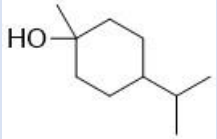
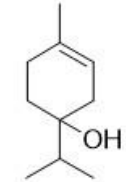
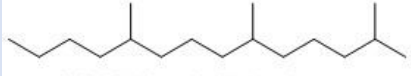
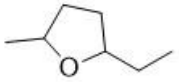
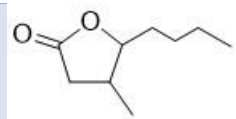
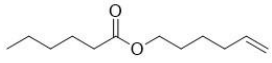
122	Piperitone	d-Piperitone	(6S)-3-Methyl-6-propan-2-ylcyclohex-2-en-1-one	6091-50-5	859	53,80 %	MT	 Piperitone
123	*p*-Menthan-1-ol	Cyclohexanol, 1-methyl-4-(1-methylethyl)-	1-Methyl-4-propan-2-ylcyclohexan-1-ol	21129-27-1	939	13,80 %	MT	 p-menthan-1-ol
124	Terpinen-4-ol	*p*-Menth-1-en-4-ol	4-Methyl-1-propan-2-ylcyclohex-3-en-1-ol	562-74-3	814	36,00 %	PL	 Terpinen-4-ol
125	Tetradecane, 2,6,10-trimethyl-	-	2,6,10-Trimethyltetradecane	14905-56-7	788	7,84 %	VG	 2,6,10-trimethyltetradecane
126	Tetrahydrofuran, 2-ethyl-5-methyl-	-	2-Ethyl-5-methyloxolane	931-39-5	842	37,70 %	Peach	 2-ethyl-5-methyloxolane
127	Whiskey lactone	*cis*-3-Methyl-4-octanolide	5-Butyl-4-methyloxolan-2-one	39212-23-2	705	10,40 %	MT	 Whiskey lactone
128	5-Hexenyl hexanoate	Hexanoic acid, 5-hexenyl ester	Hex-5-enyl hexanoate	108058-81-7	800	15,30 %	RB	 5-hexenyl hexanoate

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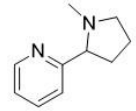
129	alpha-Nicotine	*o*-Nicotine	2-(1-Methylpyrrolidin-2-yl)pyridine	23950-04-1	866	56,20 %	RB		 2-(1-methylpyrrolidin-2-yl)pyridine	

4.3 LOD and LOQ

All LOD and LOQ values were in µg/mL heptane trapped aerosol range. LOD and LOQ were calculated using Equation 3 and Equation 4 and presented in Table 7.

Four components, decane, heptacosane, isovanillin and pentadecane were not detected (ND) in chromatograms of analytical standards, therefore LOD, LOQ and linear regression were not calculated for these components.

Table 7. LOD and LOQ for analytical standards in aerosol from e-cigarettes. All values were in range µg/mL solvent.

NO	Trivial name	LOD [µg/mL]	LOQ [µg/mL]
15	Decane	ND	ND
82	Ethyl butyrate	0,9	3,1
11	d-Limonene	0,2	0,7
34	Eucalyptol	0,1	0,5
66	3-Heptanone	0,2	0,6
23	2-Heptanone	0,1	0,3
28	Hexyl acetate	0,2	0,7
27	*cis*-3-Hexenyl acetate	0,1	0,4
2	1,3-Ditert-butylbenzene	0,1	0,2
83	Hexyl butanoate	0,1	0,2
8	Linalool	0,4	1,3
111	Linalyl acetate	0,2	0,8
75	Benzaldehyde	0,04	0,12
16	Heptacosane	ND	ND
42	Pulegone	0,2	0,6
10	Benzyl acetate	0,1	0,2
84	Carvone	0,3	0,9
4	Butylated hydroxytoluene	0,1	0,3
22	beta-Damascone	0,6	2,0
6	Dodecyl acrylate	0,4	1,4
101	Guaiacol	0,8	2,7
1	Nicotine	0,2	0,7
1	Nicotine (Subtracted blank)	0,2	0,7
104	Hexanoic acid	ND	ND
73	alpha-Ionone	0,4	1,2
30	beta-Ionone	0,2	0,6
29	*p*-Anisaldehyde	0,2	0,7
19	Triacetin	0,4	1,2
9	gamma-Nonalactone	0,5	1,6
107	Hydrocoumarin	0,3	0,9
24	delta-Decalactone	0,2	0,8
20	gamma-Undecalactone	0,3	1,1
119	Pentadecane	0,5	1,6
64	3,4-Dimethoxy-benzaldehyde	0,4	1,2
77	Isovanillin	ND	ND

4.4 Quantification

The external standards in Table 5 were analysed with different concentrations to make calibration curves (Appendix I). Concentrations corresponding to areas outside the linear range could not be determined with linear regression models. Peach was therefore further diluted 1:2 with heptane and CV was diluted 1:5.

If not otherwise specified, aerosol concentrations are calculated using heptane traps.

From 34 tentative identified components 30 components were identified in at least one of the trapped aerosols. 21 components were further quantified in one or more trapped aerosols. Table 8 shows identified, detected or quantified components in heptane trapped e-cigarette aerosol.

*Table 8. Identified, detected or quantified components in heptane trapped aerosol. The concentration of trapped aerosols in heptane were all in µg/mL range. Sorted after components RT. Concentrations with * are calculated using a chloroform trap.*

NO	Trivial name	PL [µg/mL]	RED [µg/mL]	Peach [µg/mL]	BB [µg/mL]	MT [µg/mL]	VG [µg/mL]	LM [µg/mL]	UB [µg/mL]	EMP [µg/mL]	RB [µg/mL]	CV [µg/mL]	Standard deviation [µg/mL]
82	Ethyl butyrate			15,3									1,5
11	d-Limonene					<LOD		29,0	31,5	6,5			3,9
34	Eucalyptol	<LOD				<LOD							5,1
66	3-Heptanone			<LOD									1,9
23	2-Heptanone			<LOD						<LOD			1,8
28	Hexyl acetate			<LOD							9,7		2,2
27	*cis*-3-Hexenyl acetate			7,7							15,4		1,8
2	1,3-Ditert-butylbenzene		3,3		3,2	6,1	2,1	27,9	3,1	3,9	3,4	2,8	1,0
83	Hexyl butanoate			3,5									1,3
8	Linalool	<LOD		11,1		<LOQ		36,7	<LOQ				4,3
111	Linalyl acetate			2,9	<LOQ								1,1
75	Benzaldehyde			1,4									0,8
42	Pulegone					<LOD		10,0					1,9
10	Benzyl acetate		<LOD	7,2		<LOD					262,1		5,3
84	Carvone					<LOD							2,1
4	Butylated hydroxytoluene	2,5*		3,2	26,6	1,2	2,2				1,2	0,8	0,1
22	beta-Damascone			<LOD							10,5		1,1
6	Dodecyl acrylate		<LOD	3,3		<LOQ			<LOD		<LOQ	<LOD	1,5
101	Guaiacol		<LOD										5,1

Table 8. Identified, detected or quantified components in heptane trapped aerosol. The concentration of trapped aerosols in heptane were all in µg/mL range. Sorted after components RT.

1	Nicotine	6,4	37,8	413,4	314,9	134,1	133,0	309,0	79,8	193,2	140,1	313,0	4,6
1	Nicotine (Adjusted for blank)	<LOD	32,3	403,2	309,4	128,6	127,5	303,5	74,3	187,7	134,6	307,5	4,6
73	alpha-Ionone										24,8		3,0
30	beta-Ionone			1,1							8,5		0,2
29	*p*-Anisaldehyde	<LOD									<LOQ		0,6
19	Triacetin			10,4							2,9	123,8	0,7
9	gamma-Nonalactone	5,4						<LOQ			<LOD	39,4	1,9
107	Hydrocoumarin											<LOD	1,7
24	delta-Decalactone			21,4		2,2							0,3
20	gamma-Undecalactone			4,3					1,7				0,4
119	Pentadecane			<LOD	<LOD								3,3
64	3,4-Dimethoxybenzaldehyde	<LOD											2,5

d-limonene was quantified in LM, UB and EMP, and identified in MT. Concentrations of d-limonene is presented in Figure 8.

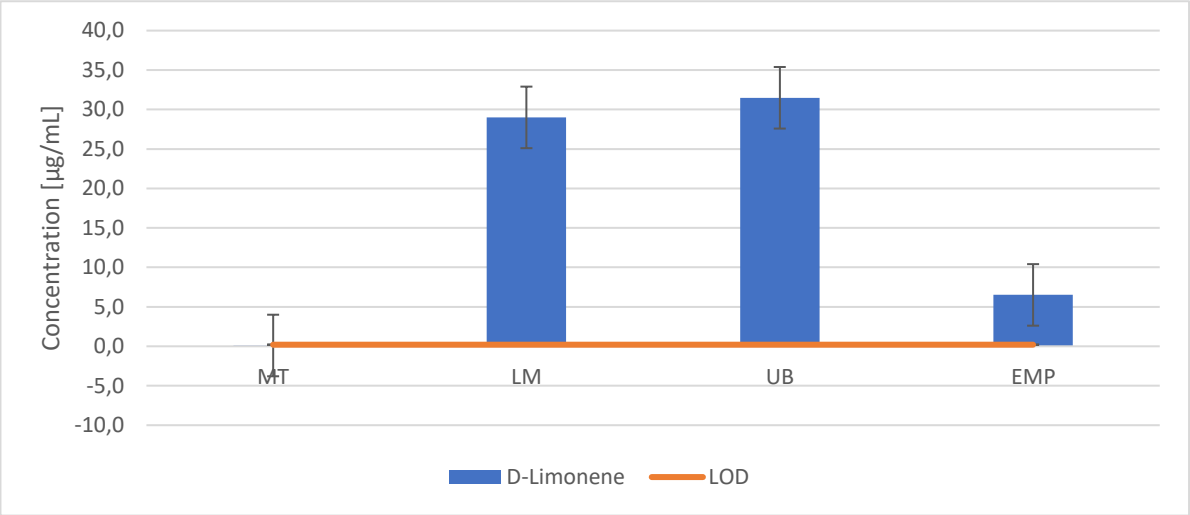


Figure 8. Bar graph of d-limonene in trapped aerosolised e-liquid with LOD and standard deviation, n = 3.

Bar graph of 1,3-ditert-butylbenzene, identified, detected and quantified in 9 different aerosols. Figure 9, present the concentration with standard deviation ±1,0 µg/mL and LOD at 0,1 µg/mL.

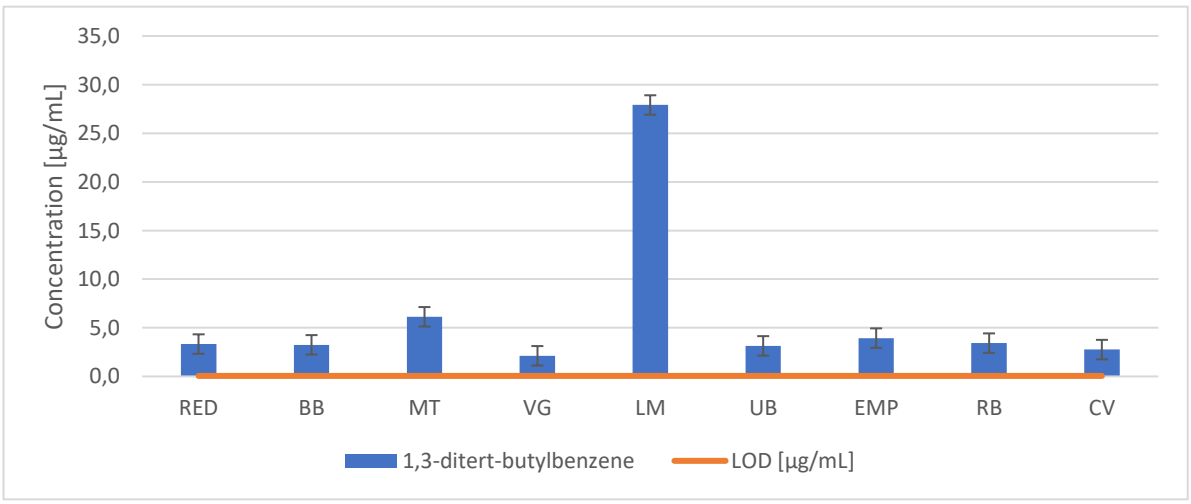


Figure 9. Bar graph of 1,3-ditert-butylbenzene in trapped aerosolised e-liquid with LOD and standard deviation, n = 3.

Dodecyl acrylate was quantified in Peach, detected and identified in five other aerosols shown in Figure 10.

