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Study on Different Applications of Volatile Organic Compounds Using Thermal Desorption with Gas Chromatography

DOCTORAL DISSERTATION

Natural sciences,
Chemistry (N 003)

VILNIUS 2022

This dissertation was written between 2017 and 2021 at the Center for Physical Sciences and Technology.

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This doctoral dissertation will be defended in a public meeting of the Dissertation Defence Panel:

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The dissertation shall be defended at a public meeting of the Dissertation Defence Panel at 11 a.m. on 3rd May 2022 in meeting room C202 of the Center for Physical Sciences and Technology.

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VILNIAUS UNIVERSITETAS
FIZINIŲ IR TECHNOLOGIJOS MOKSLŲ CENTRAS

Adrián
VICENT CLARAMUNT

Termo desorbcijos ir dujų chromatografijos panaudojimas įvairių lakiųjų organinių junginių taikymų tyrimui

DAKTARO DISERTACIJA

Gamtos mokslai,
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VILNIUS 2022

Disertacija rengta 2017– 2021 metais Fizinių ir technologijos mokslų centre.

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Agraïments

M'agradaria que una xicoteta part de este treball siga en valencià, perque és una part important de les meues arrels.

Aquest treball ha sigut realitzat en un període de la meua vida prou complicat. A més del treball que comporta fer una investigació per a un doctorat, hem viscut una pandèmia global, he experimentat l'estrès d'un canvi de pis, la ràbia d'haver de renunciar a un esport preferit per culpa d'una lesió greu, la gran tristesa de perdre una persona molt amada i l'enorme alegria de portar-ne una altra al món.

No vull agrair aquest treball a cap grup de gent en concret, sinó a tots aquells que m'han ajudat a afrontar aquest difícil període de manera més fácil. Probablement, si estàs llegint això sigues una d'elles. A tots vosaltres, moltes gràcies

Per últim, vull donar-me les gràcies a mi mateix, per no rendir-me i aconseguir acabar aquesta treball.

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I would like a small part of this work to be writte in Valencian, because it is an important part of my roots.

This work has been done in a rather complicated period of my life. In addition to the work involved in doing research for a PhD, we have experienced a global pandemic, I have experienced the stress of changing a flat, the rage of having to give up a favorite sport because of a serious injury, the great sadness of losing a loved one and the great joy of bringing anotherone to the world.

I don't want to thank this work to a particular group of people, but all those who have helped me cope with this difficult time more easily. Probably, if you are reading this right now, you are one of them. To all of you, Thank you very much.

Finally, I want to thank myself for not giving up and getting this job done.

Thanks.

LIST OF ABBREVIATIONS

BTX – benzene/toluene/xylene
FAO – Food and Agricultural Organization
FFA – furfuryl alcohol
FT-IR – Fourier transform infrared spectroscopy
HIS – hyperspectral imaging
HPLC-DAD – high performance liquid chromatography with diode-array detection
HS – head-space
IA – Invasive aspergillosis
ICP – inductively coupled plasma
IMS – ion mobility spectrometry
JECFA – Joint FAO/WHO Expert Committee on Food Additives
LDPE – low density polyethylene
LOD – limit of detection
MRPs – Maillard reaction products
NAFLD – non-alcoholic fatty liver disease
NIST – national institute of standards and technology
NMR – nuclear magnetic resonance
O_3 – ozone
OFP – ozone formation potential
OSHA – occupational safety and health administration
PAHs – polycyclic aromatic hydrocarbons
PCA – principal component analysis
PDO – protected denomination of origin
PEL – permissible exposure limit
PGI – protected geographical indication
PM – particulate matter
PTR-MS – proton transfer reaction mass spectrometry
Py – pyridine
SIFT-MS – selected ion flow tube mass spectrometry
SPME – solid-phase microextraction
SPE – solid-phase extraction
SOAF – secondary organic aerosols formation
TB – pulmonary tuberculosis
TD – thermal desorption
TD/GC-MS – thermal desorption coupled to gas chromatography mass spectrometer
TWA – time-weight average
VOCs – volatile organic compounds
WHO – World Health Organisation

TABLE OF CONTENTS

Aim and tasks of the doctoral dissertation

The aim of this dissertation is the collection, study and understanding of Volatile Organic Compounds (VOCs) from sources of different origin using gas chromatography with thermal desorption (TD) technique and the possible applications for their use. To achieve that, we focused on the collection systems already available and try to build our own system. Once this step is achieved, and together with available analytical tools (TD/GC-MS systems) we proceed with the collection of VOCs and their study. The main tasks for the research:

- To develop our own sampling system to collect VOCs in breath samples;
- To optimize the conditions for volatile sampling using thermal desorption, and their analysis employing gas chromatography coupled with mass spectrometry;
- To collect breath samples and identify possible biomarkers for disease, food ingestion or environmental exposure;
- To collect VOCs from food emissions and identify markers for possible origin determination;
- To collect environmental samples and identify markers for possible location discrimination;
- To analyse the results finding relations between the three different fields (human breath – food – environment).

Scientific novelty

We have developed a system for sampling of VOCs in breath during this research. Even though this system is similar to the found in the market, we managed to create our system with cheap and easy to obtain materials and simple process to make it. As we were going to be handling human breath samples it was important to avoid cross-contamination. Because of that our system was adequate for single-use analysis. The system is easy to build and obtains reliable results. In addition to that, we have validated the system for the analysis of solid samples with successful results.

For the first time, we have tried to analyse the links between environmental, human breath and food VOCs.

We have developed a specific calibration method for the quantification of VOCs in gaseous matrix, which could be employed also for solid samples.

For the first time, we have employed the profiling of VOCs from breath for the detection of toxic compounds in food.

Statements to be defended

1. Our developed breath collection methodology is suitable for the study of VOCs in human breath.
2. The same sampling system could be used for the collection of VOCs from solid samples.
3. Specific VOCs could be used as markers for different sources.
4. VOCs in the environment influence VOCs profile of human breath, as well as VOCs composition in food.
5. The VOCs composition can be a tool for discrimination of factors like origin, exposure, or disease.

1. INTRODUCTION

Volatile organic compounds (VOCs) are chemical compounds constantly surrounding us in our everyday environments. Exposure to a high concentration of those compounds could cause concerning health and environmental problems. Their study and constant monitoring are important for air quality control, both indoors and outdoors. In addition to that, the study of their emissions from food can be useful to determine possible fraud or contamination. Moreover, the study of VOCs emitted from human breath could be used as a tool for disease diagnosis.

During our research, we studied the currently available devices for VOCs collection, and developed our own cheap, easy to use, and reliable system. Self-modified low density polyethylene (LDPE) bags were selected as the perfect device for the purpose of human breath VOCs collection.

Thermal desorption coupled to gas chromatography mass spectrometry (TD/GC-MS) system was the methodology used for the analysis and identification of the collected VOCs.

Human breath samples were collected and analysed to understand the VOCs profile and its variations depending on several factors, like smoking or food habits, exposure to fragrances or traffic contamination, practise of sports or even presence of a disease.

An innovative calibration method was created for quantification of toxic compounds present in breath, coming from food ingestion.

The same developed system for human breath was employed for the collection of VOCs from food emissions. The study of food VOCs was successfully achieved for the discrimination of different species of peanuts and coffee blends.

To finalise our research, air samples from several environments were analysed to study the diverse VOCs profiles. Different sources, especially from fossil fuels, vegetation and fragrances, can give a distinctive VOCs pattern for recognition of specific locations. In addition, people exposed to those locations could modify their VOCs breath profile.

1.1. Development of VOCs collection device

The main instrumentation we have used for the doctoral thesis was TD/GC-MS. However, we firstly needed to collect the VOCs into thermal desorption (TD) tubes. Air samples were relatively easy to collect using an air sampling pump by just connecting the tube to the pump and leave it in the spot you want to collect the VOCs from. Nevertheless, VOCs from breath samples were more challenging to collect because we wanted to collect the compounds coming from human breath, avoiding water vapour and external contamination. For that purpose, we investigated the literature for methods to collect and analyse human breath VOCs. The most widely method used are polymer bags, particularly Tedlar® bags. Polymer bags are a very good option for the collection of breath samples, due to their simplicity, and low price.

Recently, a breath collection device was developed by Owlstone medical, a portable mask with a clean air supply and a department for TD tubes. We built and investigated several devices to find the cheapest, easiest and most reliable system to collect breath samples.

The main objective of this section was the development of a device, capable of collecting the VOCs from human breath. This device should be constructed with affordable materials, enough sensitivity to identify as many compounds as possible, and robust enough to obtained reliable results and good repeatability.

1.2. VOCs from human breath

The main objective of this chapter was to be able to understand the VOCs present in human breath, identify as many compounds as possible, their origin and variations. Once we achieved that, we could find some specific biomarkers for different situations (exposure to contaminants, eating habits or even disease diagnosis).

Once our breath collection system was fully developed, we started collecting samples from several volunteers. Breath samples were obtained from volunteer from different work sector, located in different offices within the city. In some cases, two samples were collected from each volunteer, one in the morning, and a second one in the afternoon. In addition to that, a short questionnaire was filled by each of the volunteers [Annex A]. Moreover, we

also collected breath samples for the detection of fragrance and food toxins exposure.

1.3. VOCs from food

The need to determine the authenticity of the food makes essential the implementation of new reliable methodologies. Even though many methods are already available, most of them are very expensive or highly qualified people to perform the analysis are needed. And alternative method for food authenticity determination could be done through their VOCs composition. Furthermore, while analysing the VOCs emitted from food, we could identify possible toxic compounds present in the products we consume.

The main objective of this chapter was the analysis of VOCs emitted by food. To collect the compounds emitted from food we employed the same system we developed for human breath samples collection. Our interest focused on the identification of specific compounds to provide food authentication. In addition to that, we also identified and quantified toxic VOCs produced in thermally processed food.

1.4. VOCs from environment

1.4.1. Indoor samples

Many VOCs sources are present indoors. From food products and cooking processes, to cleaning products and fragrances. The presence of fragrances in homes and offices are very common practices as a concept of cleanness, freshness and comfort. However, an overexposure to the chemicals present on those fragrances can rise a health concern. In this part of our research, we analysed several air-fresheners to find their composition, and the impact in the people's breath composition. We exposed several volunteers to a place with a high concentration of air-freshener; we then collected their breath to find specific fragrance compounds.

1.4.2. Outdoor samples

In this chapter we collected VOCs from several points within Lithuania. Several samples in an industrial area in Klaipeda were collected and compared at different times of the day during several days. In addition, air samples were collected from Vilnius city centre, within a relatively small area, to be able to find some specific marker for each different point. We wanted to probe that even within a small area, we could find different compounds, which are specific from different emissions.

1.5. Links between VOCs in human breath, food and environment

To finalise our research, once we collected all the data, we analysed the possible relation between VOCs sources. The presence of some specific compounds in breath could tell us if we are sick, which products we are using (cosmetics) or consuming (food or drinks), in which environment we live (or spend most of our time), and if we are doing some sports. Through the determination of VOCs content, we could determine the origin of food products, as well as the possible toxics present in them. Finally, because of the specific VOCs coming from food, or the anthropogenic VOCs produced within our bodies, we could establish if a specific location is frequented by people or if food is present there.

2. LITERATURE REVIEW

2.1. What are volatile organic compounds?

Volatile organic compounds (VOCs) are compounds with high vapor pressure and low solubility in water. Any organic compound having an initial boiling point less than or equal to 250 °C (482 °F) measured at a standard atmospheric pressure of 101.3 kPa is considered a VOC [1]. Thousands of VOCs have been identified from a wide range of sources [2, 3]. Benzene, toluene and xylenes (BTX) [Figure 1] are the most common VOCs to which we are exposed to everyday. BTX are aromatic hydrocarbons considered cancerogenic which are produced in petroleum refining industry. As those compounds are a concern for human health their levels are one of the main parameters when considering environmental contamination, together with particle matter, CO_2, ozone (O_3) and nitrogen oxide [4]. There are many studies about VOCs as pollutants in cities [5-7], as well as indoor pollution [8, 9]. Those studies show the high levels of contaminant VOCs due to car emissions, and the danger of not having a proper ventilation when being indoors. Recent studies show the influence of the Covid-19 pandemic in the levels of contaminants, as the traffic decreased drastically, lowering the emissions of toxic VOCs [7]. Most of those contaminants VOCs, especially the most toxic ones, are produced in human activities (anthropogenic source), mainly from fossil fuel emissions.

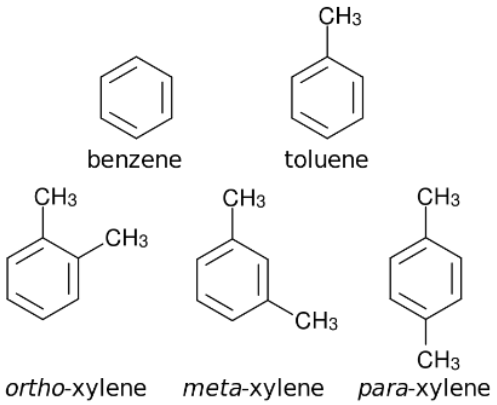


Figure 1. Volatile Organic Compounds: BTX

VOCs not only have an anthropogenic origin, but they can also have a biogenic origin. Terrestrial vegetation is the dominant source of atmospheric

VOCs accounting for about 90 % of the total emission globally [10]. Isoprene and monoterpenes are the most abundant species among the biogenic VOCs. Both isoprene and monoterpenes are mainly emitted in the tropical region (88 % and 83 % of the global total, resp.) Other typically found VOCs in high amounts in the environment are limonene and α-pinene [Figure 2]. Those compounds, among other volatiles from plants, have a nice smell, and therefore used in the fragrance industry. They are also considered to have antibacterial and antifungal properties [11, 12]. Essential oils extracted from plants have a big quantity of VOCs [13, 14]. Because of that, an abundant amount of those VOCs is added to cosmetics, household cleaning products, as well as food as flavouring agents. Not only plants emit endogenous VOCs, but also bacteria and fungi [15-17].