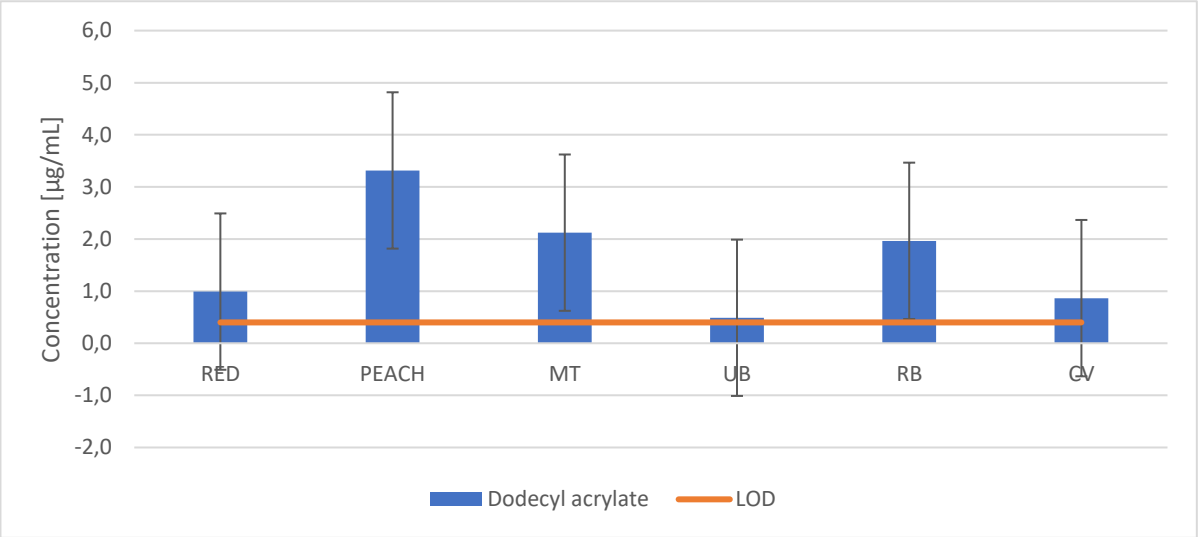


Figure 10. Bar graph of dodecyl acrylate in trapped aerosolised e-liquid with LOD and standard deviation n = 3.

Nicotine was quantified in all aerosols. After correction of possible carry-over from blank samples, nicotine was quantified in every aerosol except PL, shown in Figure 11.

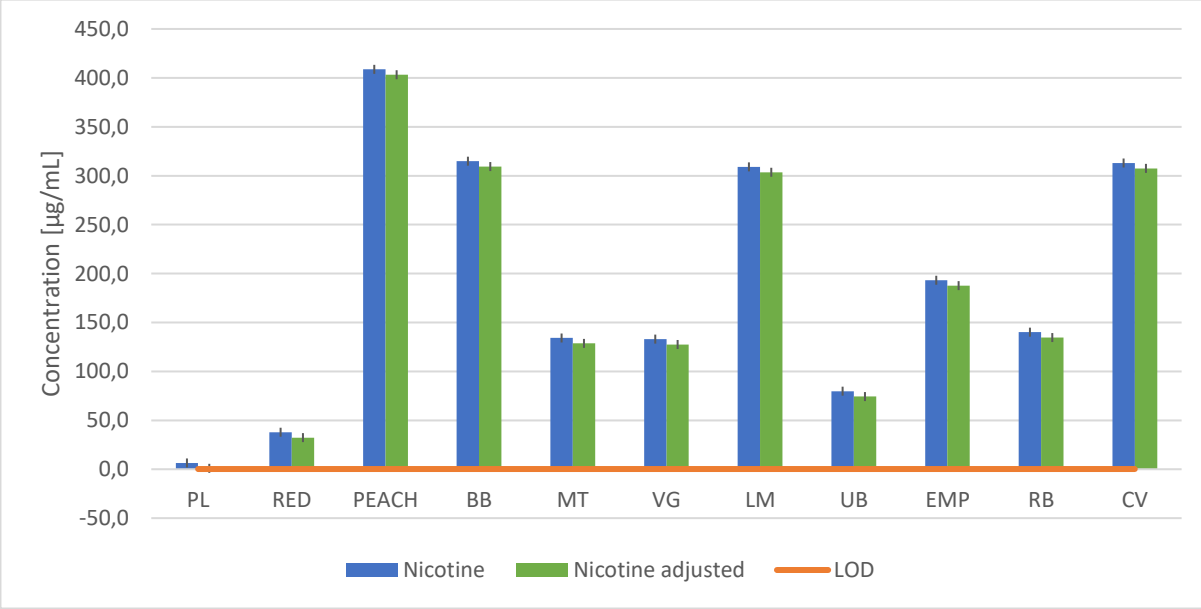


Figure 11. Bar graph of nicotine and nicotine adjusted for the blank, in trapped aerosolised e-liquid with LOD and standard deviation, n = 3.

Butylated hydroxytoluene was quantified in 6 heptane trapped aerosols and one chloroform trapped aerosol, with concentrations shown in Figure 12.

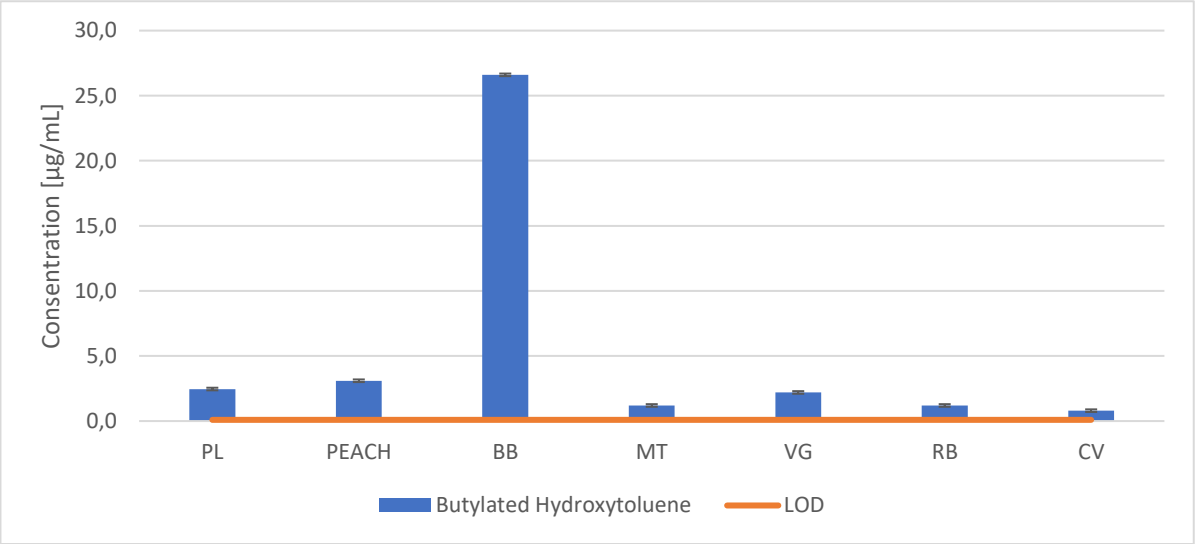


Figure 12. Bar graph of butylated hydroxytoluene in heptane trapped aerosol with LOD and standard devation, n = 3. In PL butylated hydroxytoluene was trapped using chloroform trap.

5. Discussion

With the objective of identifying the nonpolar components in the aerosol generated by e-cigarettes, a method to trap the aerosol was developed. This method was originally meant to trap aerosol to perform a screening of nonpolar component in the trap, that further evolved to a quantification method. The method was tested at three temperatures, -10 ºC, 0 ºC and room temperature with UB. Room temperature was chosen as the temperature to trap aerosol due to more realistic aerosolization patterns. The puff frequency and length do not represent natural vape patterns, it is ideally a method to quantify the maximum concentration of nonpolar constituent in the aerosol from 30 puffs with 10 second duration. The method will be further discussed in terms of blanks, LOD/LOQ, and which solvents that were used. Then components found will be discussed as a potential health risk both alone and in a mixture.

5.1 Blanks

Blank solutions without the e-cigarette attached indicate that no interference or carry-over was observed from the gas wash bottle or in the air. The components present in the blanks with the e-cigarette attached might come from e-liquid not vaporised or carry-over from the hoses.

Since the e-cigarette was attached during aerosol sampling, some contamination was inevitable. The main contaminant is nicotine $5{,}5 \pm 4{,}6$ µg/mL in heptane and chloroform trapped aerosol; the other contaminants were under LOQ in all solvents. The standard deviation from nicotine $\pm 4{,}6$ µg/mL was a result of the diverse range of nicotine concentration. Nicotine was present in every e-liquid and aerosol tested, even in PL which was said to be nicotine free. With the correction of possible nicotine carry-over, nicotine was merely identified (<LOD), in PL. The carry-over of nicotine was determined before the trap was introduced to the system and might, therefore, be overestimated.

5.2 LOD and LOQ

Four components, decane, heptacosane, isovanillin and hexanoic acid, were not detected in external standard solutions. Co-elution can be a reason for not detecting these compounds. RIC was performed selecting several main *m/z* from these components, to test if co-elution was a problem. With RIC, decane was identified, but only in the highest concentration of the external standard. For the other concentrations, decane was not detected so the linear regression model, LOD and LOQ were not possible to calculate. Since decane was confirmed in one standard solution, it can be used to identify decane in trapped aerosol samples. Using RIC, heptacosane

was identified in the two highest concentrations. The peaks were not consistent with the tentative identified component in the trapped aerosol. Heptacosane was identified at RT 50,34 in external standard, but in trapped aerosol samples, it was suggested tentative identified at RT 8,92 by NIST library in VG. Since these RT's are different heptacosane was tentatively identified. Isovanillin and hexanoic acid were not detected in any external standard sample, with neither TIC nor RIC, they remain tentative identified.

5.3 Solvents

The nonpolar solvent heptane with dielectric constant 1,9 (VEGA 2018) was tested on every e-liquid. Chloroform with higher polarity (dielectric constant 4,8) was tested to trap aerosol from RB, Peach, MT, PL and VG. PL aerosol was also trapped with the even more polar solvent ethyl acetate (dielectric constant 6,0) (Murov 1998; Snyder 1974). The different solvents with their distinct polarity might trap different components. For nonpolar components all solvents are miscible (NCBI 2018b; NCBI 2018c; NCBI 2018e). Ethyl acetate did not trap any unique components in PL.

5.4 Components

All components identified on any level were discovered using least one of the solvents heptane, chloroform and ethyl acetate. If not otherwise specified, it will be the heptane trapped aerosol that is discussed. The heptane trap is used throughout the study and tested with all e-liquids.

Components will be discussed after their chemical group, alcohols, aldehydes and ketones, alkaloids, alkanes, esters, terpenes and other components. Further to be addressed will be the most common components and then the unique components in each trapped aerosol will be addressed. Every linear regression model, used to qualitatively determine concentrations, are explained further in Appendix I.

5.4.1. Alcohols

Seventeen different alcohols were collected and tentatively identified in trapped aerosol from e-cigarettes. Alcohols are added to e-liquids to change its taste, odour and volatility.

Butylated hydroxytoluene (Peak no **4**, Table 6) is often used as an additive to prevent autoxidation and formation of free radicals. Quantified in 7 trapped aerosols in the range $0{,}8 \pm 0{,}1$ µg/mL to $26{,}6 \pm 0{,}1$ µg/mL. Butylated hydroxytoluene had the highest concentration in the heptane traps for all aerosols except PL where the chloroform trap had the highest concentration. This compound was classified as not carcinogenic by WHO in 1987. Butylated

hydroxytoluene has been suggested unstable in both soil and water samples and studies have found over ten degradation products, where 3,5-di-tert-butyl-4-hydroxybenzaldehyde (**65**) was one of them (Fries & Püttmann 2002; Inui *et al.* 1979a; Inui *et al.* 1979b; Mikami *et al.* 1979a; Mikami *et al.* 1979b; Nieva-Echevarría *et al.* 2015). 3,5-di-tert-butyl-4-hydroxybenzaldehyde was tentatively identified in trapped aerosol from VG and might originate from butylated hydroxytoluene.

Linalool (**8**) has a pleasant scent and often occur in conjunction with linalyl acetate. Linalool was quantified with a concentration of $36{,}7 \pm 4{,}3$ μg/mL in LM, $11{,}1 \pm 4{,}3$ μg/mL in Peach and identified and detected in PL, MT and UB. Linalool is suspected allergen and can cause severe skin irritations (Lewis 2005) in higher concentration than obtained as an odour additive.

2,4-ditert-butylphenol (**14**) a known food additive (Nemoto *et al.* 2001), used as an anti-oxidation agent and reported as skin and eye irritating in animal studies (Varsha *et al.* 2015). It was tentatively identified in trapped aerosols of RED, LM and VG.

PG (**41**) was tentatively identified in MT and PL chloroform trapped aerosol. Since PG is one of the main constituents in e-liquids, it is natural to think it would be identified in more than two aerosols. According to the list of ingredients, Table 1, MT contained 50 % PG and PL 40 % PG, in comparison Peach and BB contained 70 %, but PG was not identified on any level in neither aerosol. PG has very polar attributes, which can be the reason for this compound to only appear in two trapped aerosols.

Alpha-terpineol (**74**), the most occurring structural isomer (65 %) of terpineol, was tentatively identified in UB. Other isomers of terpineol are 1-terpineol (**55**), beta-terpineol (**81**) and terpinen-4-ol (**124**), also tentatively identified in PL. These terpineols are food flavouring ingredient with fresh, pleasant minty odour (NCBI 2018f; Terpineol 2018). Six other alcohols, shown in Table 9, are known food additives with no toxic properties to humans

Table 9. Tentatively identified alcohols in different trapped aerosol, denoted e-liquids, and their application.

Peak no	Trivial name	E-liquid	Attribute
49	2-phenyl-1,3-dioxan-5-ol	PL	Flavouring agent
78	Benzyl alcohol	RED	Flavouring agent
94	Ethyl maltol	PL	Flavouring agent
109	Isomenthol	LM	Flavouring agent
110	Isoneomenthol	RED	Flavouring agent
123	*p*-menthan-1-ol	MT	Food additive

NG (**100**) is a main component in e-liquids and widely used as a humectant in e-liquids. Even though every e-liquid was said to contain at least 30 % NG, it was only tentative identified in chloroform trap from PL aerosol. NG has very polar attributes, and might not be trapped in the nonpolar solvents used. Since PL was said to contain the highest concentration of NG, at 60 %, it might be the reason for the tentative identification in PL only.

Guaiacol (101) is known for its burnt and smoky wooden flavour (NCBI 2018d) and was identified in RED. The calibration curve for guaiacol was evaluated using q-test, Appendix III, and the extreme maximum value was deemed not significant. This resulted in a new calibration curve with no linear fit to the regression model and guaiacol could not be detected or quantified. The polyalcohol neopentyl glycol (115), tentatively identified in PL, might be a contamination in e-liquids from the diverse industrial use as a coating or paper product (NCBI 2018g).

5.4.2. Aldehydes and ketones

Nineteen aldehydes and ketones were tentatively identified, identified, detected or quantified in trapped aerosols. Both groups contain components with probable use as additives to enhance flavour, durability and taste. Known food additives with no known toxic effect as food additives are shown in Table 10. Some components can result in irritation on human skin and mucous membranes. 2-heptanone (**23**), identified in both Peach and EMP, can irritate eyes, nose and throat in high concentrations (Hawley & Lewis 1993; U. S. Coast Guard 1985). How high the concentration must be to evoke these effects have not yet been obtained, in one oral animal study of acute poisoning the limit was 730 mg/kg for mice (Lewis 2005). 3-heptanone (**66**), on the other hand, is GRAS as a food additive and was identified in the same Peach sample as 2-heptanone.

Beta-Damascone (**22**) was identified in Peach aerosol and quantified in trapped aerosol from RB with a concentration of 10,5 ± 1,1 µg/mL (R^2 > 0,95). There are no safety concerns using beta-damascone as an apple flavour additive according to WHO (2002). In trapped aerosol of PL and RB, *p*-anisaldehyde (**29**), a minty flavour agent, was identified and detected. It is a known additive in e-liquids, previous identified in 3 of 28 e-liquids by Hutzler *et al.* (2014). WHO (2001) reported *p*-anisaldehyde safe as a food additive.

Beta-ionone (**30**), a flavouring agent, was quantified (R^2 > 0,99) in RB, 8,5 ± 0,2 µg/mL, and Peach, 1,1 ± 0,2 µg/mL. It can in higher concentrations (5 % on petrolatum) cause skin irritation (Belsito *et al.* 2007; Frosch *et al.* 1995; Lalko *et al.* 2007). Alpha-ionone (**73**) was quantified in trapped aerosol from RB at concentration 24,8 ± 3,0 µg/mL; it is GRAS at current levels and a known flavouring ingredient.

Table 10. Aldehyde and ketones found in trapped aerosol, possible application and peak number.

Peak no	Trivial name	E-liquid	Attribute
40	*p*-menthan-3-one	MT and LM	Flavouring agent
60	4-Oxoisophorone	RED	Flavouring agent
64	3,4-dimethoxybenzaldehyde	PL	Flavouring agent
68	3-Methylcyclohexanone	MT	Flavouring agent
87	Beta-Damascenone	RED	Flavouring agent
122	Piperitine	MT	Flavouring agent

Furfural (**36**), tentatively identified in Peach and PL trapped aerosol, is reported to be a possible carcinogenic substance by FDA (NTP 1990b), and the daily intake should not exceed 0,5 mg/kg body weight (bw) (WHO 2000). 2,4,4-Trimethyl-3-(3-methylbutyl)cyclohex-2-enone (**59**), a volatile aromatic compound and tentatively identified in VG has unknown properties in e-cigarette aerosol. It has previously been found in oolong tea by solid-phase microextraction (SPME) analysed with GC-MS (Sheibani *et al.* 2016) and might be a food additive.

In PL aerosol 2-hydroxychalcone (**63**) was tentatively identified. 2-hydroxychalcones characteristic in e-liquid and aerosol are yet to be determined. In animal studies, 2-hydroxychalcone was successfully tested as an inhibitor for oral carcinogenesis and inhibitor of aggressive breast cancer (Kim *et al.* 2013; Makita *et al.* 1996).

Benzaldehyde (**75**) had a concentration of 1,4 ± 0,8 µg/mL in trapped Peach aerosol. Benzaldehyde is normally used as an odour additive with possibility to irritate skin and eyes

and a daily intake over 5 mg/kg bw should be avoided (WHO 2001). In trapped aerosol of PL, isovanillin (**77**), a phenolic aldehyde that inhibits aldehyde oxidase production, was tentatively identified. Isovanillin has very polar attributes, which may be the reason it was not detected with the external standard dissolved with heptane.

D-Isomenthone (**86**), often used to lessen the odour of its stereoisomer menthone (**90**), both having a peppermint-like odour tentatively identified in MT and RED.

From trapped aerosol of LM beta-citral (**79**) was tentatively identified, it is used as a lemon flavour enhancer. Inhaling beta-citral can cause a severe cough, and no more than 0,5 mg/kg bw should be consumed each day (WHO 2003).

Carvone (**84**) a possible allergen was identified in MT. Carvone was also identified in another study of e-liquids and aerosol; in 2 of 28 e-liquids analysed by Hutzler *et al.* (2014). The allergen can cause inflammatory response in skin and lips (Hausen 1984; Quertermous & Fowler 2010). It has also been reported to inhibit nitrosamine carcinogenesis in animal experiments (Wattenberg *et al.* 1989).

5.4.3 Alkaloids

The most known alkaloid in e-liquids is nicotine (**1**), with its addictive trait. Nicotine was identified in every aerosol trapped, even in PL, which was said to be nicotine free. Nicotine amount could not be determined in PL but was calculated for the remaining aerosols spanning from $32{,}3 \pm 4{,}6$ µg/mL to $403{,}2 \pm 4{,}6$ µg/mL. The bar plot in Figure 11 of nicotine visualize the different concentration of trapped nicotine. VG was said to contain the most nicotine (24 mg/mL). Trapped concentration of four aerosols said to contain less nicotine (BB, LM, CV and EMP at 6 mg/mL) were quantified to higher concentrations than in VG. One possibility is that VG do not contain 24 mg/mL nicotine. Another possible reason was that concentration of VG from gas wash bottles to GC-MS-samples was interrupted too quickly. The concentration of components in VG will then be underestimated. Tentatively identified nicotyrine (**80**) in VG was tested as a nicotine analogue for experimental cigarettes by Philip Morris. Nicotyrine was said to be different from the other cigarettes, but not deemed an acceptable substitute in Sanders' test (1984).

5.4.4 Esters

42 components found in trapped aerosol were esters. Esters are primarily used as food additives to enhance taste or odour in e-liquids and aerosol. Tentative identified food additives with no known toxic health effects to humans are listed in Table 11. Some esters can cause allergic

reactions or irritations to parts of the human body. Dodecyl acrylate (**6**) was identified, detected and quantified in 6 aerosols. In Peach trapped aerosol dodecyl acrylate had a concentration of $3{,}3 \pm 1{,}5$ μg/mL and it can be irritating to eyes and skin (Ash 2007). The standard deviation of dodecyl acrylate was high, even with a good linear model ($R^2 > 0{,}95$), visualised in Figure 10. A way to improve the linear model is to increase the number of runs or add more representative concentrations of external standard, this could achieve a more fit linear regression model.

In trapped aerosol of Peach, 2-hydroxypropyl acetate (**45**), 1-hydroxypropan-2-yl acetate (**46**) and 2-acetyloxypropyl acetate (**47**) were tentatively identified in the chloroform trap. Their polar properties are probably the reason the heptane trap sample did not contain either of these components. Most likely are these components added to e-liquids for their taste or odour characteristics. 2-acetyloxypropyl acetate was GRAS by FEMA (2009).

Gamma-nonalactone (**9**) was quantified ($R^2 > 0{,}95$) in CV aerosol to $39{,}4 \pm 1{,}9$ μg/mL and $5{,}4 \pm 1{,}9$ μg/mL in PL. Gamma-decalactone (**7**) tentatively identified in five trapped aerosols, has a peach like flavour (Braga & Belo 2016). A test with 10 % gamma-decalactone on petrolatum gave no reactions from the 25 subjects exposed (Opdyke 1979). Both flavouring additives were also identified by Hutzler *et al.* (2014) and have been GRAS by FEMA since 1998 (Doull et al.).

Acceptable daily intake of benzyl acetate (**10**) is 0-5 mg/kg bw. At current levels as a flavouring agent, benzyl acetate with a pear-like odour has no human health risks (WHO 2001). Hutzler *et al.* (2014) identified benzyl acetate in a screening of hazardous component in e-liquids and aerosol. The odour additive was quantified ($R^2 > 0{,}99$), in RB, with concentration of $262{,}1 \pm 5{,}3$ μg/mL.

Triacetin (**19**) a food additive GRAS and identified in 7 of 148 e-liquids in Sassano *et al.* (2018) and quantified in RB, CV and Peach. Concentration of triacetin was $123{,}8 \pm 0{,}7$ μg/mL in trapped CV, $10{,}4 \pm 0{,}7$ μg/mL in Peach and $2{,}9 \pm 0{,}7$ μg/mL in RB. Triacetin can be used as flavour solvent and might indirectly be added in e-liquids and aerosol.

Gamma-undecalactone (**20**) used as an insecticide, registered by the FDA, is toxic towards many insects but GRAS by FEMA (1998) as a food additive for humans. Gamma-undecalactone was quantified ($R^2 > 0{,}99$) in both Peach and UB, $4{,}3 \pm 0{,}4$ μg/mL and $1{,}7 \pm 0{,}4$ μg/mL respectively. The known flavour additive was identified in 3 of 3 aerosols, filter pads and e-liquids tested by Eddingsaas *et al.* (2018).

Delta-decalactone (**24**) was quantified (R^2 > 0,99) in both trapped Peach aerosol and MT aerosol, $21{,}4 \pm 0{,}3$ μg/mL and $2{,}2 \pm 0{,}3$ μg/mL respectively. It is GRAS by FEMA (1998) with a coconut-like flavouring profile. Since delta-decalactone and hydrocoumarin co-eluated at RT 48,50, delta-decalactone might be overestimated.

Cis-3-hexenyl acetate (**27**) was quantified (R^2 > 0,99) in the trapped aerosol of RB, $15{,}4 \pm 1{,}8$ μg/mL, and Peach $7{,}7 \pm 1{,}8$ μg/mL, with no safety concerns reported from WHO (1997). *Cis*-3-hexenyl acetate was also tentatively identified in chloroform trapped aerosol samples.

Hexyl acetate (**28**) is a known flavouring additive, with the potential to cause eye and skin irritations in higher concentration than normally used as an additive (Lewis 2005). Hexyl acetate was quantified in RB with concentration $9{,}7 \pm 2{,}2$ μg/mL.

WHO evaluated *trans*-3-hexenyl acetate (**67**) as a safe flavouring agent. FEMA (1996) describe 3-methylcyclohexanone as GRAS with a minty, cool flavour, it is also in EUs list of food flavourings.

Table 11. Esters tentatively identified in trapped e-cigarette aerosol. These esters are known food additives with no toxic effect. Peak number and possible application are listed.

Peak no	Trivial name	E-liquid	Attribute
7	gamma-Decalactone	MT, PL, Peach, UB and LM	Flavouring agent
9	gamma-Nonalactone	RB, LM, CV and PL	Flavouring agent
10	Benzyl acetate	RB, Peach, MT and RED	Flavouring agent
19	Triacetin	CV, Peach and RB	Food additive
20	gamma-Undecalactone	Peach and UB	Flavouring agent
24	delta-Decalactone	Peach and MT	Flavouring agent
27	*cis*-3-Hexenyl acetate	RB and Peach	Flavouring agent
37	Cyclohexyl hexanoate	RB and MT	Flavouring agent
38	Isomenthol acetate	MT and LM	Flavouring agent
54	Isoamyl acetate	Peach	Flavouring agent
57	gamma-Octalactone	Peach	Flavouring agent
58	gamma-Caprolactone	PL	Flavouring agent
61	Methyl dec-2-enoate	LM	Flavouring agent
67	*trans*-3-Hexenyl acetate	MT	Flavouring agent
82	Ethyl butyrate	Peach	Flavouring agent
83	Hexyl butanoate	Peach	Flavouring agent
85	beta-Terpinyl acetate	Peach	Food additive
88	Ethyl decanoate	PL	Flavouring agent
89	delta-Dodecalatone	PL	Flavouring agent
93	Ethyl elaidate	PL	Flavouring agent
96	Ethyl octanoate	PL	Flavouring agent
97	Ethyl oleate	VG	Flavouring agent
98	Ethyl palmitate	VG	Flavouring agent
99	gamma-Dodecalactone	PL	Flavouring agent
105	Ethyl caproate	PL	Flavouring agent
106	Methyl hexanoate	PL	Flavouring agent
108	Isoamyl isovalerate	PL	Flavouring agent
112	Methyl cinnamate	UB	Flavouring agent
113	Methyl isobutyrate	PL	Flavouring agent
117	Ethyl octadecenoate	PL	Flavouring agent
127	Whiskey lactone	MT	Flavouring agent
128	Hex-5-enyl hexanoate	RB	Unknown

Ethyl butyrate (**82**) was quantified with a concentration, $15{,}3 \pm 1{,}5$ μg/mL, in Peach trapped aerosol, the second highest concentration found in Peach aerosol, after nicotine $403{,}2 + 4{,}6$ μg/mL. In 7 of 30 e-liquids evaluated by Tierney *et al.* (2016) ethyl butyrate was quantified with concentrations from 0,1 mg/mL to 11,1 mg/mL, a significant difference from trapped ethyl butyrate collected in Peach.

Hexyl butanoate (**83**) was quantified in a Peach trapped aerosol. The linear regression model ($R^2 > 0{,}95$) was made with 3 external standard concentrations, the minimum amount to draw a linear regression. This result in a poor linear model and the concentration quantified in Peach aerosol, $3{,}5 \pm 1{,}3$ μg/mL, can vary from the true value.