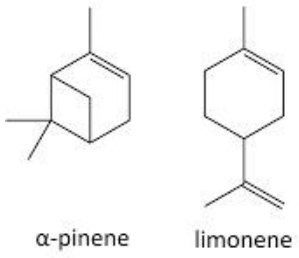


Figure 2: Volatile Organic Compounds: Limonene and α-Pinene

As we can see, we are exposed to a big number of VOCs in our everyday life [18]. Most of them are coming from anthropogenic sources: car pollution, household products like cleaning agents, cosmetics, fragrances, solvents from paints, food products that we could find also in our working places among plenty of many more processes [9]. Because of that, many of those VOCs are incorporated inside our bodies. Thousands of VOCs are also emitted from the human body daily [19]. Their composition is affected from the exposure to the environment, but also from the many processes inside our bodies. Many VOCs or deviations in their levels have been linked to specific diseases. Hence, the VOCs are considered biomarkers and are used for disease diagnose and monitoring [20].

2.2. VOCs sampling systems

Depending on the application we can use several techniques that will allow the sampling of VOCs. When collecting VOCs from the environment some studies collect the total amount of volatiles by trapping them into a sorbent for later analysis in the laboratory. Using this technique, air samples are pumped through a tube containing a specific sorbent, which collects all the volatiles present in air. Those tubes are then thermally desorbed and, most of the time, analysed by gas chromatography (TD/GC-MS) [21, 22]. A more sophisticated option is using proton transfer reaction mass spectrometry (PTR-MS) or selected ion flow tube mass spectrometry (SIFT-MS) in which VOCs are analysed on-line in real-time, however these techniques are highly costly to purchase. Other techniques can track on-line levels of specific VOCs using instruments with sensors called Continuous Ambient Air Monitoring Systems (CAAQMS) at Photochemical Assessment Monitoring Stations (PAMS); however, only a few volatiles (mainly BTX) are recorded [23, 24], and not the whole range of volatiles. This last type of VOC monitoring is useful for checking the levels of toxic BTX in real-time.
When the VOCs collected are from solid or liquid samples (cosmetics, food and beverages, plants, soil, and other surfaces), the most common method for analysis is solid-phase microextraction (SPME) [11, 25]. Similar to TD, this technique collects the volatiles into a sorbent which is directly desorbed into the chromatographic system.

Finally, when collecting human breath emissions, several techniques are also used for the collection and analysis of VOCs [Figure 3]. To collect breath samples, polymer bags are the most common. Many different polymers have been used for the collection of breath samples. Tedlar® (PTFE-polytetrafluoroethylene), Nalophan® (PET-polyethylene terephthalate), Cali-5-Bond™, FlexFoil® (PET/NY/AL/CPE-polyethylene terephthalate/nylon/aluminium foil/chlorinated polyethylene) and Teflon® polymers are the most used, especially due to their simplicity and low price [26]. Furthermore, many studies discuss about the suitability for breath samples [27, 28]. In addition to that, glass vials (for SPME), thermal desorption tubes (different adsorbents, used in TD/GC-MS), micropacked sorbent traps or metal canisters are used. An instrument recently developed by Owlstone medical (ReCIVA® breath analyser) also focuses on the collection of breath VOCs. Polymer bags and ReCIVA® breath analyser collect the breath VOCs that later will be analysed using TD/GC-MS technique. Other techniques used for the analysis of VOCs in breath are SIFT-MS [29], PTR-MS [30], laser absorption spectroscopy (LAS), ion

mobility spectrometry (IMS) and electronic noses containing a variety of gas sensors and semiconductor-based sensor arrays, although gas sensors are often much less sensitive, usually lack specificity, and are prone to drift [31].

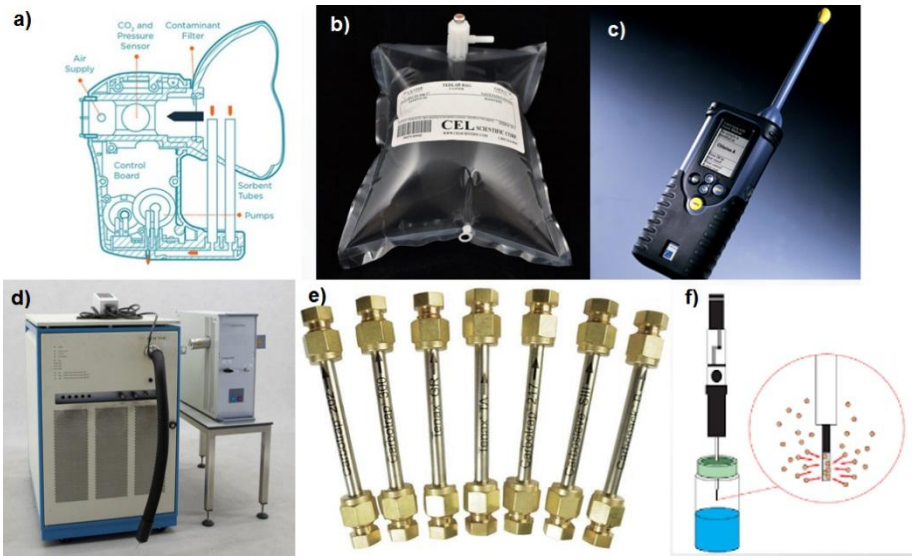


Figure 3: VOC collection devices: (a) ReCIVA® Breath sampler. (Owlstone Medical, UK) (b) Tedlar Bag (CEL Scientific); (c) Cyranose Electronic nose (Sensigent); (d) PTR-MS (Gemini Lab); (e) Thermal desorption tube (Markes International Ltd., Llantrisant, UK); (f) Head-space SPME

2.3. VOCs in publications

The study of VOCs can be found since in Ancient Greece during Classical period, when Aristotle used the smell of people's breath (volatiles) to diagnose diseases. We can find many scientific publications since several decades ago [32] until nowadays; the number of annual publications on the topic of VOCs has doubled in the last decade [Figure 4]. Because VOCs are an important element in our environment, together with an improvement in analytical technologies, their study has increased considerably. As we can find in the literature, there are 3 main applications of VOCs analysis.

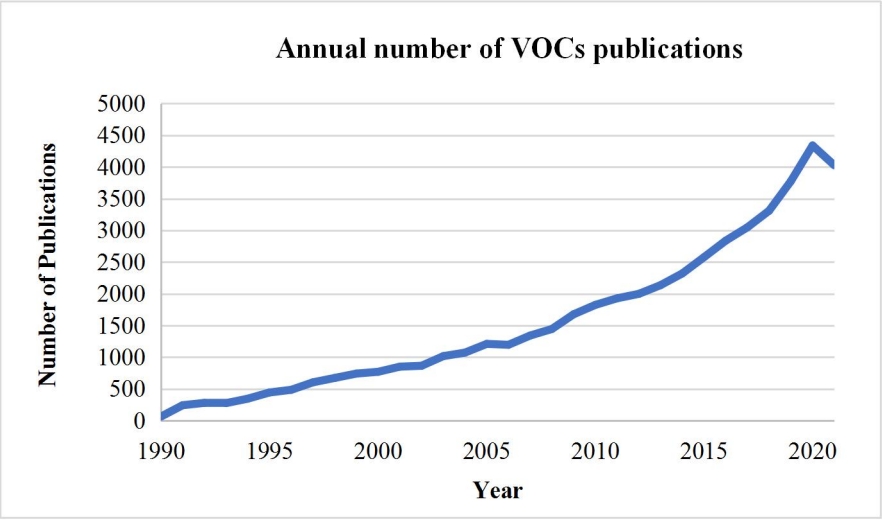


Figure 4: Increase in annual publications in the area of VOCs. Graph shows the results of a Web of Science search for publications including the term "volatile organic compound"

2.3.1. Analysis of contaminants in the environment.

Aromatic hydrocarbons are the most common VOCs to which we are exposed to everyday in urban locations. People living in big cities are usually concern about the quality of the air and guidelines are available to control it [33]. When being outdoors, we are exposed to many contaminants coming from the fumes of cars exhaust and burning of fossil fuels. Air pollution is mainly caused by anthropogenic sources, such as traffic emissions and industrial activities, which release harmful or excessive quantities of noxious substances including toxic gases and particulates in many atmospheric regions of the world. The World Health Organization (WHO) has revealed that 4.2 million deaths each year worldwide are caused by polluted odorous air [34]. There is evidence that VOCs from polluted odorous air have persistent adverse health effects, such as chronic diseases in extreme cases, increase of respiratory diseases (asthma) [35] and elevation of cancer risk [36]. Furthermore, those toxic VOCs are important precursors to O_3 and secondary organic aerosols formation (SOAF) in the atmosphere [37, 38]. BTX (or BTEX if Ethylbenzene is included) [Figure 1] are the major VOC species usually found on the cities, especially toluene, which is the dominant potential contributor to ozone formation potential (OFP) and SOAF [23, 39]. However, we can find a lot of research studying the levels of many other volatiles, low carbon components (C2 to C4), such as ethane,

propane, acetylene and butane, VOCs (C5–C12), including alkanes, alkynes, halogenated hydrocarbons, aromatics and VOCs with high carbon number [38]. It is common to compare the levels of VOCs in different points of the country or within cities [6, 37, 39-41], as well as studying the variations of VOCs depending on the time of the day or the season [5, 40]. As traffic is the main source of contaminants, recent studies show of during pandemic lockdown, the levels of contaminants coming from car emissions decreased drastically [7, 23]. Some articles show how meteorological parameters played an important role in the concentration variation of pollutants [42].

Nowadays people spend most of their time indoors; sleeping at home, working in the offices, eating in canteens or restaurants, or spending free time in cinemas, shopping centres and other stores [Figure 5]. It is in those places where we are exposed to a high content of VOCs [43].

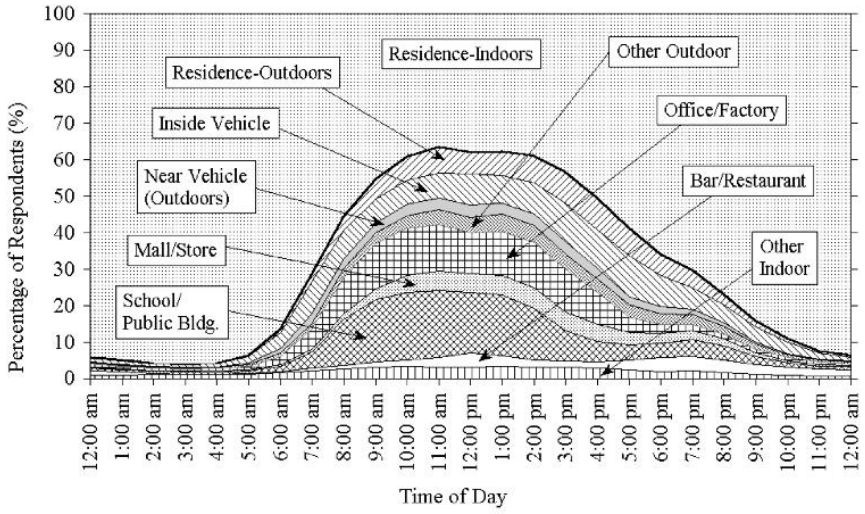


Figure 5: Location of people at different times of the day [From (43)]

The source of those compounds are mainly fragrances from cosmetics, household cleaning products, paints, solvents, food, furniture, air fresheners, outdoors contamination, tobacco smoke and even our own body emissions [8, 9, 33].

2.3.2. Analysis of VOCs in fragrances and in food composition.

2.3.2.1. Fragrances

Plants, flowers and other vegetation emit a great number of VOCs [10]. These VOCs are involved in many functions, the smell of those VOCs can attract insects for their own purposes (pollination) or repel them to avoid their attack or invasion [44]. In addition to that, certain plants will emit different VOCs due to external or internal wounds [45]. Many metabolic pathways are associated with the synthesis of volatiles in plants [Figure 6]. Biological systems use these VOCs for sharing information within their environment and outside the habitats, we could use those VOCs as markers for quality assessment [45, 46], When we produce extracts from vegetation, we are collecting those VOCs, most of the time because of their nice smell (Essential oils). Furthermore, some of those VOCs have antibacterial properties [11]. The composition of VOCs in essential oils can be counted in hundreds of different compounds [13]. We use those essential oils to add a nice smell to cosmetics and fragrances, sometimes as food additives. However, being exposed to fragranced products has been associated with health effects such as asthmatic exacerbations, headaches, mucosal symptoms, and contact allergy [47].