Hydrocoumarin (**107**) was identified in CV aerosol. WHO have no concerns towards hydrocoumarin used as a flavouring agent, it is also listed as a known flavouring agent in EUs list of flavourings and by the FDA. To identify hydrocoumarin RIC was used, since it co-eluted with delta-decalactone.

In the trapped aerosols tested was linalyl acetate (**111**) only detected together with linalool in Peach aerosol, they usually occur in conjuntion. Their close RT at 8,59 min for linalool and 8,79 min for linalyl acetate might cause co-elution and make identification, detection and quantification difficult. TIC was used to detect both components. Linalyl acetate can cause severe skin irritations in higher doses (Lewis 2005).

O-acetylcitric acid triethyl ester (**116**) was tentatively identified in LM and commonly used as a plasticiser that can indirectly be found as a food additive (NCBI 2018a). The toxicity of O-acetylcitric acid triethyl ester is yet to be determined when inhaled or consumed even though it was deemed safe to use in cosmetics by Johnson (2002).

5.4.5 Ethers

Ethers are added to food and e-cigarettes usually for their ability to enhance odour, taste or durability. Tentatively identified ethers used as food additives with no severe toxic effects to humans are shown in Table 12.

Table 12. Ethers used as food additives with no known toxic health effects toward humans.

Peak no	Trivial name	E-liquid	Attribute
50	Anisaldehyde propylene glycol acetal	RB	Flavour agents
56	Veratraldehyde propylene glycol acetal	PL	Flavour agents
76	Benzaldehyde propylene glycol acetal	PL	Flavour agents
52	*p*-Methoxyanisole	PL	Flavour agents

One component 2-ethyl-5-methyl-1,4-dioxane (**53**) was tentatively identified in RB aerosol. The properties of 2-ethyl-5-methyl-1,4-dioxane in e-liquid or aerosol are unknown, and its origin in the aerosol is yet to be determined. It might be a constituent in the e-liquid or a contamination from the hose. A suspected carcinogenic compound, methyleugenol (**114**), was tentatively identified in UB aerosol. The compound is used as a food flavouring ingredient and the carcinogenic attributes are based on an animal study by Johnson *et al.* (2000). 2-ethyl-5-methyloxolane (**126**) has unknown properties in the Peach aerosol.

5.4.6 Terpenes

Terpenes often occur naturally in plants, trees even insects. They are widely used as flavour additives to enhance taste and odour (Dewick 2009). Six terpenes were tentatively identified, identified and quantified in e-cigarette aerosols, were two GRAS food additives are shown in Table 13.

In LM, UB and EMP concentrations of d-limonene (**11**) were $29{,}0 \pm 3{,}9$ µg/mL, $31{,}5 \pm 3{,}9$ µg/mL and $6{,}5 \pm 3{,}9$ µg/mL respectively. National Toxicology Program (NTP) found, in 1990, carcinogenic activity in rats originated from d-limonene. In 1999 insufficient evidence of carcinogenicity, placed d-limonene in a group of compounds which were classified as not carcinogenic in humans, by International Agency for Research on Cancer (IARC). D-limonene is now GRAS by the FDA (2011). LOD, standard deviation and concentration of d-limonene are shown in Figure 8. It clearly shows the diverse concentrations of d-limonene used as a flavouring agent.

In MT and RED, caryophyllene (**31**) was tentatively identified, a known flavouring ingredient with wood like odour and no safety concerns as a flavouring agent, GRAS by FEMA (2011).

Eucalyptol (34), cyclic ether and monoterpenoid, used as a flavouring agent to enhance camphor-like odour and a cool, minty taste. It is GRAS by FEMA and identified in PL and MT. From the linear regression model, Appendix I Figure 15, the standard deviation of eucalyptol was ± 5,1 µg/mL trapped aerosol, a high standard deviation that might originate from a bad external standard sample. The q-test, Appendix III, performed on the extreme values, deemed both min and max values significant, Appendix I.

Pulegone (**42**) was identified in trapped aerosol from MT and LM, quantified in LM 10,0 ± 1,9 µg/mL. Pulegone is the main component (62-97 %) of pennyroyal oil and reports of pennyroyal oil consumption (over 10 mL) have led to severe toxic effect and even death (European Commission 2002; National Toxicology Program 2011). Pulegone was also identified in 3 of 28 e-liquids in Hutzler *et al.* (2014). In normal concentration used as a minty flavour enhancer pulegone is GRAS by FEMA (1996).

Table 13. Flavour enhancing terpenes tentatively identified in e-cigarette aerosol.

Peak no	Trivial name	E-liquid	Attribute
51	1,4-cineole	PL	Flavour agents
91	D-sylvestrene	MT	Flavour agents

5.4.7 Alkanes

The alkane chains tetracosane (C24), pentacosane (C25), eicosane (C20), docosane (C22), nonadecane (C19), heptacosane (C27), decane (C10), hexadecane (C16), tetradecane (C14) and dodecane (C12) were identified in 2-9 different trapped aerosols. The commercial use of long chained alkanes is limited, their odour span from odourless to fuel-like, they are all phytochemical in several plants (Duke 2000). Branched alkanes like farnesane, 2-methylundecane, 2-Methylicosane, 2,6,11-Trimethyldodecane, 2-methylnonadecane, 2-methyloctadecane have limited known use in e-cigarettes. One possible origin of these alkanes is the rubber hose connection the e-cigarette to the gas wash bottle and the trap. During aerosol collection, the rubber wire will be exposed to hot aerosol, which will heat up the wire, and be a possible origin for the alkanes. These components will not be detected in the blank samples since the blank samples were collected without any aerosol going through the hose. A possible step to check for contaminations from the hose would be to mix humectants, PG and VG, and test the e-cigarette without any other components involved, this was not done in this thesis.

5.4.8 Other components

Components not associated with any of the chemical families previous discussed will be discussed in this chapter, eight components will be addressed, and GRAS food additives are listed in Table 14.

1,3-ditert-butylbenzene (**2**) was quantified in every trapped vape, except PL and BB. It might have found its way to the e-liquid and aerosol through food packaging, where it is commonly used (de Oliveira *et al.* 2012). Concentrations of 1,3-ditert-butylbenzene, shown in a bar plot in Figure 9, are in range $2{,}1 \pm 1{,}0$ μg/mL to $6{,}1 \pm 1{,}0$ μg/mL for all but one aerosol. In LM the concentration was four times higher, $27{,}9 \pm 1{,}0$ μg/mL, which might indicate that LM have had longer shelf life with more contact with the food packaging.

One component was tentatively identified 4-methyl-2-phenyl-1,3,2-dioxaborolane (**48**), which is not listed in either EUs Lists of Food Flavourings nor list of known additives in cigarettes. With no known functions of 4-methyl-2-phenyl-1,3,2-dioxaborolane, it can indicate that it is either a contaminant from outside the system or wrongly interpreted by the NIST library and GC-MS system.

Table 14. Food additives tentatively identified in e-cigarette aerosol, not characterised by the chemical families previous discussed.

Peak no	Trivial name	E-liquid	Attribute
71	Sulfurol	PL	Flavour agents
72	Pratol	Peach	Food additive
92	2-chloro-1,1-diethoxyethane	PL	Unknown
104	Hexanoic acid	RB	Flavour agents

Ethyl N-cyclohexylcarbamate (**95**) not previous known as an additive in the e-liquid or aerosol with unknown attributes in the vapour and organic carbamates are a group of chemicals more and more used in medicinal chemistry and drug design (Ghosh & Brindisi 2015).

O-cymene, most likely a food additive, tentatively identified in PL. *O*-cymene is an isomer of the natural aromatic compound *p*-cymene which is a flavouring agent (NCBI 2018h).

Until now, health risks of the components have been discussed only by the components alone, in consistency with Sassano *et al.* (2018) description of the e-liquid as a heterogeneous solution. Even though e-liquids are heterogeneous, the aerosol will expose the user of every component in the aerosol at the same time. Some components are possible carcinogenic, toxic and allergens while other are GRAS as food additives alone. In a mixture these components might have a different characteristic. Lerner *et al.* (2015) reported different oxidative character of different e-liquids depending on their flavour ingredient. Sweet and fruity e-liquids were stronger oxidizers than tobacco flavoured e-liquids. The same study exposed human lung cells to e-cigarette aerosol which resulted in oxidative and inflammatory response, which is further corroborated by Aug *et al.* (2015). A different study on human lung cells exposed to e-cigarette aerosol decreased the cells viability, but not as much as mainstream smoke (Scheffler *et al.* 2015). It is still important to remember that long-term effects of e-cigarette use are yet to be determined so the current assessment of health risk from e-cigarettes may not be the final answer.

6. Conclusion

E-cigarettes potential to reduce smoke-related health risks worldwide is immense. Since the product is under little regulation; many components are unknown to the user. The objective of this thesis was therefore to characterise nonpolar components from e-cigarettes. A screening analysis of trapped aerosol was done using GC-MS. From a total of 129 tentatively identified components 30 were identified with external standards. Quantification gave a maximum concentration of exposure from constituents in e-cigarette aerosol in 30 puffs of 10 second for 21 components. Due to the diverse constituents in the aerosol and the lack of long-term health risks, a total toxicity risk is difficult to formulate. Chemical groups as esters, alcohols, aldehydes and ketones were represented in the aerosols. Over seventeen potential harmful nonpolar components were found in the aerosols from 11 different e-liquids. The potential carcinogen components methyleugenol and furfural were tentatively identified in the nonpolar phase. The use of e-cigarette to help with smoke cessation looks like a positive health benefit. Even though harmful components were identified in this thesis e-cigarette aerosol produces less potentially toxic component than the adversary tobacco smoke.

6.1 Future research

The study of e-liquid constituents should continue to map every component in the aerosol and e-liquid. Potential health risks and benefits should be further studied. Health risks include long-term effects, single components effect and the effect of the mixed solution. A regulator system should be integrated for every manufacturer and regulator of e-cigarettes and to do so, future research should focus on potential regulating methods of e-liquid and aerosol content.

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Appendix I

Quantitative analysis of components in aerosols from e-liquids.

Ethyl butyrate

Ethyl butyrate was found in one sample (Peach) with a NIST score 891, retention time (RT) 5,34 and probability 51,70%. Five known concentrations of ethyl butyrate were analysed on GC-MS. Linear regression of ethyl butyrate gave $y = 54136x + 161{,}77$, shown in Figure 13. From the unknown sample an area of 982 was integrated that corresponds to a concentration of 15,2 ± 1,5 µg/mL.

$$y = 54136x + 161{,}77$$

$$x = \frac{(y - 161{,}77)}{54136}\frac{mg}{mL}$$

$$x = \frac{(982 - 161{,}77)}{54136}\frac{mg}{mL}$$

$$x = 0{,}01515\frac{mg}{mL} = 15{,}15\frac{\mu g}{mL}$$

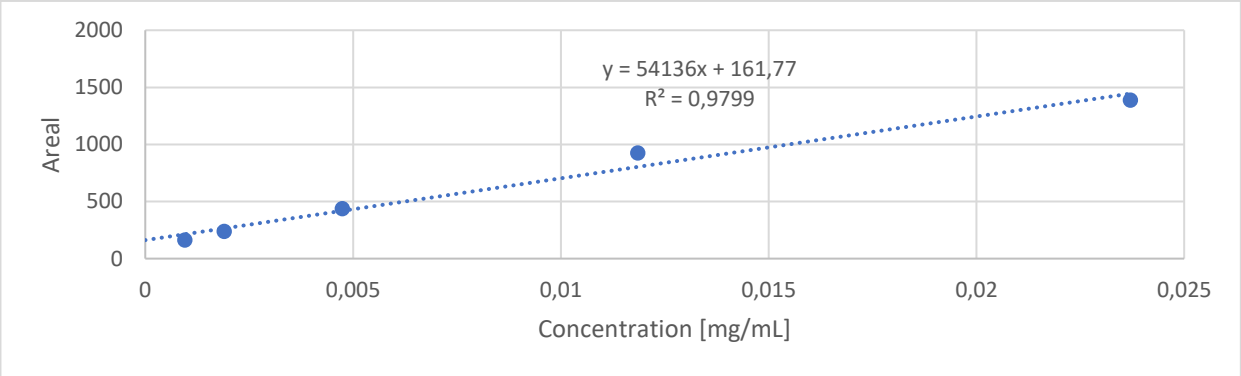

Figure 13. Calibration curve of Ethyl butyrate with function y = 54136x + 161,77 and R^2 = 0,9799

D-Limonene

Calibration curve, shown in Figure 14, had a poor linear model with R^2 = 0,8013. Both extreme values (min and max) were deemed significant with a q-test. Concenstrations of D-limonene in heptane trapped aerosol is given in Table 7 for LM, UB and EMP.

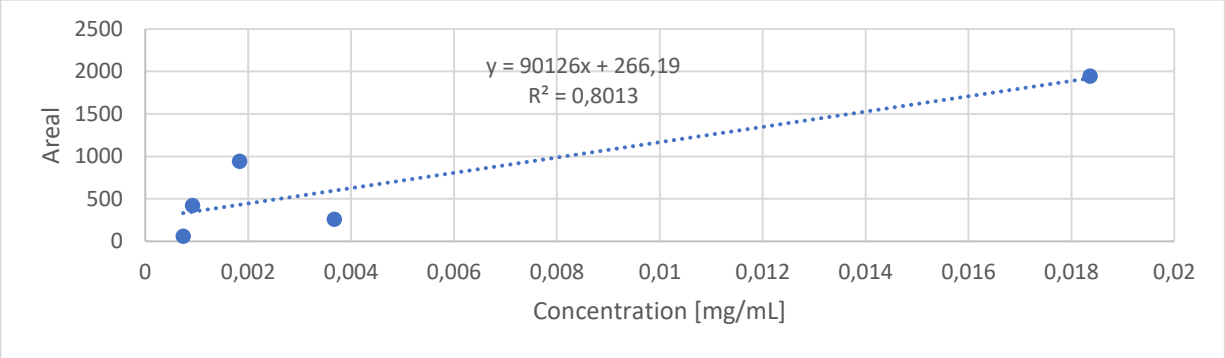

Figure 14. Linear regression of D-Limonene with t = 90126x + 266,19 and R^2 = 0,8013

Eucalyptol

From MT RT 6,62 calibration curve, Figure 15, had poor linearity with R^2 = 0,8802. Both extreme values (min and max) were deemed significant with a q-test so the model was kept. Eucalyptol was identified in PL and MT, shown in Table 7.