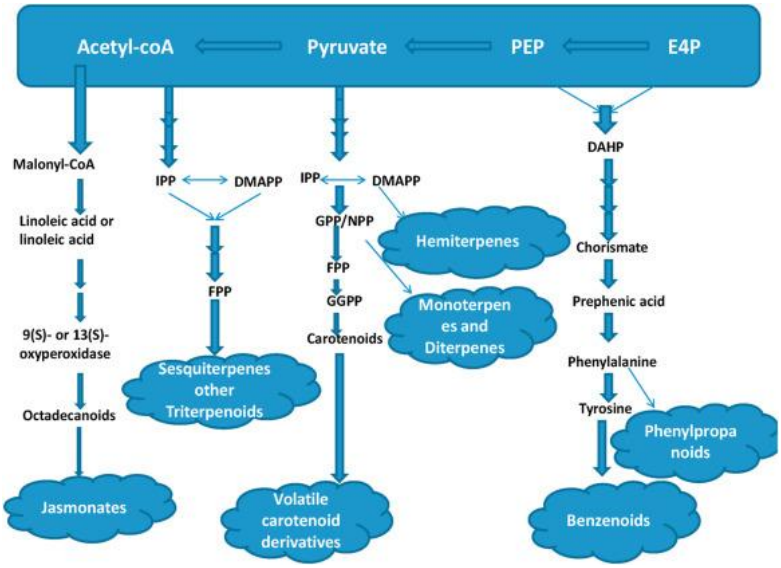


Figure 6: Biosynthetic pathways of plants for VOCs [from [45]]

Fragranced products refer to products with a fragrance or scent, such as air fresheners, deodorizers, laundry detergents, fabric softeners, dishwashing detergents, hand sanitizers, personal care products, baby shampoo, and cleaning supplies. These products are widely used by individuals, industries, and institutions.

As well as fragrances, for many foods that undergo thermal treatment produce the formation of a large number of volatile compounds that affect their quality. The generation of numerous process-induced compounds has strong implications for food quality and safety [21]. In addition to that, the emission of those VOCs could create a toxic environment, either coming from the tools used for cooking, from the process of cooking itself [48], or coming from bacterial and microbial emissions from the food waste [46, 49]. Furthermore, we could stablish the quality of the products depending on their aroma and flavour, therefore, by their VOC emissions [50]. We can find in the literature much information about VOCs from different types of food, their specific aroma and flavour [51].

It is also common to investigate the food and drink origin by analysing its metal composition and isotopic ratio using inductively coupled plasma (ICP) technique [52, 53]. This authentication of origin is useful in order to avoid fraudulent practises [54]. There is an alternative approach for origin authentication by comparing their VOC profile [55, 56], which we will study during this research.

2.3.2.2. Food authenticity

Many consumers are willing to pay extra for products that they consider to be of superior quality, such as organic, protected denomination of origin (PDO), protected geographical indication (PGI) products. The labelling of food products is essential to inform consumers what kind of products they are buying. Food products that are of high value and undergo a number of processing steps are often target of fraudulent labelling. The substitution, in part or whole, of high-cost foods for cheaper and inferior food products in order to defraud the consumer is a problem nowadays [Table 1].

Table 1: Examples of Food Authenticity Issues

Commodity	Issue
Herbs and spices	Adulteration with water to increase weights
	Incorrect botanical declaration
	Intentional addition of low value materials
Fruit and vegetable	Undeclared water, sugar, and acid addition to fruit juice
	Undeclared pulpwash or peel extract addition into fruit juice
	Incorrect declaration of fruit type
Grains	Basmati rice replaced with nonbasmati rice
	Undeclared replacement of durum wheat with common wheat and impurities in wheat flour
	Discrimination of viable-germinating corns and soybeans from dead seeds
	Incorrect declaration of geographical and cultivar origin of cereal rice and wheat
Oils and fats	Undeclared addition of other vegetable oils to single seed oils
	Undeclared addition of poorer quality oils to extra virgin olive oil
	Butter adulterated with hydrogenated oil and animal fat
Milk and dairy	Undeclared addition of water to milk
	Cow milk in sheep, goat or buffalo milk yoghurt, or cheese
	Distinction between cheese made from raw or heat-treated milk
	Discrimination of milk and cheese from different regions, varieties and manufacturing processes
	Addition of melamine or nonmilk fat/oil into dairy products
	Mislabelling of conventional milk as a product from organic farming
Meat and fish	Incorrect declaration of species
	Labelling previously frozen meat as fresh
	Undeclared water addition to meat and fish in excess of legally permitted amounts
	Distinction between fresh and thawed meat
Beverages	Single malt whisky replaced by blended one
	Inappropriate sugar addition to increase alcohol content in wine
	Incorrect declaration of vintage or geographical origin of wine
Miscellaneous	Incorrect declaration of floral or geographical origin of honey
	Undeclared sugar addition to honey
	Undeclared use of genetically modified food

For this kind of products there is a need to determine the authenticity of the food. Products like olive oil, fish, honey, dairy products and meat are being the target of fraudulent activities [57].

Many technologies and methods to detect food fraud are available; spectroscopic techniques including Fourier transform infrared (FT-IR) spectroscopy, Raman spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and Hyperspectral imaging (HIS) are described in their great

potentials in authentication of various food products [58]. As well as other techniques based on isotopic analysis, chromatography, DNA analysis, and enzymatic analysis.

The analysis of volatiles from food as a method for authenticity has been studied for animal milk [59]; they suggested that flavour/aroma differences in cheeses produced from animals grazing in different areas and seasons might be due to a different content of terpenoids from plants. In addition, by using α-pinene as a marker compound, it was possible the detection of adulteration of poppy seed oils with sunflower oils at different levels [60].

Peanuts

The peanut kernel contains 24–36 % protein, 44–54 % fat, 10–23 % carbohydrate, 4–6 % of fibre and mineral content of 2-3 %. These quantities can differ greatly between different peanuts. Peanuts are also rich in vitamins, including niacin, vitamin E, B1, B2, B6, pantothenic acid, and folic acid. It contains minerals, saponins, resveratrol, proanthocyanidins, flavonoids, and other bioactive components. In addition to that, a specific composition of VOCs can be found for the different species of peanuts.

The peanut is widely planted in the world, throughout Asia, Europe, Africa, Americas, and Oceania, etc. According to the statistics of United Nations Food and Agricultural Organization (FAO), the peanut acreage was 29,596,969 ha and the yield was 48,756,790 tonnes in 2019 and is still increasing year by year [61]. The top five countries in terms of peanut yield are China, India, Nigeria, Sudan, and the United States.

With the rapid development of the peanut processing industry, scientific research in the peanut processing field has been undertaken by people all over the world.

The United States' peanuts mainly comprise four types, namely, Virginia type, Valencia type, Runner type, and Spanish type, and the main production area is in the southeast United States [Figure 7].

Most of the research about peanuts in the literature mainly include studies on near-infrared rapid non-destructive testing techniques for the nutritional components in peanuts, such as fatty acid, vitamin E, and sterol, as well as technical studies on the processing features and quality evaluation for the special peanut varieties dedicated to the production of peanut butter and peanut candies [62].

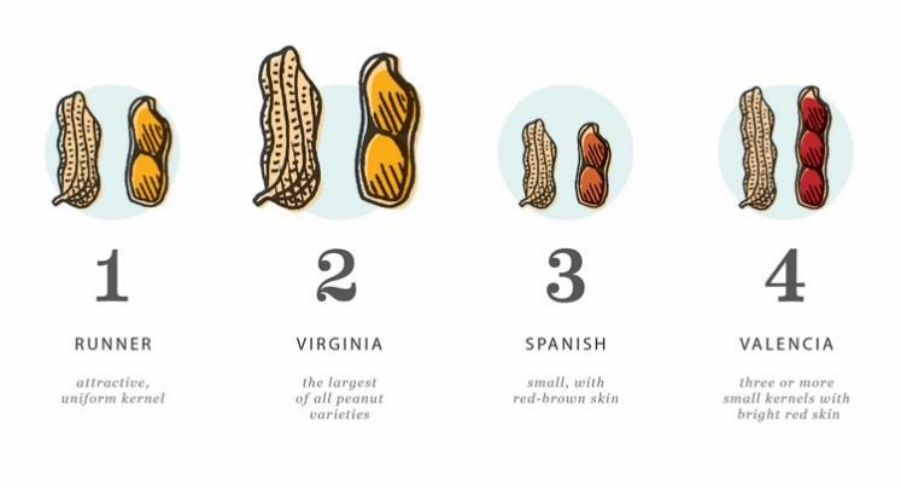


Figure 7: Types of peanuts. From Whitley's Peanut Factory [www.whitleyspeanut.com/funfacts]

Because a different type of peanut might give a specific final taste and quality to the product, it is important to ensure that there is no fraud by using a different type, or the quality of the peanut is optimal.

Coffee

Coffee beans are cultivated mainly in the equatorial areas all around the world in over 70 countries, with a global production of 10,035,576 tonnes in 2019 [61]. The two most common grown coffee bean species are *Coffea arabica* and *Coffea robusta*. *C. arabica* [Figure 8] is the dominant species cultivated as its taste is less acidic. However, mixes of both species are commonly found in the market.

Figure 8: Coffea Arabica plant. From [63]

Drinking coffee is a common cultural practice among adult population across the globe. In 2019/2020, around 168.5 million 60-kilogram bags of coffee were consumed worldwide [64], making coffee one of the most widely consumed beverages. Northern European countries consume the highest quantities of coffee. Finland is the top consumer country with an average of 12 kg of coffee consumed per person a year [65].

Coffee contains over 1500 chemical components, most of which (over 800) are VOCs created after the coffee roasting process, consequently providing the specific taste and smell [66, 67]. Finding a specific blend of coffee beans is a challenge for coffee roasters that want to mesmerize their clients with specific tastes.

2.3.2.3. Contaminants in coffee

Roasting coffee beans facilitates chemical reactions between amino acids and reducing sugars lead to the formation of Maillard reaction products (MRPs). Depending on the way the food is being processed, both beneficial and toxic MRPs can be produced. Pyridine (Py) and Furfuryl alcohol (FFA) are two products found in roasted coffee beans produced by Maillard reaction [68, 69]. Even though coffee is not classifiable as carcinogenic to humans, Py and FFA are classified as group 2B (Possible carcinogenic) [70, 71]. The current occupational safety and health administration (OSHA) airborne permissible exposure limit (PEL) for Py and FFA are 15 and 50 mg/m3 time-weight average (TWA) respectively [72, 73]. The Joint Expert Committee on Food Additives (JECFA) (Joint FAO/WHO Expert Committee on Food Analysis [74] has allocated an Acceptable Daily Intake (ADI) of 0.5 mg/kg bw (bodyweight) for FFA and 0.002 mg/kg/day for inhalation exposure for Py by the U.S. EPA [75].

Many studies have been performed studying health implication of drinking coffee, mostly focusing on the benefits of antioxidants content (polyphenols, chlorogenic acids, caffeine, and melanoidins) [67, 76]. The studies show an apparently beneficial association of consuming three cups of coffee a day. But it might depend on the type of beans used, degree of roasting, preparation method, extra addition of ingredients (sugar or milk), and the tolerance from the person [77]. Even though drinking coffee does not seem to have definitive harmful outcomes for health, the presence of toxic MRPs in high levels could raise health concerns [78-80]. Specially with the presence of MRPs like acrylamide and furan (classified as 2A and 2B carcinogenic) which have raised a big concern after their high levels in processed food [81, 82], being coffee one of their important dietary sources [83, 84]. Previously, determination of volatile compounds in coffee have been studied employing the methods of head-space (HS)/GC-MS [85], dispersive solid phase extraction (SPE) with GC-MS [86] and solid phase microextraction (SPME)/GC-MS [87].

An alternative method for detection of toxic MRPs in food could be breath analysis. Breath analysis focuses on the collection and identification of emitted VOCs in breath after ingesting a specific product. Breath analysis is widely used as a non-invasive method to diagnose and monitor various diseases like cancer, pulmonary tuberculosis (TB), asthma, liver disease and diabetes [88-92]. In addition, Py has been found in the breath of active

smokers [93]. In this study we aimed to identify and quantify Py and FFA in human breath after coffee ingestion and compared the quantities of the same compounds in the coffee. Development of such a method could be adapted as a tool for detection of food intoxication.

2.3.3. Analysis of human breath samples for the diagnosis of diseases.

Human exhaled breath consists mainly of unmodified nitrogen and argon, oxygen, carbon dioxide and water vapour. Together with those compounds there are many gaseous metabolites present at trace levels. Thousands of VOCs are emitted from the human body daily [19]. The sources of those VOCs might be endogenous or coming from external contamination. Endogenous VOCs are produced by metabolic processes that take place in different body parts and, through the blood stream, are transported to the lungs where they spread into the exhaled breath flow by the alveolar gas exchange mechanism [94]. Most of the VOCs found inside our bodies are influenced by many parameters [95][Figure 9]. The levels detected in breath will depend on many factors, including the concentration in ambient air, the duration of exposure, the solubility and partition co-efficient into tissues, the mass and fat content of the individual, as well as the underlying endogenous concentration [31].

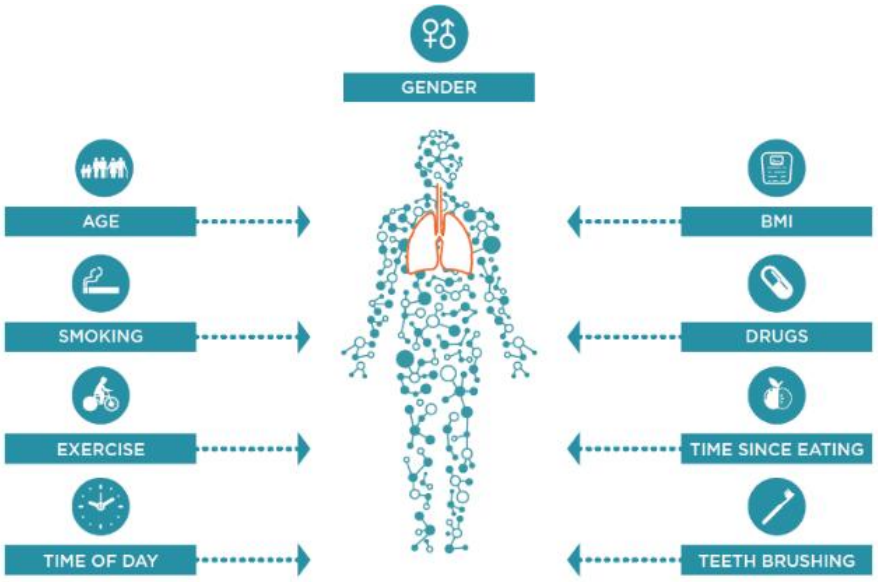


Figure 9: Parameters affecting VOCs breath composition (from [96])

One of the main purposes for studying VOCs emission from humans is the detection of biomarkers for disease diagnosis and monitoring [97]. The presence of unexpected VOCs or even the variations of some of them could be a characteristic pattern of a disease.

Diseases like non-alcoholic fatty liver disease (NAFLD), cancer (such as lung cancer, colorectal cancer, bladder and prostate cancer) or invasive aspergillosis (IA) are very difficult to diagnose, or they are diagnosed at very late stage of the disease, when the possibilities for their treatment decrease drastically. Because of that it is very important to be able to detect those diseases in an early stage and with reliable results.

Breath analysis research has increased its popularity in recent years due to its low cost, ease of use, and it's a non-invasive method for possible disease diagnosis.

Analysis of breath samples for testing of volatiles can be performed frequently in follow-up studies, which may reflect disease progression and be helpful in monitoring therapeutic intervention. Moreover, breath tests are non-invasive and thus suitable for critically ill patients (in intensive care units) and small children. Furthermore, they have been proven to be useful for diagnosing a broad range of diseases [Figure 10] [98].

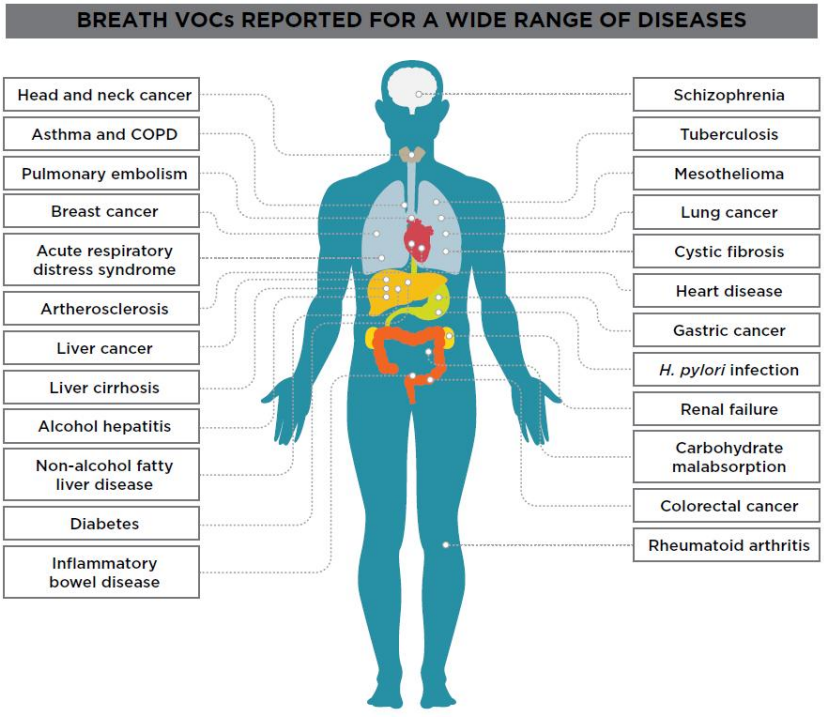


Figure 10: List of diseases detected from breath (from [96])

Acetone levels are elevated in diabetes, due to rise of blood sugar level and intensive lipolysis. In all patients from [99], the breath acetone declined linearly with blood glucose concentration. Hence, this study indicates that breath acetone does vary as glycaemia and/or metabolic status changes in type 1 diabetes. Abnormal breath isoprene levels are related to end-stage renal failure and increases in isoprene levels have been associated with oxidative stress [100]. Acetaldehyde might be responsible for the cancerogenic effect of alcohol [101]. Pattern of VOCs in patients suffering from colorectal cancer particularly levels of some specific VOCs 1,3-dimethylbenzene, 1,2-pentadiene, cyclohexene and methylcyclohexene [102].

Breast cancer is accompanied by increased oxidative stress caused by lipid peroxidation of polyunsaturated fatty acids in membranes, producing alkanes and methylalkanes, such as 3-methylundecane, 6-methylpentadecane, and 2-methylpropane, among others, potential biomarkers as suggested by [103]. Sulfur-containing compounds, such as dimethylsulfide, hydrogen sulphide, and mercaptans (e.g., methylmercaptan and ethylmercaptan) are proposed as liver cancer biomarkers [104].

Infectious diseases like TB is caused by Mycobacterium. TB. potential biomarkers have been found by breath sampling, such as methyl phenylacetate, methyl *p*-anisate, methyl nicotinate, and *o*-phenylanisole [105]. Contradictory studies presented different marker compounds, possible due to the fact that *Mycobacterium tuberculosis* is a slow growing organism. This means that if VOCs are produced, they may be released or modified by the host, hence, they may well be present at low concentration and not be detected.

Among those diseases, many more like asthma, cystic fibrosis, liver cirrhosis, NAFLD or schizophrenia have been studied for the presence of possible VOCs biomarkers [106].

As we can see, the research of biomarkers coming from breath could be a great tool for diagnosis of several diseases. However, many parameters could influence in the results. Therefore, the understanding of the different backgrounds and sources of VOCs is essential for the success in this area.

After checking the literature, we have learned about all the different available devices for VOCs collection, analysis and identification. We have found many different applications for the study of VOCs and their importance in our everyday life. By getting all this knowledge we have developed our own VOCs collection device and study many different VOCs sources.

3. INSTRUMENTATION AND METHODOLOGY

3.1. Thermal desorption tubes and sorbents

The main element in our TD system is a stainless-steel sorbent tube (8.9 cm long, 0.635 cm outer diameter) filled with a sorbent. Many different sorbents are available in the market. Their variations depend on the affinity for different components and could be chosen depending on specific needs [Annex B].

The compounds trapped in the sorbents are released by heating them to high temperatures. Because of that, they can be reused many times. In addition to that, the sorbents must be conditioned before use to avoid contamination. Due to their constant use and the high temperatures, they are exposed to, it is common to observe degradation of the sorbents. When the sorbent is degrading by heat or by reaction with other compounds, they produce artifacts that can be detected by the GC-MS system, giving misleading signals that can interfere with the collected samples. To avoid the degradation of the sorbents it is important to follow the manufacturer instructions and not surpass their limit temperatures. Many different artifacts have been identified for sorbents like Tenax® TA and Porapak Q [107, 108].
One way to reduce the influence of artifacts in our results is by collecting blanks before our samples. By analysing empty TD tubes, we will get the signals produced by the artifacts. After that we could subtract the signal from our samples [Figure 11]

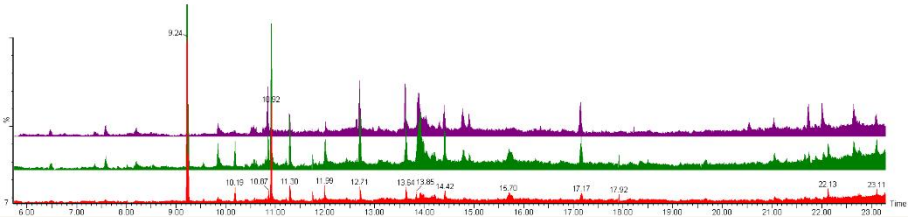


Figure 11: Subtracting interferences from sorbent artifacts. Purple: blank; green: air sample; red: artifacts signal removed from the sample signal.

When the quantity of artifacts is too big it is recommended to renew the sorbent.

Tenax® TA (Markes International Ltd., Llantrisant, UK) sorbent [Figure 12] was already used for the study of toxic volatile PAH in the atmosphere as early as 1967 [109]. And it is mostly used still nowadays because of its wide range of applications and stability.

During this research, two other sorbents were also compared Carbograph 1 TD, and Carboxen 1003. However, those 2 sorbents retain very strongly VOCs, and are meant for very volatile compounds. Because the aim of our study is to obtain the biggest spectra possible of compound, Tenax® TA sorbent would fit better for our needs. Furthermore, we want to focus also on breath samples, which have a high humidity content and will significantly interfere with our system, it will get strongly retained in the sorbent, and could potentially spoil the GC column and detector. Because Tenax® TA sorbent is hydrophobic, we will avoid the high humidity related problems.

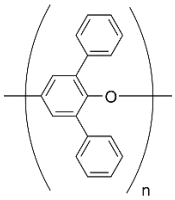


Figure 12: Poly(2,6-diphenyl-p-phenylene oxide) resin. Mainly known as Tenax® TA

The sorbents are loaded inside a stainless-steel tube, which will be used to collect and analysed the trapped VOCs [Figure 13]. Several sorbents could be loaded inside the same tube, making the study even more specific depending on the research.

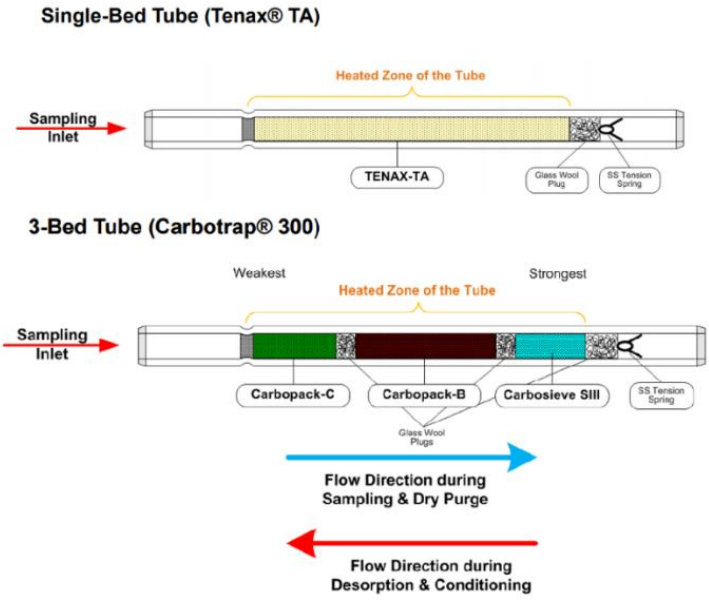


Figure 13: Thermal Desorption tubes

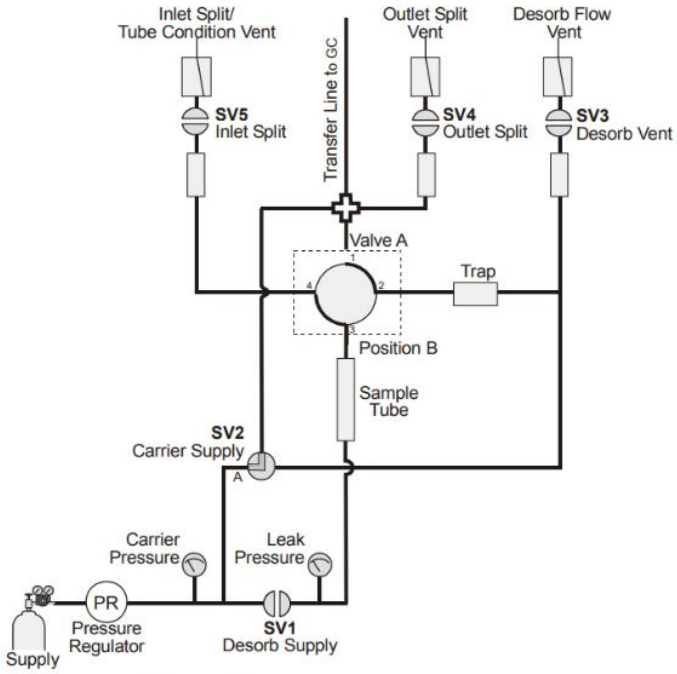
For our research we decided to use only Tenax® TA sorbent TD tubes. Mainly, because of the big range of VOCs compatibility (C6 to C30) and its stability.