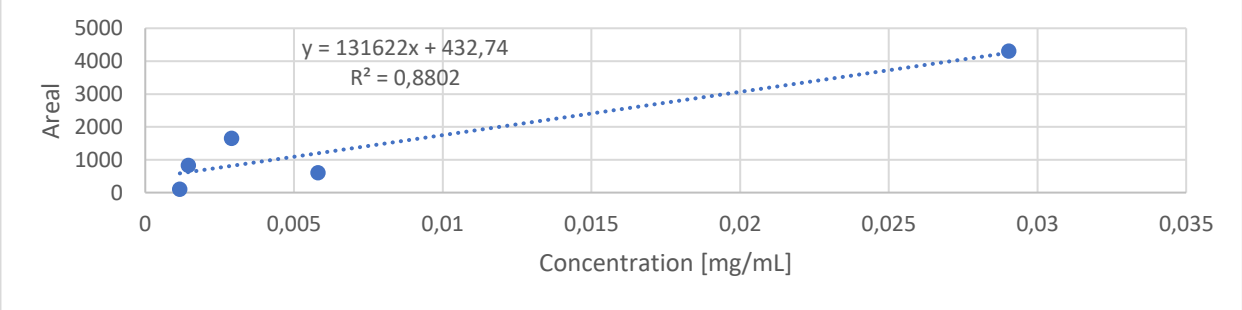

Figure 15. Linear regression of Eucalyptol with calibration curve y = 131622x + 432,74 and R^2 = 0,8802

3-Heptanone

In the aerosol from Peach, 3-heptanone was found from NIST library with 835 score and probability 44,9% at RT 6,65. An area was integrated to 74 corresponds to a concentration of $0{,}53 \pm 1{,}5$ µg/mL from calibration curve y = 80361x-15,006, from Figure 16. This gives a concentration interval under LOD and 3-heptanone could only be identified.

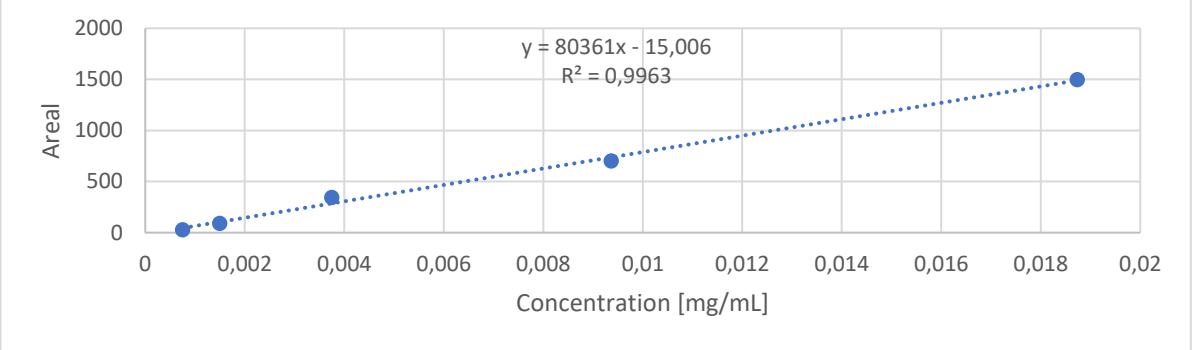

Figure 16. Calibration curve of 3-heptanone with y = 80361x-15,006 and R^2 = 0,9963

2-Heptanone

In the same Peach trapped aerosol as 3-heptanone, 2-heptanone was identified, with a NIST score of 936 and probability 69,8 % at RT 6,84. The area was integrated to 154, which corresponds to a concentration of $1{,}1 \pm 1{,}8$ µg/mL from calibration curve in Figure 17, this result in a concentration under LOD. 2-heptanone was also identified in EMP, also under LOD. A test on the highest point with Dixon's q-test deemed the data significant

$$Q_{data} = \frac{|1640 - 1116|}{|1640 - 26|} = 0{,}324$$

From Dixon's q-test, Appendix III Table 16, the critical value $Q_{95\,\%,N=5} = 0{,}710$ resulting in $Q_{data} < Q_{critical}$ and the suspected value can not be classified as an outlier.

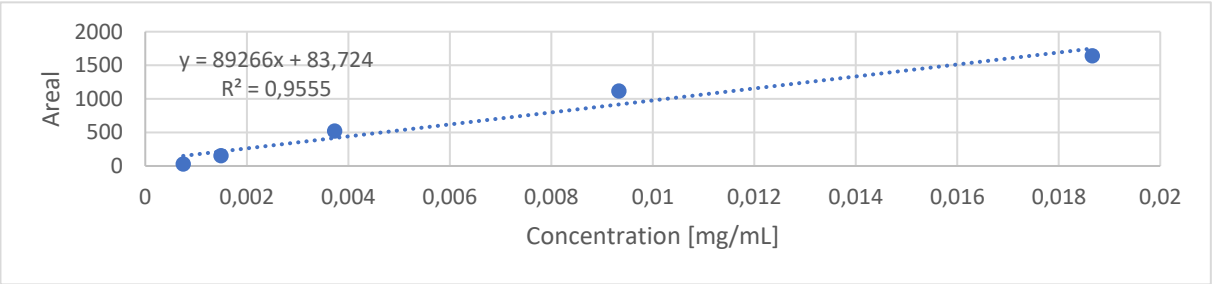

Figure 17. Calibration curve of 2-heptanone with y = 89266x+83,724 and R^2 = 0,9555

Hexyl acetate

Found in chloroform and heptane trap at RT 6,97, with 909 NIST score in the heptane sample and a probability at 83,7 %. Aerosols containing hexyl acetate was RB and Peach. The concentration was determined using calibration curve from the heptane trap sample. The concentration was calculated to 9,7 $\pm$ 2,2 µg/mL with calibration curve in Figure 18.

$$Q_{data,max} = 0{,}288 \text{ and } Q_{data,min} = 0{,}042$$

From Dixon's q-test, Table 16, the critical value $Q_{95\,\%,N=5} = 0{,}710$ resulting in $Q_{data} < Q_{critical}$ and the suspected value can not be classified as an outlier.

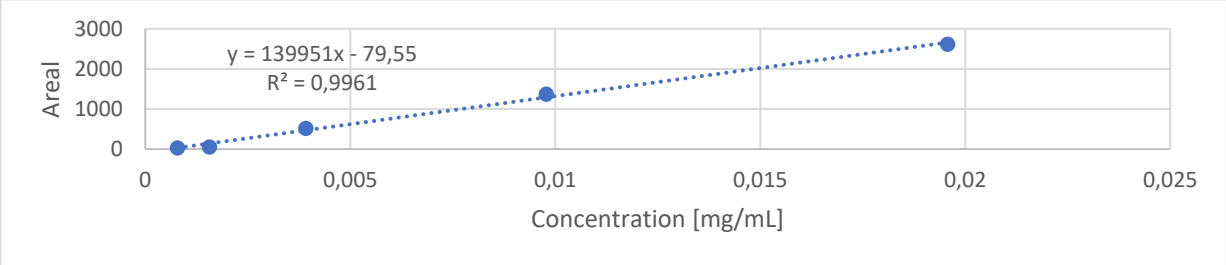

Figure 18. Calibration curve of hexyl acetate with y = 139951x-79,55 and R^2 = 0,9961

cis-3-Hexenyl acetate

Found in chloroform and heptane trap of Peach and RB at RT 7,30, NIST score 866 and 27,0 % probability. Calibration curve, Figure 19, for cis-3-Hexenyl acetate gave concentrations shown in Table 7, from area of 3597 in Peach and 6063 in RB.

$$Q_{data,max} = 0{,}5967 \text{ and } Q_{data,min} = 0{,}070$$

From Dixon's q-test, Appendix III Table 16, the critical value $Q_{95\,\%,N=5} = 0{,}710$ resulting in $Q_{data} < Q_{critical}$ and the suspected value can not be classified as an outlier.

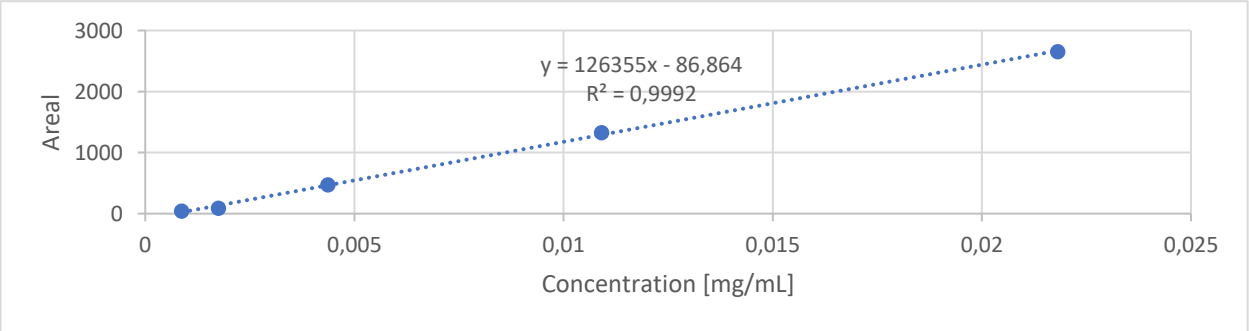

Figure 19. Calibration curve of cis-3-hexenyl acetate with y = 126355x – 86,864 and R² = 0,9992

1,3-ditert-butylbenzene

Found in chloroform and heptane traps of aerosol from RED, BB, VG, RB, MT, RED, LM and CV. Also found in 11 W and 23 W samples of UB. Highest NIST match was in RB with 936 and 78,4 %, at RT 7,85. Consentration of 1,3-ditert-butylbenzene in the different trapped aerosols are given in Table 7.

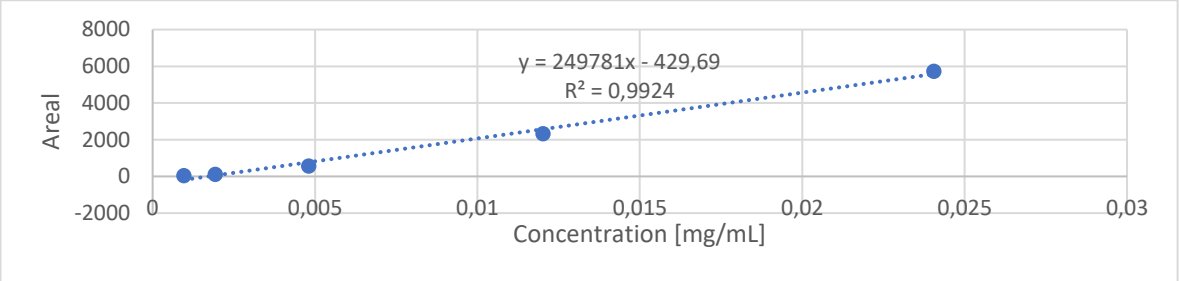

Figure 20. Calibration curve of 1,3-ditert-butylbenzene with y = 249781x – 429,69 and R² = 0,9924

Hexyl butanoate

Found in both chloroform and heptane trap of peach aerosol at RT 7,92, with NIST score 956 with 79,8 % probability. From calibration curve in Figure 21, and area of 2200 a concentration of 3,5 ± 1,3 µg/mL was calculated (Peach vacuum). Only three concentratons were detected which is the minimum for a linear regression.

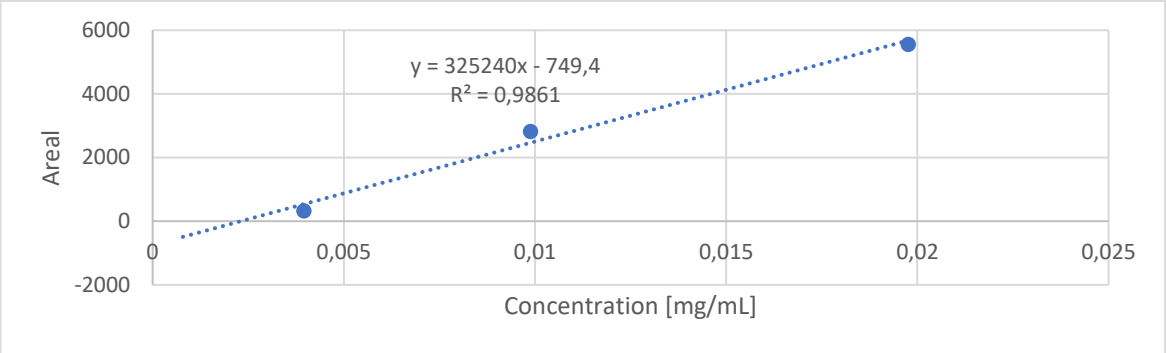

Figure 21. Calibration curve of hexyl butanoate with y = 325240x – 749,4 and R² = 0,9861

Linalool

Linalool was identified in five different aerosols. From both heptane and chloroform sample, linalool was tentatively identified in MT, Peach, LM and PL. Additionally was linalool found in UB and LM from the heptane sample only. It had a peak in the chromatogram at RT 8,59 with NIST score 918 and probability 66,7 %. From the calibration curve, Figure 22, the concentration of linalool was calculated and given in Table 7.