3.2. TD/GC-MS

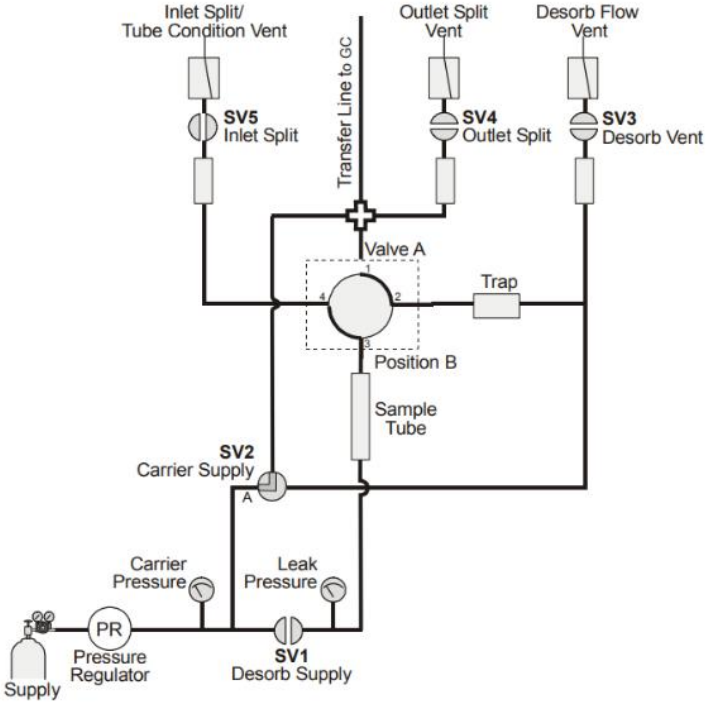
Samples are pumped through the TD tubes using an air pump (SKC Inc. Aircheck Sampler 224-44XRM model) [Figure 14]. When samples are passing through the sorbent, the main components get retained inside until heated.

Figure 14: SKC Inc. Aircheck Sampler 224-44XRM model

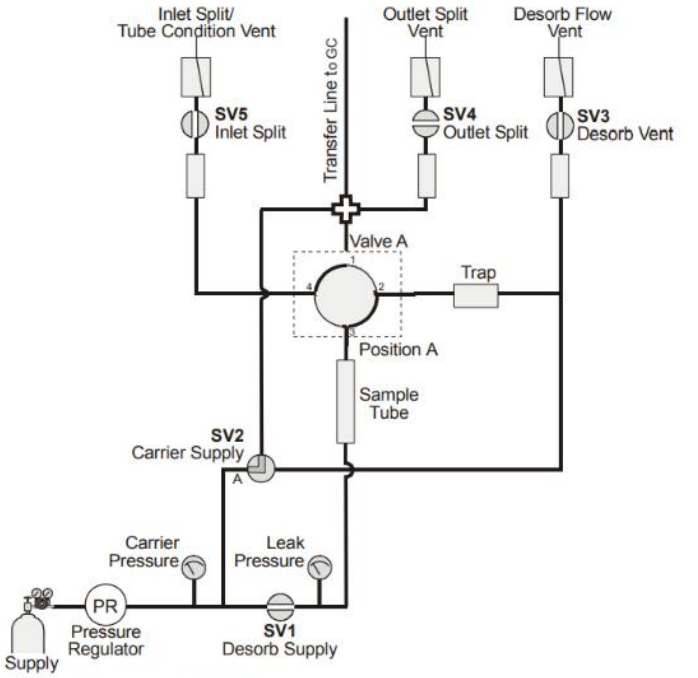
A TD system consist of a heating point for the sample and a cooled trap for compound focusing. TD tubes are loaded into the TD system (ATD 400; Perkin Elmer) where the tubes are heated, releasing the retained compounds into the GC system. There are 4 main TD configurations during our work [Figure 15].



1 - Standby Configuration



2 - Tube Conditioning



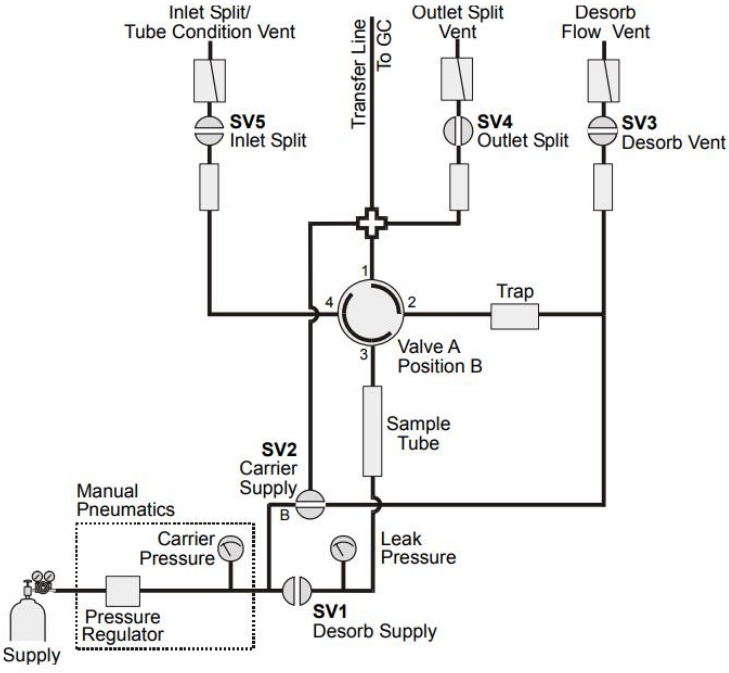


Figure 15: Scheme of Thermal Desorption System. Main 4 step configurations

The first configuration is the “Standby mode”, where the carrier gas goes through the TD directly to the GC system. During “Tube conditioning mode”, the TD tubes are heated, and the eluted components get out of the system through the Tube Condition vent, and about entering the GC system. By doing that, we are removing possible contamination present in the sorbent, and we ensure the TD tube is clean before sampling. During the “primary desorption mode”, the TD tube carrying the sample is heated to release the sample analytes into the cold trap, where they get retained and focused into another sorbent. In the last step, the “trap desorption mode”, the cold trap is quickly heated, and all the compounds are introduced into the GC system.

Once the compounds leave the TD, they get separated an identified using a GC-MS system (GC Agilent 6890N coupled to mass spectrometer Waters/Micromass AutoSpec Premier) [Figure 16].

Figure 16: Gas Chromatography high resolution mass spectrometry system

The capillary column DB-5MS, 30 m, 0.25 mmID, 0.25 mm (Agilent Technologies) was used in our GC system. For most of the analysis performed during this research a good separation of the compounds was achieved by holding the column temperature at 40 °C for 3 min and then increased by 5 °C/min to 120 °C, followed by ramping at 10 °C/min to

220 °C. Once the separated compounds reach the detector, the molecules are ionized in the source chamber by electron impact [Figure 17].

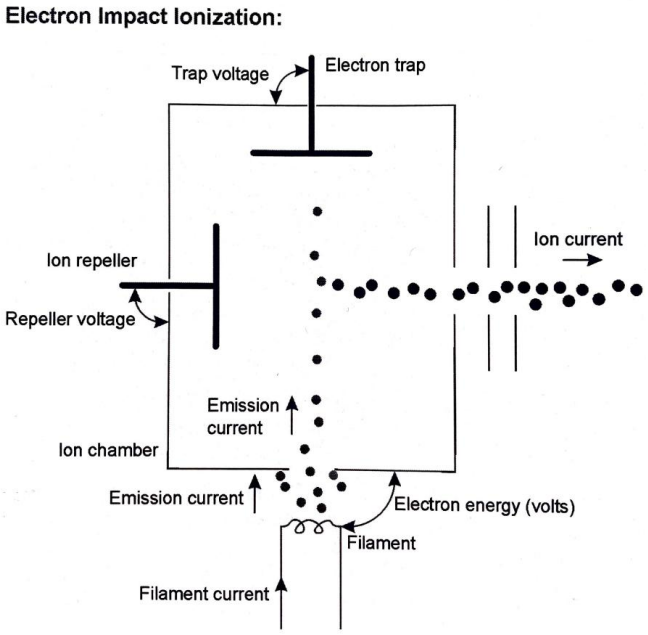


Figure 17: Mass spectrometer ionization source

Inner source chamber had to be cleaned at least once every six months, or when a drop in sensitivity is detected. The outer source assembly was cleaned every 2 years.

When the sample analytes are ionized, they are directed and focused through a series of slits and lenses to a powerful magnet that will discard the ions outside the selected range of analysis. Those last compounds will go through a second set of lenses until reach the detector [Figure 18].

The MS analyses were performed in full-scan mode, using a scanning range of *m/z* 50–200. The ion source was maintained at 250 °C, and ionization energy (EI+) of 70 eV was used for each measurement. Compound identification was performed by library match using national institute of standards and technology (NIST) MS Search 2.0 (2005) mass spectral library.

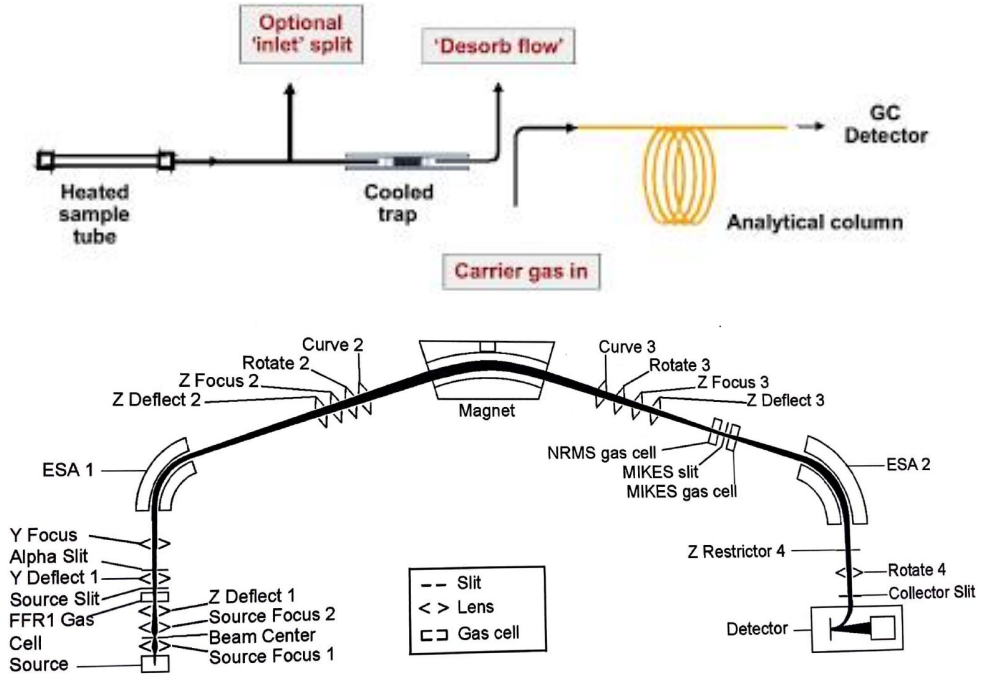


Figure 18: TD/GC-MS system configuration

3.3. Optimization of thermal desorption parameters

The main parameters when using thermal desorption system are, firstly, the heating temperature of the TD tube; secondly, the time the TD tube will be heated; and finally, the gas desorption flow. To optimise those parameters, we studied the difference in VOCs signal, looking for the highest possible. The studied conditions are summarised in Table 2.

Table 2: Thermal desorption parameters for optimization

<table>
<tr><th>Sorbent</th><th>Temperature, (°C)</th><th>Desorption flow (mL/min)</th><th>Heating time (min)</th></tr>
<tr><td rowspan="11">Tenax® TA</td><td rowspan="6">300</td><td rowspan="3">100</td><td>1</td></tr>
<tr><td>3</td></tr>
<tr><td>5</td></tr>
<tr><td>80</td><td rowspan="4">10</td></tr>
<tr><td>60</td></tr>
<tr><td>40</td></tr>
<tr><td>280</td><td rowspan="3">100</td></tr>
<tr><td>250</td></tr>
<tr><td>220</td></tr>
<tr><td rowspan="11">Carbograph 1TD</td><td rowspan="6">340</td><td rowspan="3">100</td><td>1</td></tr>
<tr><td>3</td></tr>
<tr><td>5</td></tr>
<tr><td>80</td><td rowspan="4">10</td></tr>
<tr><td>60</td></tr>
<tr><td>40</td></tr>
<tr><td>320</td><td rowspan="3">100</td></tr>
<tr><td>300</td></tr>
<tr><td>280</td></tr>
</table>

We checked the parameters in the previous table for two different types of sorbents. The sampling flow was set at 250 ml/min to avoid sample breakthrough and collected for 28 minutes. By doing that we collect a total of 7 litres of air. To get a consistent source of VOCs to compare the results we collected air samples from the laboratory. As it was discussed previously, the sorbent selected for the rest of the study was Tenax® TA, as humidity from breath samples will severely interfere with Carbograph 1 TD. The results are summarized in Figure 19.

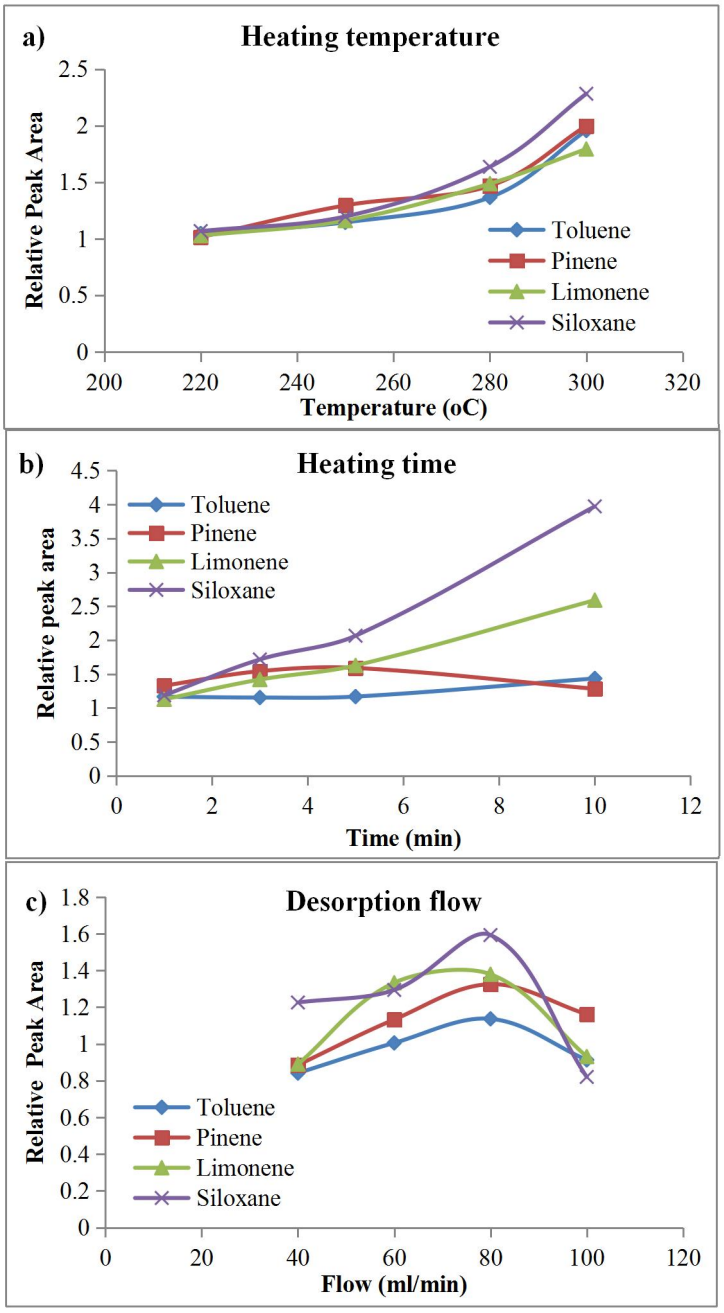


Figure 19: Parameter optimization for Tenax® TA sorbent. a) heating temperature; b) heating time; c) desorption flow

Regarding heating temperature, the more we heat the tube, a higher quantity of the components will get desorbed. However, at high temperature,

the sorbent will start degrading, producing artefacts that will interfere with our results [Figure 11]. Furthermore, the higher the temperature, the cost of the analysis will increase as more energy is needed. Because of that, we decided to select the temperature of 220 ^{o}C, as there is not big difference between 220 ^{o}C and 280 ^{o}C, and 300 ^{o}C might be too close to the limit temperature of the sorbent (350 ^{o}C).

When we analyse the heating time, we need to consider that, longer we hold the temperature, longer the analysis will take place, what we would like to avoid. In addition to that, the analysis will be more costly. Looking at the results, we decided to set our system to 5 minutes. When checking the desorption flow, it's important that all our compounds will be eluted from the sorbent, but the usage of gas should be as low as possible. Because of that, we decided to set the instrument to 60 ml/min. All the main parameters are summarized in Table 3.

Table 3: Optimized parameters for Thermal Desorption System (ATD 400)

Carrier Gas	Helium 5.0	**Oven Temp.**	220 ^{o}C
Desorption Flow	60 ml/min	**Oven Hold**	5 min
Outlet Split	6 ml/min	**Trap Low Temp.**	-10 ^{o}C
Inlet Split	No	**Trap High Temp.**	280 ^{o}C
		Trap Hold	3 min

Other parameters we need to set to run our analysis were: the outlet split, which should be 10 % of the desorption flow (i.e. 6 ml/min); the temperatures of the cold trap, which were set to -10 ^{o}C (low) and 280 ^{o}C (high), hold for 3 minutes.

3.4. Chemicals and reagents

Standards of Py (ReagentPlus, ≥99 %, CAS: 110-86-1) and FFA (≥98 %, CAS 98-00-0) were purchased from Sigma-Aldrich. Mobile phase solvents Methanol CHROMASOLVTM (Gradient grade, for HPLC ≥99.9 %, Honeywell) were used when running the HPLC system, and deionized water obtained using deionizer system NANOpure Infinity (Barnstead/Thermolyne, USA). Acetic acid glacial (USP, BP, Ph. Eur.) pure, pharma grade (Applichem) was used to acidify water mobile phase (0.1 %). Alkane Standard mixture for performance test of GC-systems (Sigma-Aldrich) was used for LDPE bag stability study.

4. DEVELOPMENT OF SAMPLING SYSTEM

4.1. Device prototypes

As it was discussed in the introduction, there are several methods for collection of VOCs. However, the collection of VOCs from human breath is a bit more challenging. We believe the analysis of breath VOCs has a great application for disease diagnosis. Consequently, we wanted to focus first on the development of a device to collect breath samples. Our fist aim was to replicate the same design as ReCIVA® breath analyser (Owlstone Medical, UK). However, we tried to make the system easier and more affordable. We purchased some masks, bags and connections from UAB "Intersurgical" company [Figure 20] and constructed several prototypes.

Figure 20: Face mask with 4 sampling tubes (top left); self-made TD tube connector (top right); T-piece directional valve (bottom).

4.1.1. Prototype (P1) of the breath sampling system - Face mask with 4 sampling tubes

The first prototype (P1) of the system was basically a face mask (Intersurgical EcoLite™) connected to 4 thermal desorption tubes through a self-made connector and a T-piece directional valve (UAB "Intersurgical") [Figure 20].

The device was connected to an air sampler to pass the collected breath through the system. The flow of air from the pump was set to 1000 ml/min in order to avoid breakthrough of the TD tubes, as the flow would be too fast for the sorbent to capture all the VOCs. However, as the average person breathing is 5 l of air/minute, the collected breath passing through the tubes was not fast enough. The excess breath produced had to be collected into an adjacent 2 l reservoir bag (UAB "Intersurgical") so that the person could keep breathing normally.

This first system was tried out for several volunteers from whom their breath samples were collected. Breathing through the mask was uncomfortable for all of them, difficulty of breathing. The quantities of VOCs collected into the 4 tubes were not reproducible [Table 4]. This suggests that the flow that goes through each tube was different, probably because the flow was not individually controlled. After several minutes of breath collection, the adjacent reservoir bag was filled out, making even more difficult the breathing process as the pumping system couldn't handle the high flow rate.

Several blanks were measured by leaving the system connected left on the table, and many different compounds were collected coming from the background, which would interfere with the breath VOCs, many plasticizers and aromatics were coming from the plastics and tubing, as well as from the adjacent bag.As we want to avoid cross-contamination, all the tubing, mask, and bag should be either cleansed and conditioned, or new components shall be used for each new participant, making the process even longer and/or expensive.

Table 4: Peak areas of the compounds collected using 4 separate tubes at the same time and their relative standard deviation (RSD).

	Toluene	Xylene	Limonene	Menthol
		Day 1		
Tube 01	3153	6196	1125	1061
Tube 02	3819	7340	1686	1045
Tube 03	6835	10956	1249	1038
Tube 04	956	1482	0 (N.D.)[1]	0 (N.D.)[1]
Average	3691	6494	1015	786
RSD (%)	**66%**	**60%**	**71%**	**67%**
		Day 2		
Tube 01	4147	18340	25040	5140
Tube 02	6927	21912	35898	4754
Tube 03	5879	30853	56652	8769
Tube 04	9624	31862	37554	8771
Average	6644	25742	38786	6859
RSD (%)	**35%**	**26%**	**34%**	**32%**
		Day 3		
Tube 01	4047	11784	11046	5213
Tube 02	9813	33966	21715	7877
Tube 03	5367	20859	19733	6489
Tube 04	8131	20153	16789	5099
Average	6840	21691	17321	6170
RSD (%)	**38%**	**42%**	**27%**	**21%**

[1] N.D.: Not detected (<LOD)

4.1.2. Four vs one TD tube

Using 4 tubes for breath collection would allow us to take a bigger amount of sample in a faster way. Because the maximum recommended flow for each TD tube is around 250 ml/min, using 4 tubes at the same time would allow us to collect 1000 ml/min of breath sample. However, as we can control only the total flow from the pump, we cannot know for sure if the flow is divided equally between the 4 tubes, as we could prove in Table 4. That will make the sampling inaccurate and imprecise, as some tubes will be collecting smaller amounts of sample while others will take a flow higher than the recommended. In addition to that, the sample will be divided into 4 different tubes, needing an extra volume of sample in order to increase the concentration of possible trace compounds. Because of that we considered the option of using only 1 TD tube instead. Even though the time of sampling will be higher, all the compounds will be trapped in a single TD tube, allowing us to detect many more compounds of interest.

4.1.3. Prototype (P2) of the breath sampling system – "Face mask with 1 sampling tube

In order to facilitate the breathing collection process for the patients and the reproducibility of the samples, the volunteers were asked to breath until adjacent bag was filled, which is connected to a bypass system in which all the air coming from the volunteer will go straight into the thermal desorption tube. All the air collected into the bag will pass through the TD tube as well, because it can only flow forward thanks to an Anti-pollution T-piece directional valve (UAB "Intersurgical") [Figure 21].

Figure 21: Sampling system - Face mask with 1 TD tube

The breathing system was still very uncomfortable for volunteers, as it was hard to breathe. Contamination coming from the bag was not solved as blanks will show different compounds every time. Because it is not a stable background, it cannot be subtracted from the sample. System should be still cleaned and conditioned, or discarded for each person.

4.1.4. Prototype (P3) of the breath sampling system - Mouth tip with 1 tube breathing system

To make the breathing even easier and more comfortable, the mask was exchanged with a mouth tip [Figure 22]. Volunteers were asked to inhale through their nose and exhale through their mouth this way filling the bag with exhaled air.

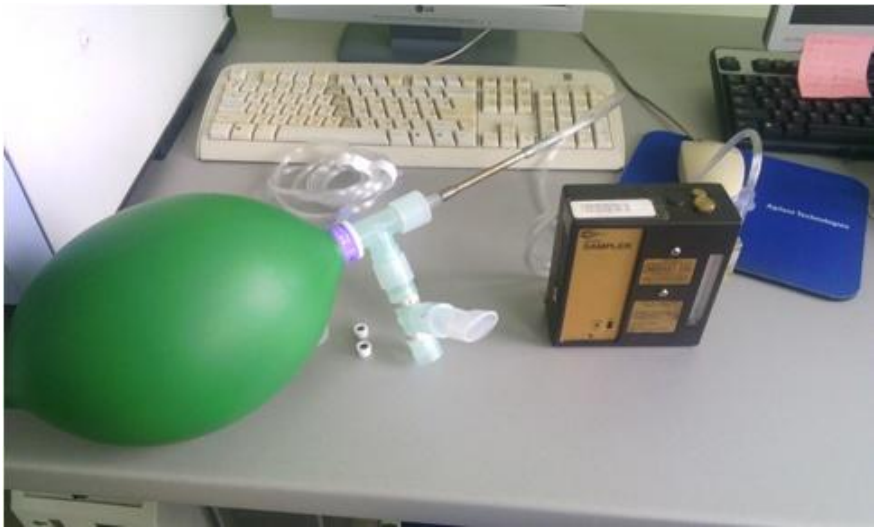


Figure 22: Prototype (P3) sampling system

This breathing system is more comfortable for the volunteers. But many compounds were still interfering from the bag, which must be cleaned or discarded afterwards.

4.2. Prototype (P4) of the breath sampling system - LDPE minigrip bags

As we solved the problem of comfort, we had still the problem of contamination coming from the system. After the sample collection, the adjacent bag should be thoroughly cleaned or simply discarded, making the process very long or expensive. In order to fix that, we decided to exchange the adjacent bag, and all the tubing for a simpler system.

The system we developed is like using Tedlar® bags [Figure 23], which are widely used for breath collection for its simplicity, reproducibility, easy to transport and relatively cheap (around 10 € / 3 litre bag). Those single-use bags are made of polymer polyvinyl fluoride, which was patented by Dupont™.

Figure 23: Tedlar® Bag (Left) and developed Minigrip LDPE plastic bag collection system (Right).

Other polymer bags have been studied as good candidates for gas analysis, as Nalophan®, Cali-5-Bond™, FlexFoil® and Teflon® polymers. Even though those materials prove to have very good performance and little contamination coming from the materials, they need to be conditioned before collecting the sample. Furthermore, reusability of the bags generates many possible problems for most of the polymers, making them single-use bags. Like those polymer bags, we employed a simple Minigrip LDPE plastic bag to collect breath samples.

4.2.1. Materials needed

The materials needed for the construction of the breath sampling system [Figure 24].

- Minigrip LDPE bag 200 x 250 mm (it works also for any other size);
- Vacuum Manifold plastic stopcock valve;
- PVC tubing Cristall Extra (5 mm inner Ø / 7 mm outer Ø) 15 mm long;
- PVC tubing Cristall Extra (2 mm inner Ø / 4 mm outer Ø) 30 mm long.