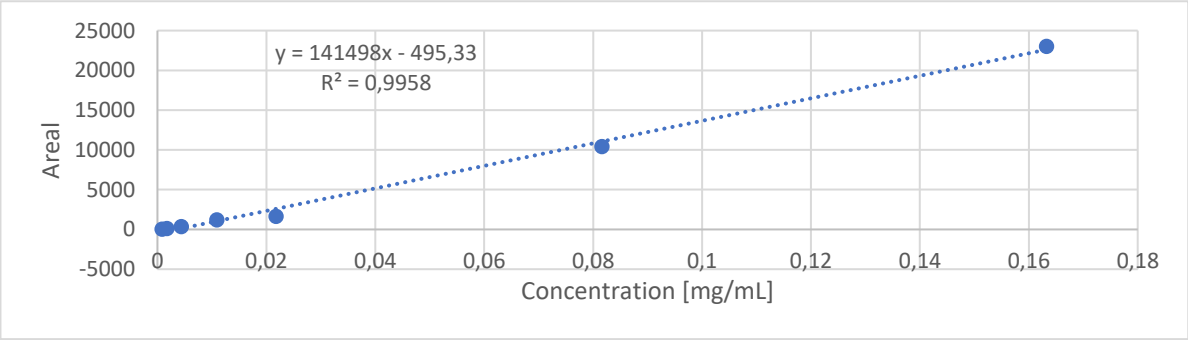

Figure 22. Calibration curve of linalool with y = 141498 – 495,33 and R² = 0,9958

Linalyl acetate

From Peach vape, an area of 244 was integrated and identified with 842 in NIST score, 28,6 % probability at RT 8,75. From calibration curve in Figure 23 concentration for linalyl acetate were calculated to 2,9 ± 1,1 µg/mL in Peach aerosol. One concentration, C1, was taken out as an outlier. It was deemed an outlier due to the value not corresponding to the rest of the data. There might have occurred a dilution error or a calculation error when looking at the data sample.

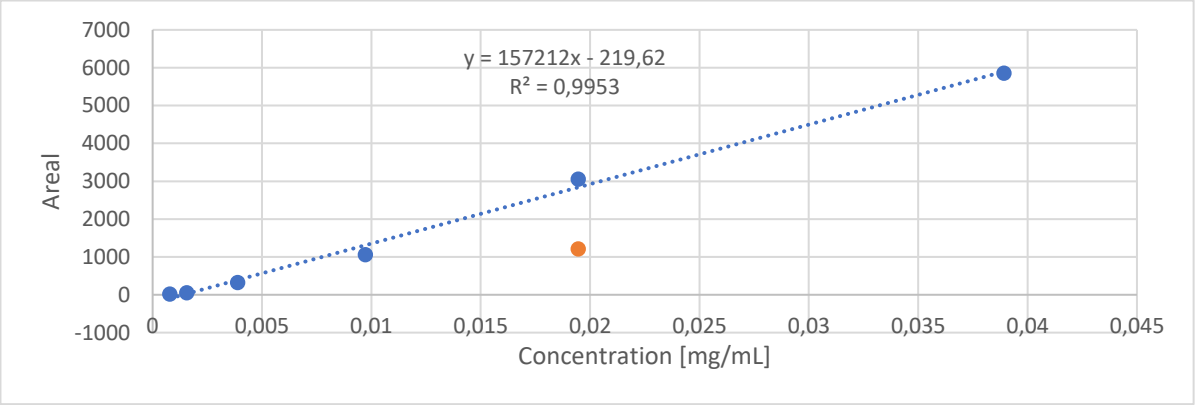

Figure 23. Calibration curve of linalyl acetate with y = 157212x – 219,62 and R2 = 0,9953 and an outlier market orange.

Benzaldehyde

Identified in both chloroform and heptane sample of Peach, with NIST score 939 and probability 72,50 % at RT 9,72. From calibration curve, Figure 24, benzaldehyde concentration in Peach aerosol was calculated to 1,4 ± 0,8 µg/mL. No outliers with q-test.

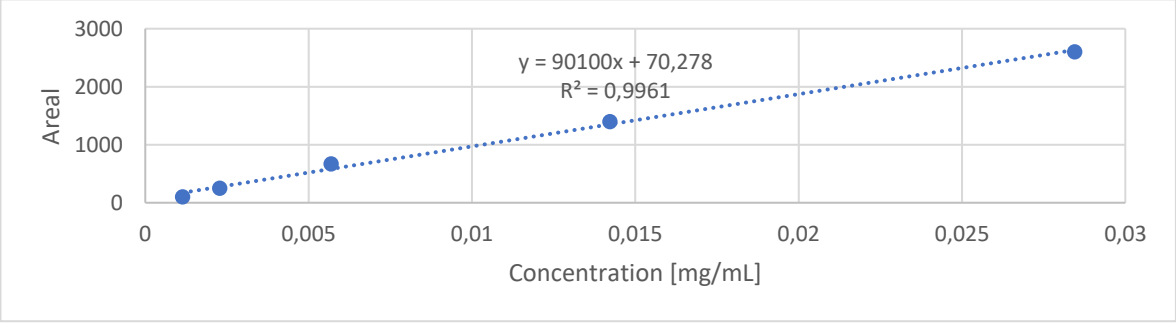

Figure 24. Calibration curve of benzaldehyde with y = 90100x + 70,278 and R² = 0,9961

Pulegone

Identified in TM and LM. Quantified in LM with calibration curve Figure 25, concentrations given in Table 7. Pulegone had a NIST score 909, probability 32,0 % at RT 11,20.

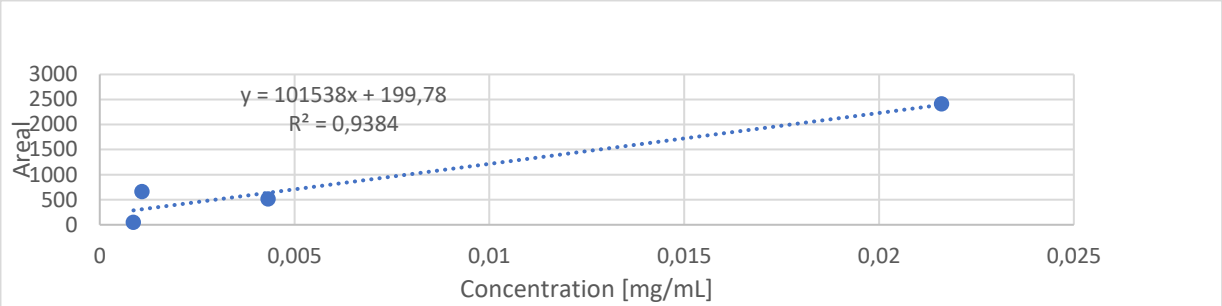

Figure 25. Calibration curve of pulegone with y = 101538x + 199,78 and R^2 = 0,9384.

Benzyl acetate

Identified in several aerosols, RB, Peach, MT and RED, at RT 12,03 in the heptane trap. In the chloroform sample benzyl acetate was identified in RB, Peach and MT. With a wide range of area integrated from aerosol traps, concentration of benzyl acetate was calculated, from calibration curve in Figure 26, and shown in Table 7.

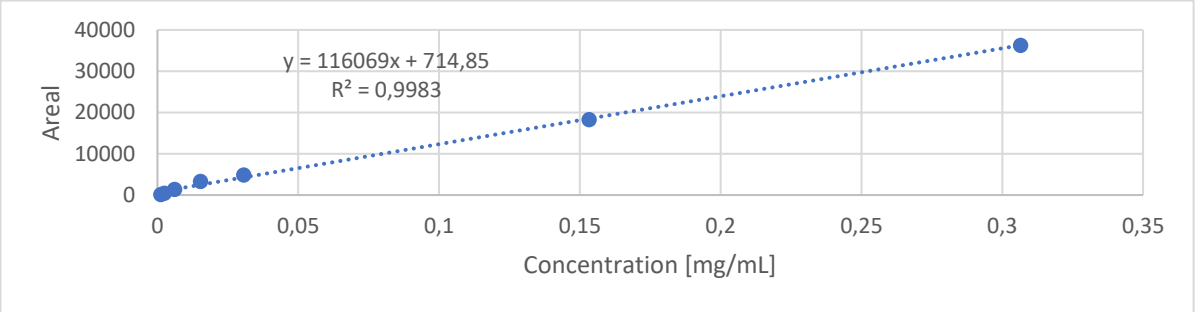

Figure 26. Calibration curve of benzyl acetate with y = 116069x + 714,85 and R² = 0,9983

Carvone

Identified in TM with a higher standard deviation than calculated concentration so carvone cannot be detected. Carvone got a NIST score 932, probability 39,4 % at RT 12,70. Calibration curve given in Figure 27.

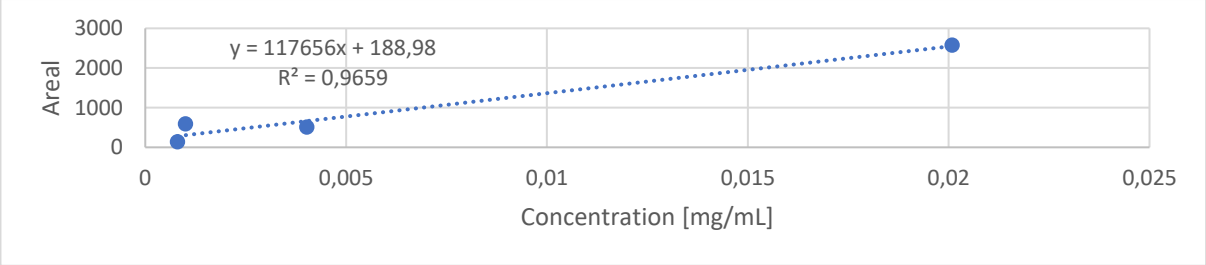

Figure 27. Calibration curve of carvone with y = 117656x + 188,98 and R^2 0,9659.

Butylated hydroxytoluene

Trapped by all three solvents (heptane, chloroform and ethyl acetate) and identified in RB, Peach, MT, PL, CV, BB and VG. From calibration curve in Figure 28, concentrations of butylated hydroxytoluene were calculated and given in Table 7. From q-test all values were deemed significant. One concentration calculated, BB, were integrated above the linear area and cannot be determined by this linear regression. On the other hand, it indicates that the concentration of butylated hydroxytoluene is far higher in BB than the other samples.

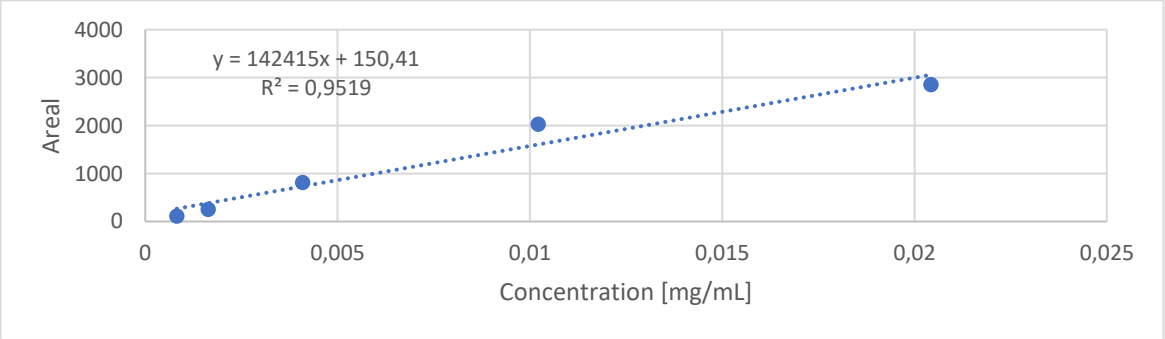

Figure 28. Calibration curve of butylated hydroxytoluene with y = 142415x – 150,41 and R2 = 0,9519.

beta-Damascone

Found in RB and Peach in both heptane and chloroform solvent with NIST score over 900 and 78,5 % probability at RT 13,75. Concentration given in Table 7, calculated from calibration curve in Figure 29.

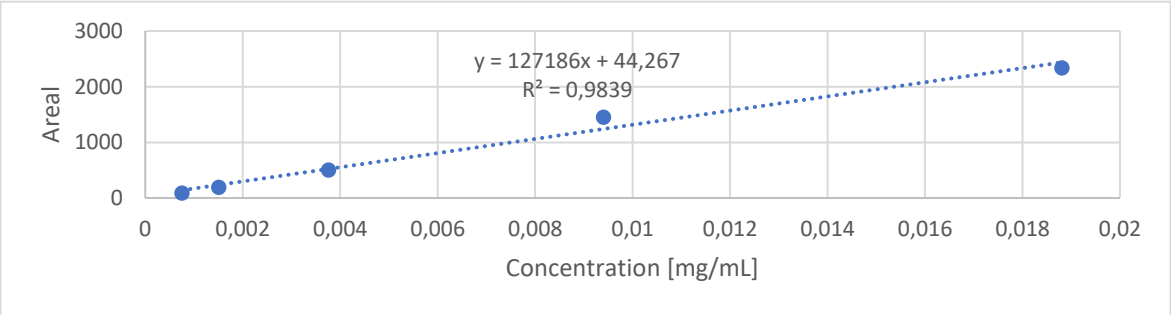

Figure 29. Calibration curve of beta-damascone with y = 127186x + 44,267 and R^2 = 0,9839

Dodecyl acrylate

MT, RED, UB, RB, CV and Peach are all e-liquids with dodecyl acrylate identified in the aerosol. NIST score 942, probability 62,4 % at RT 14,14 in Peach aerosol. With calibration curve in Figure 30 gave concentrations shown in Table 7.