Figure 24: Vacuum Manifold stopcock valve and PVC Tubing

4.2.2. Device construction

The steps to follow for the construction of the breath sampling system [Figure 25].

1. We open the Minigrip LPDE plastic bag and introduced the 15 mm long PVC tube (5 mm inner Ø). We properly seal the bag and place the small PVC tubing in the centre of the bag;
2. Holding the PVC tubing, we pierce it with the stopcock valve until fully connected. This will create a tight connection with the inside of the bag;
3. We connect the nitrogen gas system to the stopcock and open the valve to allow the flow of nitrogen fill the bag slowly;
4. Once the bag is around 80 % full, we place it inside the oven at 50 ^{o}C for at least 2 h;
5. After 2 hours we take the bag out of the oven, empty the gas content, and repeat the filling process;
6. Once we repeat the process at least twice, we empty the content of the bag and add the long PVC tubing (4 mm outer Ø) at the end of the stopcock for better filling;
7. The bag is now conditioned and ready for sample collection;
8. After usage, the plastic bag and PVC tubing are disposed; meanwhile the stopcock valve is cleaned and disinfected using iso-propanol to be reused for another bag.

Figure 25: construction steps: Step 1 (top left); Step 2 (top middle and right); Step 3 (bottom left); Step 4 (bottom middle); Step 6 (bottom right)

4.2.3. Sampling bag conditioning

When using the Minigrip LDPE plastic bags we could discard the bags after each sample due to its cheap price. However, we discovered that many compounds are coming from it [Figure 26]. And because of that, we had to condition the bags. To do that, we filled the bag with an inert gas and place them in the oven for 2 hours. After that, we collected the content into TD tubes and analyse it. The inert gases used were argon and nitrogen. Both showed similar results, therefore we kept using nitrogen due to its lower price.

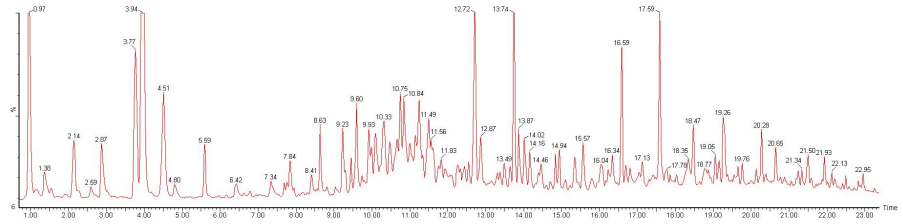


Figure 26: GC chromatogram of blank sample from Minigrip LDPE bag non-conditioned

After conditioning the bags, we could see how the number of compounds decrease dramatically, obtaining a clean enough background [Figure 27].

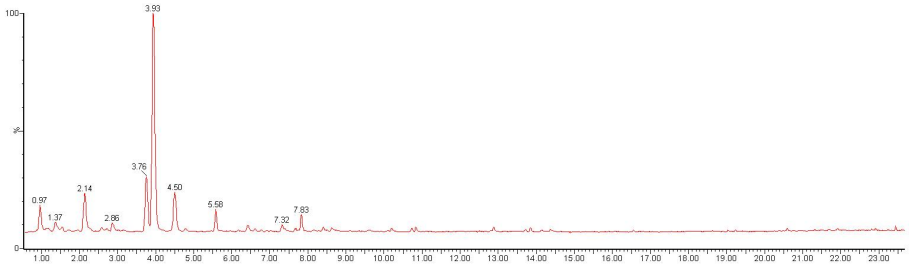


Figure 27: GC chromatogram of blank sample from Minigrip LDPE bag after 1st conditioning

Nevertheless, we conditioned the bag a second time to see if we could improve the background. We could see on Figure 28 that we need to condition at least twice to get the lowest interference from bag contaminants.

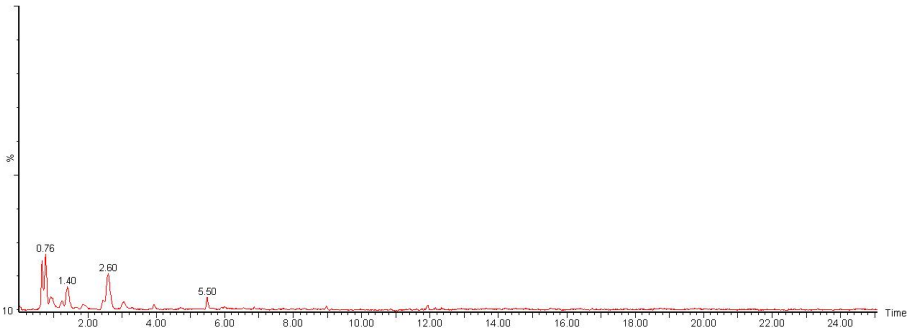


Figure 28: GC chromatogram of blank sample from Minigrip LDPE bag after 2nd conditioning. (Retention times were shifted comparing previous results due to cutting of the column)

4.2.3.1. Background study

In order to study the background emissions from the LDPE plastic bag after conditioning and collection of samples, bags were filled with nitrogen gas and left for several hours. The content was collected in order to determine any components coming from the bag.

The LDPE bags, once conditioned, can be used within at least 72h without any new interference coming from the bag materials [Figure 29].

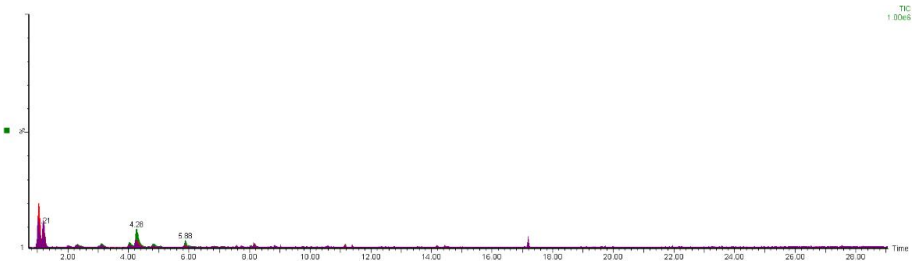


Figure 29: GC chromatograms of the blank sample of LDPE bags filled with Nitrogen after 24 - 48 - 72h (Green – Red – Purple respectively)

4.3. Breath sample parameter optimization

Before collecting the samples, we have checked that all parameters are properly optimized in order to get reliable results and good repeatability of the measurements.

4.3.1. Sampling flow

After considering the option of only one TD tube, we should then check the sampling flow of the sample. Thermal desorption tubes are supposed to handle a maximum flow of 250 ml/min. If the flow is higher some compounds might not get trapped inside. For a given volume of 3 l of breath sample, we studied the difference of using a flow of 250 ml/min and 1000 mL/min. The results are shown in Figure 30

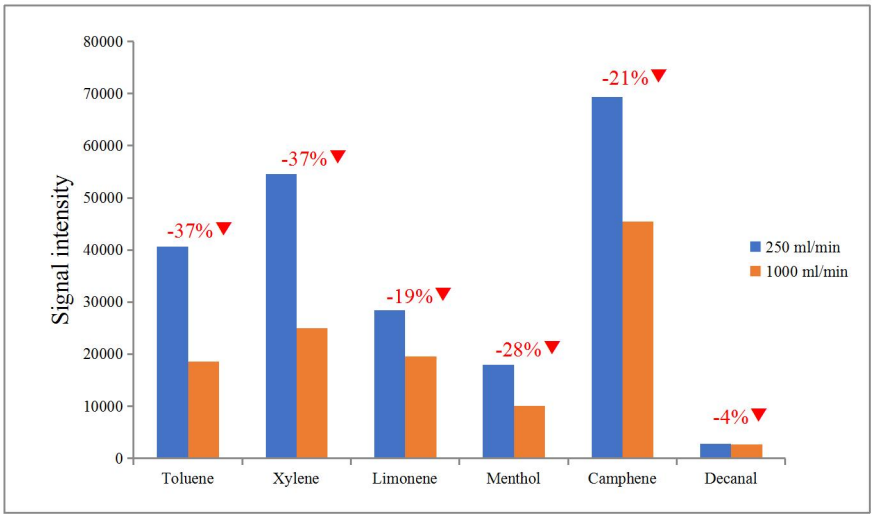


Figure 30: Average peak areas at each flow rate, and the difference of increasing the flow rate. Compounds arranged by higher to lower volatility (Left to Right).

As we can see from the results, the quantity of compounds trapped in the sorbent decreases when we increase the flow rate, especially for lighter compounds. However, we loss only around 40 % for very volatile compounds, around 20 % for heavier compounds, and only a 4 % of the heaviest, compared to 4 times shorter time to collect the samples. Therefore, we could say that it is worth collecting the sample at 1000 ml/min which will shorten the sampling time and we will still collect most of the compounds present in breath.

4.3.2. Breath sampling volume

Because we are saving time in collecting the sample, we could try to collect a bigger amount of breath sample. Bag sizes of 1 and 3 litres were compared. Refilling the sample again would increase the amount of sample, increasing the amount of trace compounds as well. Refilling the sample up to 6 times was studied, which would allow sampling up to 18 litres of breath, concentrated into the TD tube.

After seeing the results in Figure 31 we decided that the most time effective volume is 6000 ml. Because we obtained enough amount of all range of compounds without saturating the sorbent. Furthermore, we will

need to fill the 3000 ml bag for 2 times in order to get the most compounds in the fastest time.

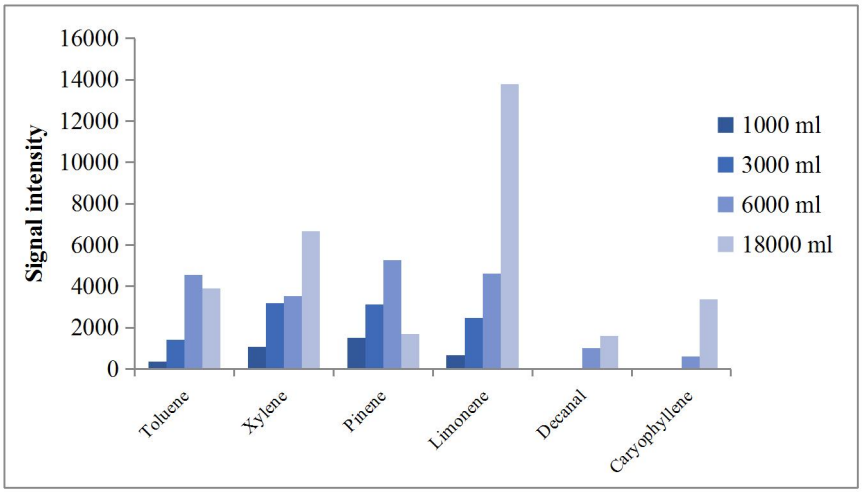


Figure 31: Average peak areas depending on sample volume. Compounds arranged by higher to lower volatility (left to right).

4.3.3. Stability of VOCs

To study how stable are the volatile compounds inside our sampling bag, three different 2-liter conditioned bags were injected with 5 µl of Alkane mix standard solution each. The content from the bag was collected after 0, 2, 4 and 6 h.

As we could see from the results [Figure 32], more volatile compounds (C7) will leave the system much faster than less volatile. It will also depend on the concentration of those compounds. However, we have found a loss of less than 20% during the first 10 minutes while holding the sample at 25 ^{o}C.

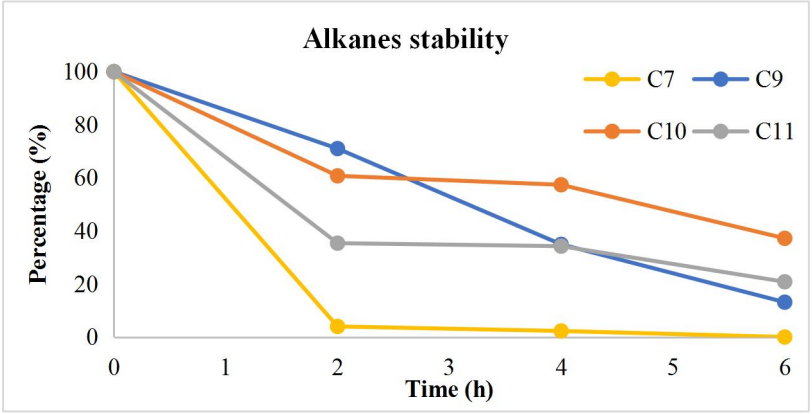


Figure 32: Percentage of alkanes left inside LDPE bags after sample collection.

In addition to that, three breath samples were collected from the same person at the same time. The content from the first bag was analysed just after collection, the rest of the bags was analysed after 4 and 24 h. The most volatile and abundant compound (Isoprene) was compared [Figure 33].

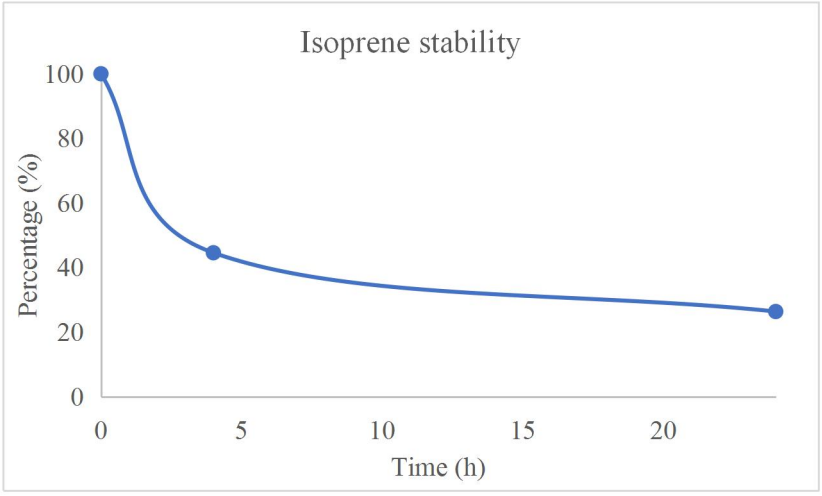


Figure 33: Percentage of Isoprene inside LDPE sampling bags after breath collection.