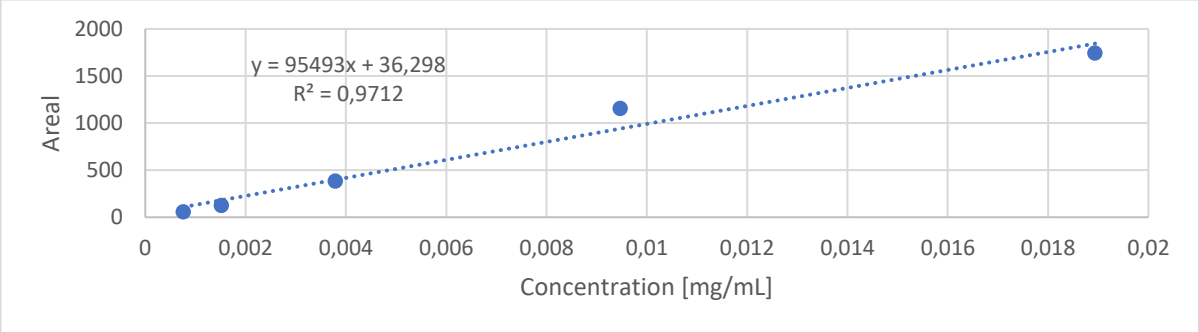

Figure 30. Calibration curve of dodecyl acrylate with y = 95493x + 36,298 and R^2 = 0,9712

Guaiacol

Only Tentativly identified in RED with NIST score 872, probability 57,5 % at RT 14,46, were linear regression gave under LOD or no fit.

With q-test guaiacol had one outlier at the highest point. $Q_{data} = 0{,}928 > 0{,}829 = Q_{critical}$ thus, the data is deemed not significant by the Q-test. Calibration curve with all point given in Figure 31.

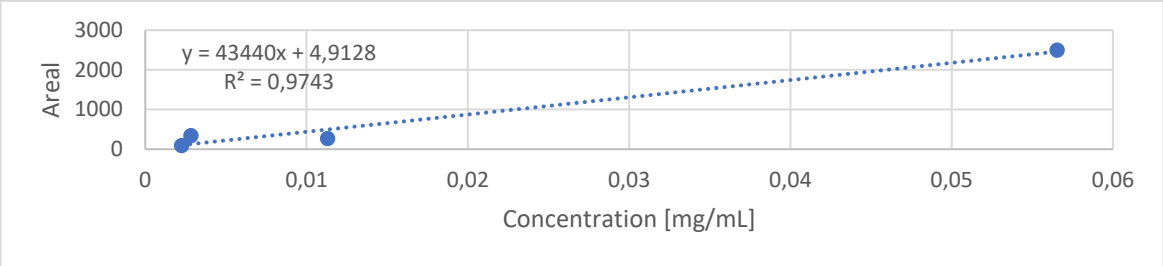

Figure 31. Guaiacol without outlier y = 43440x + 4,9128.

Removing the outlier gave calibration curve Figure 32, but no linear fit with linear regression.

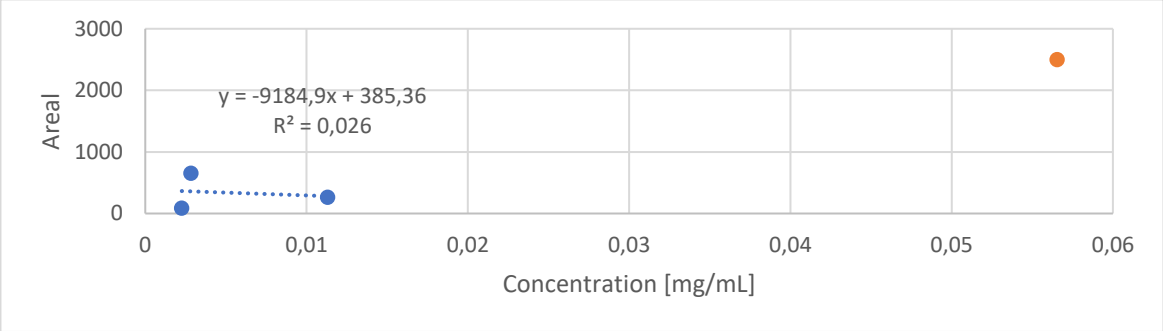

Figure 32. Linear regression without outlier. Guaiacol had no linear fit with this model.

Nicotine

Identified in all traps of aerosol with all solvents, even in PL that was advertised 0 mg/mL nicotine. Highest NIST score 958 found in RB with 77,9 % probability at RT14,8. From calibration curve Figure 33, concentrations of nicotine were calculated and given in Table 7. No outliers with q-test.

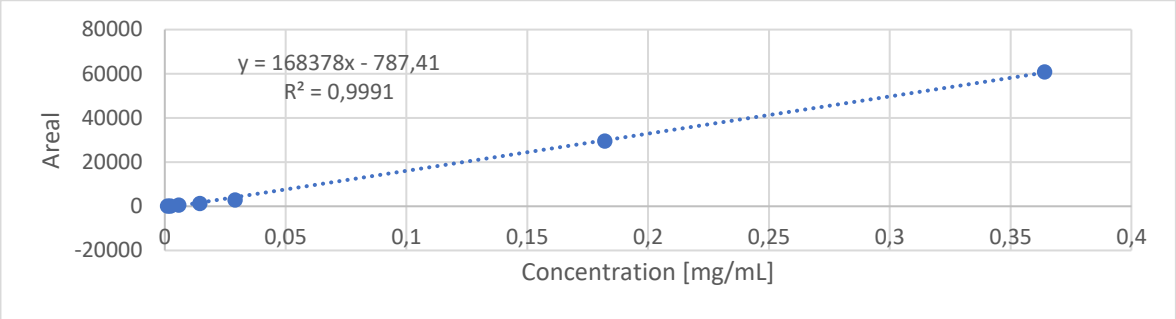

Figure 33. Calibration curve of nicotine with y = 168378x - 787,41 and R^2 = 0,9991.

Alpha-Ionone

Detected in RB with over 70 % probability and 923 in NIST score at RT 15,38. Concentration calculated to $24{,}8 \pm 3{,}0$, with calibration curve in Figure 34.

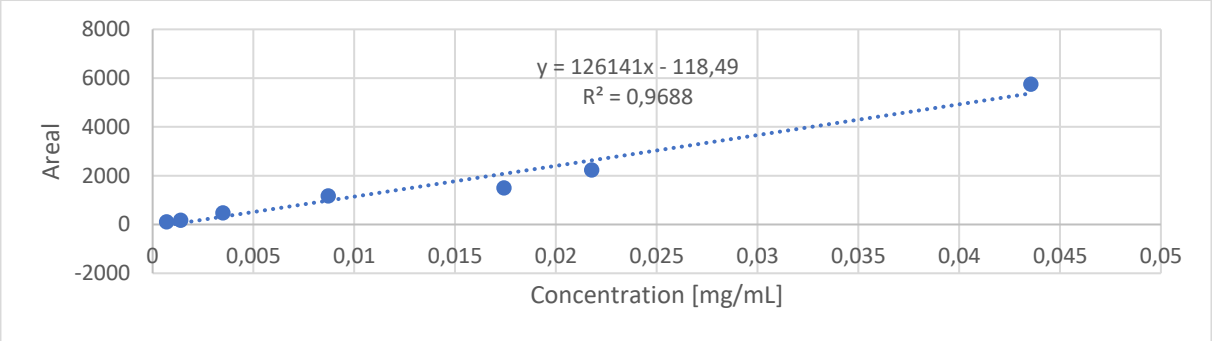

Figure 34. Calibration curve of alpha-ionone with y = 126141x - 118,49 and R^2 = 0,9688.

Beta-Ionone

Quantified in RB and Peach with concentrations given in Table 7. Identified in both heptane and chloroform solvent. At RT 17,48 beta-ionone got a NIST score 930 with 49,3 % probability from RB heptane trap. Calibration curve of beta-ionone is shown in Figure 35.

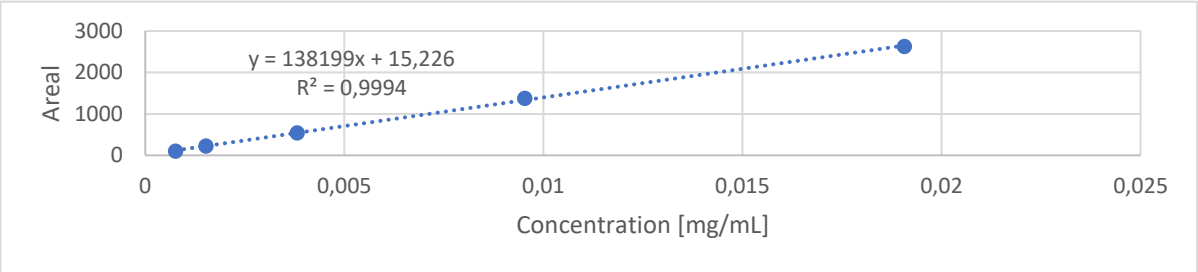

Figure 35. Calibration curve of beta-ionone with y = 138199x + 15,226 and R^2 = 0,9994

p-Anisaldehyde

p-Anisaldehyde with calibration curve in Figure 36, were identified in PL and RB, detected, but not quantified in RB. Under LOD in PL. From RT 23,56 in RB heptane trap probability at 52,0 % and 923 NIST score were calculated.

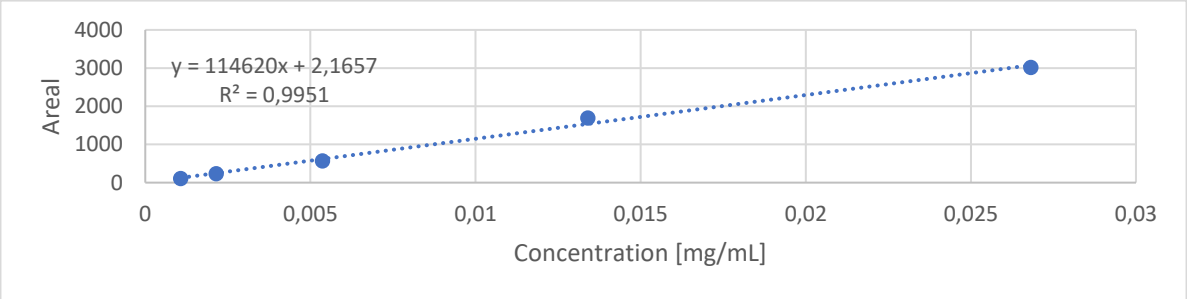

Figure 36. Calibraton curve of p-Anisaldehyde with y = 114733x and R2 = 0,9951

Triacetin

Triacetin was identified in 3 samples, CV, Peach and RB. Highest probability was 53,5 % with a NIST score 942 at RT 28,81. Concentration of triacetin in all samples are shown in Table 7 calculated from calibration curve in Figure 37.

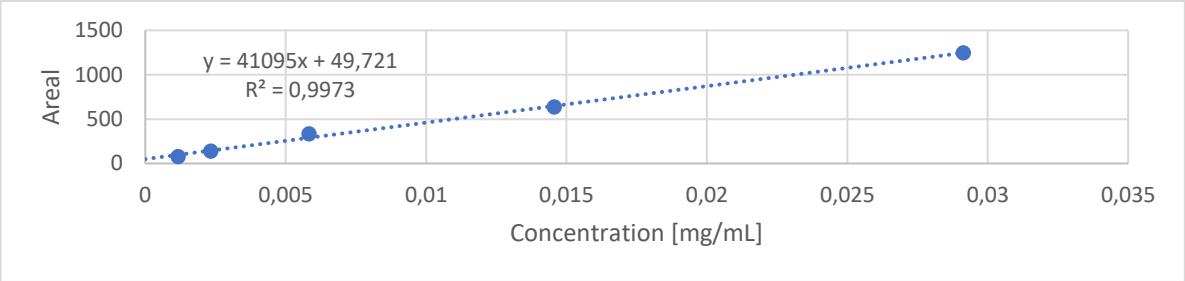

Figure 37. Calibration curve of triacetin with y = 41095 + 49,721 and R^2 = 0,9973..

Gamma-nonalactone

Gamma-nonalactone was identified in four aerosols, RB, LM, CV and PL. Highest NIST score 913 and 61,9 % probability at RT 32,85 found in CV. In CV, the concentration was calculated over the linear regression area from calibration curve Figure 38. Gamma-nonalactone was detected in LM (over LOD under LOQ), and quantified in PL. From RB sample gamma-nonalactone was deemed tentatively identified only with concentration under LOD.

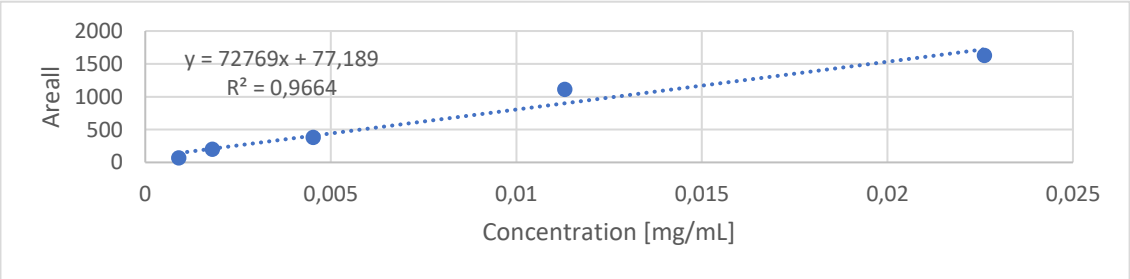

Figure 38. Calibration curve of gamma-nonalactone with y = 72769x + 77,189 and R^2 = 0,9664.

N

Hydrocoumarin

Identified in CV, with NIST score 876 at RT 48,26. Identified with calibration curve in Figure 39.

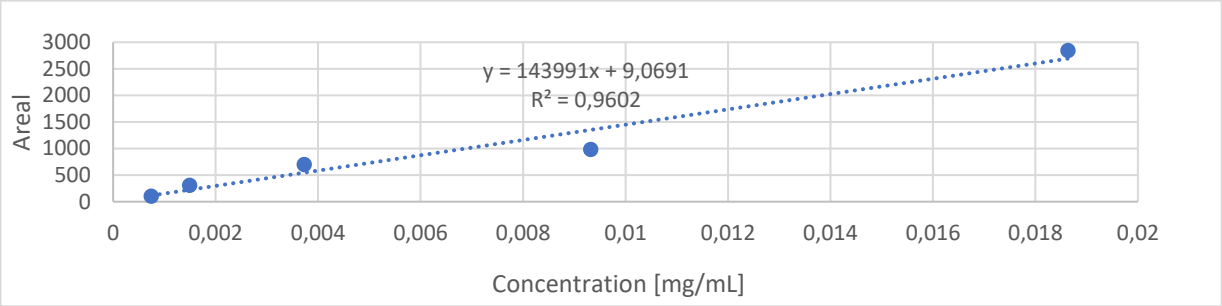

Figure 39. Calibration curve of hydrocoumarin with y = 143991x + 9,0691 and R^2 = 0,9602.

delta-Decalactone

Identified in TM and Peach, with NIST score 924 and probability 72,20 % at RT 48,52. Since this component overlapped with Hydrocoumarin in external standard chromatogram, RIC was used to find a peak for delta-Decalactone, both in the aerosol samples and standard solution. Delta-Decalactone was quantified, concentration given in Table 7, calculated from calibration curve Figure 40.

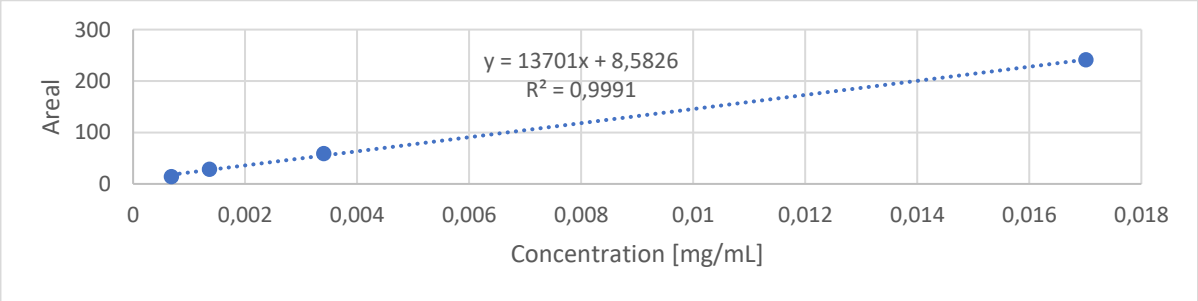

Figure 40. Calibration curve of delta-Decalactone with y = 13701x + 8,5826 and R^2 = 0,9991

O

Gamma-undecalactone

Peach and UB were the two flavours gamma-undecalactone was tentatively identified at RT 50,03. With NIST score 950 and 77,9 % probability in Peach. Quantified both aerosol samples, shown in Table 7 calculated from calibration curve in Figure 41.

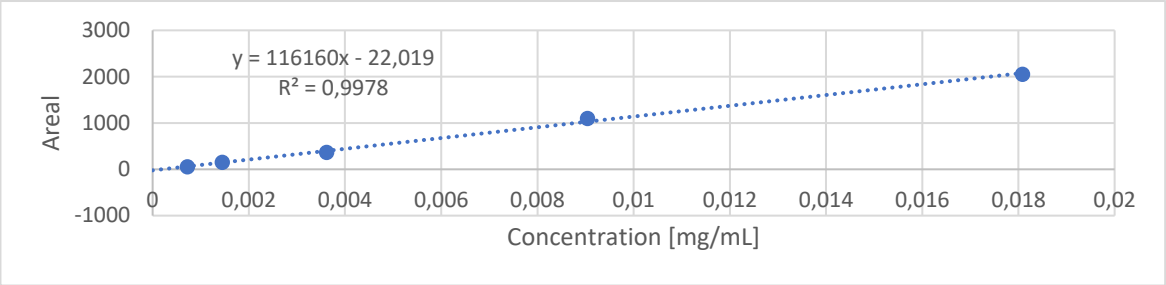

Figure 41. Calibration curve of gamma-undecalactone with y = 116160x – 22,019 and R^2 = 0,9978.

3,4-Dimethoxybenzaldehyde

Identified in PL at RT 55,44 with NIST score 936 and probability 91,7%. 3,4-dimethoxybenzaldehyde was deemed not detected as area and calibration curve, Figure 42, gave a concentration under LOD.

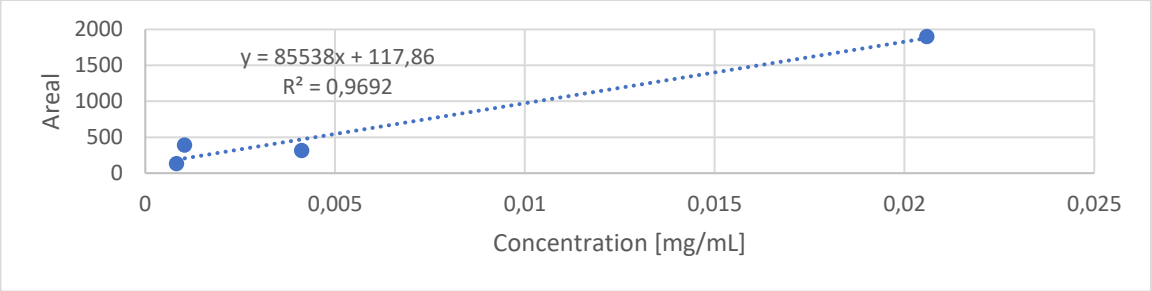

Figure 42. Linear regression of 3,4-Dimethoxybenzaldehyde.with y = 85538x + 117,86 and R^2 = 0,9692.

Pentadecane

Identified in aerosol samples of Peach and BB. In the external standard solution pentadecane co-elueted with 1,3-ditert-butylbenzene. RIC was used to separate these components in the chromatogram. With integrals from RIC, pentadecane was not detected in either aerosol and calibration curve Figure 43.

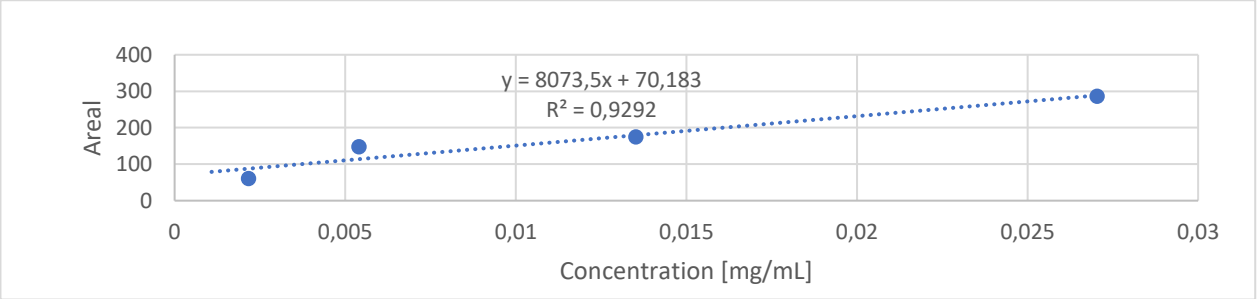

Figure 43. Calibration curve of pentadecane with y = 8073,5x + 70,183 and R^2 = 0,9292.

Q

Appendix II

Concentrations used to make calibration curves

To create calibration curves, different concentration of analytical standards was mixed and analysed on GC-MS under same settings as the other samples. Concentrations from Table 15 and the corresponding integrated area make up calibration curves in

Table 15. Concentration in different mixtures and dilutions to make calibration curves [µg/mL]

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Ethyl butyrate	23,7	4,7	0,9	1,9	0,0	0,0	0,0	0,0	0,0	0,0	11,9
3-heptanone	18,7	3,7	0,7	1,5	0,0	0,0	0,0	0,0	0,0	0,0	9,4
2-heptanone	18,7	3,7	0,7	1,5	0,0	0,0	0,0	0,0	0,0	0,0	9,3
hexyl acetate	19,6	3,9	0,8	1,6	0,0	0,0	0,0	0,0	0,0	0,0	9,8
cis-3-Hexenyl acetate	21,8	4,4	0,9	1,7	0,0	0,0	0,0	0,0	0,0	0,0	10,9
1,3-ditert-butylbenzene	24,0	4,8	1,0	1,9	0,0	0,0	0,0	0,0	0,0	0,0	12,0
Hexyl butanoate	19,8	4,0	0,8	1,6	0,0	0,0	0,0	0,0	0,0	0,0	9,9
Linalool	21,8	4,4	0,9	1,7	163,2	81,6	0,0	0,0	0,0	0,0	10,9
Linalyl acetate	19,5	3,9	0,8	1,6	38,9	19,5	0,0	0,0	0,0	0,0	9,7
Benzaldehyde	28,5	5,7	1,1	2,3	0,0	0,0	0,0	0,0	0,0	0,0	14,2
Benzyl acetate	30,7	6,1	1,2	2,5	306,6	153,3	0,0	0,0	0,0	0,0	15,3
Butylated Hydroxytoluene	20,4	4,1	0,8	1,6	0,0	0,0	0,0	0,0	0,0	0,0	10,2
beta-Damascone	18,8	3,8	0,8	1,5	0,0	0,0	0,0	0,0	0,0	0,0	9,4
Dodecyl acrylate	18,9	3,8	0,8	1,5	0,0	0,0	0,0	0,0	0,0	0,0	9,5
Nicotine	29,1	5,8	1,2	2,3	364,2	182,1	0,0	0,0	0,0	0,0	14,6
alpha-Ionone	17,4	3,5	0,7	1,4	43,6	21,8	0,0	0,0	0,0	0,0	8,7
beta-Ionone	19,1	3,8	0,8	1,5	0,0	0,0	0,0	0,0	0,0	0,0	9,5
p-Anisaldehyde	26,8	5,4	1,1	2,1	0,0	0,0	0,0	0,0	0,0	0,0	13,4

Table 15. Concentration in different mixtures and dilutions to make calibration curves [µg/mL]

Triacetin	29,1	5,8	1,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0	14,6
Gamma-nonalactone	22,6	4,5	0,9	1,8	0,0	0,0	0,0	0,0	0,0	0,0	11,3
Hydrocoumarin	18,6	3,7	0,7	1,5	0,0	0,0	0,0	0,0	0,0	0,0	9,3
Gamma-undecalactone	18,1	3,6	0,7	1,4	0,0	0,0	0,0	0,0	0,0	0,0	9,0
Heptacosane	18,7	3,7	0,7	1,5	0,0	0,0	0,0	0,0	0,0	0,0	9,4
Decane	17,9	3,6	0,7	1,4	0,0	0,0	0,0	0,0	0,0	0,0	8,9
Pentadecane	27,0	5,4	1,1	2,2	0,0	0,0	0,0	0,0	0,0	0,0	13,5
delta-Decalactone	17,0	3,4	0,7	1,4	0,0	0,0	0,0	0,0	0,0	0,0	8,5
Hexanoic acid	22,5	4,5	0,9	1,8	0,0	0,0	0,0	0,0	0,0	0,0	11,3
D-Limonene	18,4	3,7	0,7	1,5	0,0	0,0	0,0	0,0	1,8	0,9	9,2
Eucalyptol	29,0	5,8	1,2	2,3	0,0	0,0	0,0	0,0	2,9	1,5	14,5
Pulegone	21,6	4,3	0,9	1,7	0,0	0,0	0,0	0,0	2,2	1,1	10,8
Carvone	20,1	4,0	0,8	1,6	0,0	0,0	0,0	0,0	2,0	1,0	10,0
Guaiacol	56,5	11,3	2,3	4,5	0,0	0,0	0,0	0,0	5,7	2,8	28,0
3,4-Dimethoxybenzaldehyde	20,6	4,1	0,8	1,6	0,0	0,0	0,0	0,0	2,1	1,0	10,3
Isovanillin	42,5	8,5	1,7	3,4	0,0	0,0	0,0	0,0	4,2	2,1	21,2

T

Appendix III

Dixon's Q-test

A Q-test compares a point of interest in sample data to the rest of the observations and deem an observation an outlier if $Q_{data} > Q_{table}$. Dixon's Q-test assume normal distribution and should not be used more than one time per data set. To calculate Q_{data}, arrange the data set in increasing order ($x_1 < x_2 < \cdots < x_n$) then calculate $Q_{data} = \frac{gap}{range} = \frac{|x_n - x_{n-1}|}{|x_n - x_1|}$ or $\frac{|x_2 - x_1|}{|x_n - x_1|}$.

Table 16. Dixon's Q-test table with values up to 10 and confidence interval 90 %, 95 % and 99% (Rorabacher 1991).

Number of values:	3	4	5	6	7	8	9	10
$Q_{90\%}$:	0.941	0.765	0.642	0.560	0.507	0.468	0.437	0.412
$Q_{95\%}$:	0.970	0.829	0.710	0.625	0.568	0.526	0.493	0.466
$Q_{99\%}$:	0.994	0.926	0.821	0.740	0.680	0.634	0.598	0.568

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