The quantity of isoprene decreases more than 50 % after keeping the breath sample for 4 hours in the sampling bag. Therefore, we do not recommend using our system for holding the gas content for longer than a few minutes, as very volatile compounds will leak out of the bag.

4.3.4. Breathing mode influences

As bags shall be filled by the volunteers, different breath collections might be obtained for each of them. Three different ways of collecting the breath was studied. Breath collected from mouth will have more compounds produced in the gut. Whereas breath from nose might get compounds only from nasal conducts. As we can see in Figure 34, first breathing mode was a big inhale through the nose and filling the bag in a single breath (red colour chromatogram). The second mode studied was small inhale through the nose and filling the bag in several breaths (green colour chromatogram). The final mode (purple colour chromatogram) was big inhale through the nose, then discard half of the breath, and collect the final part, repeating until the bag was filled.

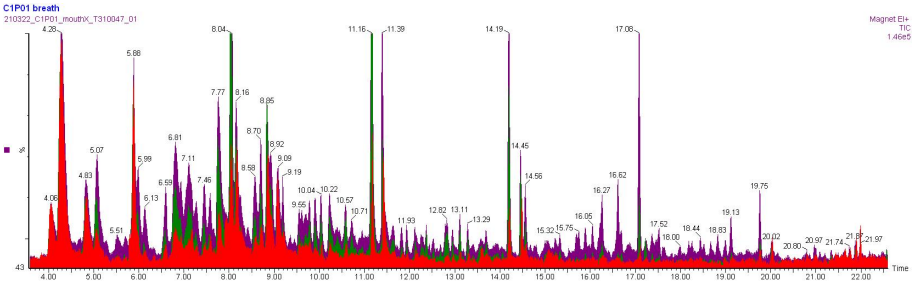


Figure 34: GC chromatogram of breath samples. Different breathing modes: Single breath nose inhale (Red), several breath mouth inhale (green), several breaths after first volume discarded (purple).

Similar number of compounds were detected using the 3 different methods. However, when discarding the first half of the breath a higher intensity of heavier (less volatile) compounds are detected. As our main objective is to identify the highest number of compounds possible to be able to study the full spectra of compounds coming from the volunteers, the last method might be preferred.

Diffusion of water vapor from LDPE bag outside the system was observed to be within 30 min after breath collection.

We have successfully developed a tool for VOCs collection from several sources. The system is a simplification of the already available polymer sampling bags. As this modification makes the cost of the bag much cheaper, we could experiment with different bag sizes for different applications. The bag is very easy and fast to build and condition, it is very comfortable to use for the collection of breath samples and easy to discard. We could fill the bag not only with gas samples (breath samples) but also with solid samples.

In addition, we have successfully obtained the best parameters for the collection, analysis and identification of VOCs by using TD/GC-MS. Sampling times were optimised to make the collection of VOCs as quick as possible, avoiding breakthrough of the compounds, as well as collecting compounds that might be present in trace levels. Desorption temperatures and times were selected in order to release all the trapped compounds in a fast way, using the lowest resources as possible. The chromatographic parameters allowed a correct separation of most of the compounds, in the fastest time to obtain a proper identification of the collected VOCs.

5. VOCs IN HUMAN BREATH

Once our sampling system is ready, we tried collecting breath samples from volunteers. Workers from four different offices agreed to volunteer in our study. Two breath samples were collected from most of our volunteers, one sample in the morning, just after arriving at their workplace, and a second sample before they left their job in the afternoon. A questionnaire was provided to all the volunteers [Annex A] to obtain important parameters for data analysis. The main idea of the study was to identify influences that could modify their breath composition, from food and smoking habits, sports, medicines or illnesses, to the influence from the indoor office environment. To probe possible influences from the working places, an air sample was collected in addition to the breath samples.

Volunteers were asked to fill the 3 l LDPE plastic bags (already conditioned) twice in order to collect a total of 6 l of breath sample. Collected breath samples are pumped through a thermal desorption tube at a flow of 1000 ml/min. After collecting and analysing the breath samples from 23 different volunteers during different times of the day [Figure 35], at least 123 different VOCs were detected and most of them identified [Table 5]. To identify the compounds, the NIST MS Search 2.0 (2005) mass spectral library was used.

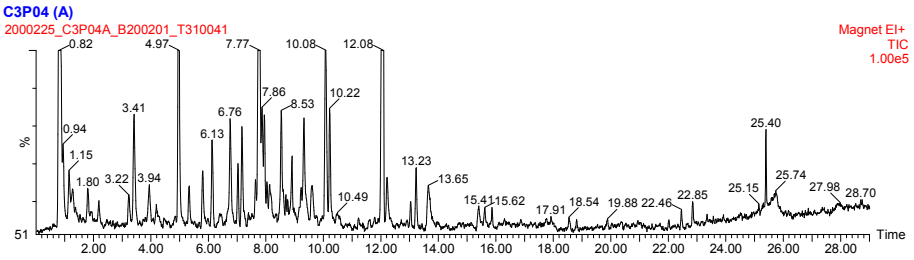


Figure 35: Breath sample chromatogram from a volunteer collected in the morning

Table 5: List of VOCs detected and identified in human breath

Nr	T_R (min)	Compound	Possible sources
1	0.86	Acetone	Anthropogenic
2	0.86	Isoprene	Anthropogenic
3	0.94	Hexane	Petrol / Car exhaust
4	1.11	Octane	Petrol / Car exhaust
5	1.21	Isopropyl acetate	Organic solvent
6	1.13	Benzene	Petrol / Car exhaust
7	1.30	Allyl methyl sulfide	Anthropogenic
8	1.38	Methyl Propyl sulfide	Anthropogenic
9	1.44	Carbonic acid	Other
10	1.52	1-(methylthio)- 1-Propene	Anthropogenic
11	1.59	Unknown	Unknown
12	1.73	Pyridine	Food MRP
13	1.80	Toluene	Petrol / Car exhaust
14	2.16	Hexanal	Anthropogenic
15	2.46	Octanal	Anthropogenic
16	2.27	Tetrachloroethylene	Organic solvent / Dry cleaning
17	2.35	Dihydro-2-methyl-3-furanone	Food MRP
18	2.41	Butyl Acetate	Organic solvent / Fragrances
19	2.47	Methyl Formate	Food MRP
20	2.65	Methylpyrazine	Food MRP
21	2.66	C_nH_n	Petrol / Car exhaust
22	2.78	Furfural	Food MRP
23	2.93	Propanoic acid	Anthropogenic
24	3.30	Furfuryl alcohol	Food MRP
25	3.20		
26	3.38	Xylene (o,m,p)	Petrol / Car exhaust
27	3.90		
28	3.77	Hexanoic acid	Anthropogenic
29	4.15	C_nH_{2n+2}	Petrol / Car exhaust
30	4.26	Aldehyde	Anthropogenic
31	4.45	Butyl Glycol	Organic solvent
32	4.52	Methyl formate	Other
33	4.56	Acetylfuran	Food MRP
34	4.71	Dimethyl Pyrimidine	Food MRP
35	4.82	Unknown	Unknown
36	4.91	α-Pinene	Vegetation / Fragrances
37	5.05	C_nH_{2n+2}	Petrol / Car exhaust
38	5.20	Hexadecanol	Cosmetics / Fragrances
39	5.23	Hexylene Glycol	Cosmetics / Fragrances
40	5.26	Camphene	Vegetation / Fragrances
41	5.52	Propyl benzene	Petrol / Car exhaust
42	5.76	m-Ethyl methylbenzene	Petrol / Car exhaust
43	5.97	psi-cumene	Vegetation / Fragrances
44	6.06	β-Pinene	Vegetation / Fragrances

45	6.26	Ethyl Toluene	Petrol / Car exhaust
46	6.39	Unknown	Unknown
47	6.58	C_nH_{2n+2}	Petrol / Car exhaust
48	6.69	Mesitylene	Vegetation / Fragrances
49	6.72	β-Myrcene	Vegetation / Fragrances
50	6.83	VinylFuran	Other
51	6.98	Decane	Petrol / Car exhaust
52	7.11	3-Carene	Vegetation / Fragrances
53	7.37	Dipropylene glycol monomethyl ether	Other
54	7.47	Unknown	Unknown
55	7.49	Dipropylene glycol monomethyl ether	Other
56	7.59	m-Cymene	Vegetation / Fragrances
57	7.73	Limonene	Vegetation / Fragrances
58	7.87	Eucalyptol	Vegetation / Fragrances
59	7.91	C_nH_{2n+2}	Petrol / Car exhaust
60	7.94	Caprylic acid	Other
61	7.98	Decanal	Vegetation / Fragrances
62	8.01	Ethylhexanol	Vegetation / Fragrances
63	8.09	C_nH_{2n+2}	Petrol / Car exhaust
64	8.13	1-Phenyl-1,2-butanediol	Other
65	8.50	C_nH_{2n+2}	Petrol / Car exhaust
66	8.64	4-Carene	Vegetation / Fragrances
67	8.71	(Methyl tridecane) C_nH_{2n+2}	Petrol / Car exhaust
68	8.87	C_nH_{2n+2}	Petrol / Car exhaust
69	8.93	Ethyl Methyl Benzene	Petrol / Car exhaust
70	9.10	(Methyl decane) C_nH_{2n+2}	Petrol / Car exhaust
71	9.23	C_nH_{2n+2}	Petrol / Car exhaust
72	9.31	Dihydro myrcenol	Vegetation / Fragrances
73	9.41	Hexahydronerolidol	Vegetation / Fragrances
74	9.51	4-Caranol	Vegetation / Fragrances
75	9.59	α-Cumyl alcohol	Vegetation / Fragrances
76	9.90	Dihydrocarveol	Vegetation / Fragrances
77	9.99	2-Butyl-1-octanol	Anthropogenic
78	10.12	Linalyl anthranilate	Vegetation / Fragrances
79	10.08	Undecane	Petrol / Car exhaust
80	10.15	Nonanal	Anthropogenic
81	10.16	Bergamiol	Vegetation / Fragrances
82	10.46	Menthadienol	Vegetation / Fragrances
83	10.62	Unknown	Unknown
84	10.83	Unknown	Unknown
85	11.18	Camphor	Vegetation / Fragrances
86	11.52	Isomenthone	Vegetation / Fragrances
87	11.71	Unknown	Unknown
88	11.86	Isomenthol	Vegetation / Fragrances

89	11.96	Unknown	Unknown
90	12.16	Menthol	Vegetation / Fragrances
91	12.20	Unknown	Unknown
92	12.18	Naphthalene	Petrol / Coal
93	12.52	Unknown	Unknown
94	12.68	Benzoic acid	Vegetation / Fragrances
95	12.87	Unknown	Unknown
96	13.00	C_nH_{2n+2}	Petrol / Car exhaust
97	13.16	Decanal	Vegetation / Fragrances
98	13.64	2-phenoxy ethanol	Other
99	14.25	D-Carvone	Vegetation / Fragrances
100	14.63	Linalyl isobutyrate	Vegetation / Fragrances
101	14.66	Oxalic acid, bis(trimethylsilyl) ester	Other
102	15.35	Isobornyl acetate	Vegetation / Fragrances
103	15.60	4-tert-Butylcyclohexyl acetate	Cosmetic / Fragrances
104	15.86	Tridecane	Petrol / Car exhaust
105	16.25	C_nH_{2n+2}	Petrol / Car exhaust
106	16.85	Oxalic Acid	Other
107	17.12	α-Terpinyl butyrate	Vegetation / Fragrances
108	17.68	β-Vinylnaphthalene	Petrol / Coal
109	17.89	C_nH_{2n+2}	Petrol / Car exhaust
110	18.48	C_nH_n	Petrol / Car exhaust
111	18.74	Caryophyllene	Vegetation / Fragrances
112	19.12	Verdyl Acetate	Vegetation / Fragrances
113	19.27	Oxalic acid	Other
114	20.33	α-Cetone	Cosmetic / Fragrances
115	20.76	C_nH_{2n+2}	Petrol / Car exhaust
116	20.87	3-Biphenylol	Other
117	22.32	Oxalic acid	Other
118	22.43	Phthalic acid	Plasticizer
119	22.78	Benzophenone	Other
120	23.03	C_nH_{2n+2}	Petrol / Car exhaust
121	23.16	C_nH_{2n+2}	Petrol / Car exhaust
122	23.23	Unknown	Unknown
123	25.36	Isopropyl myristate	Vegetation / Fragrances

Most of the compounds found in breath had possible sources from car exhaust, and fragrances. This could give us an idea of the environment of which we are exposed to every day for people mostly working in office spaces in a big city.

Other interesting VOCs found in breath are isomenthone, isomenthol and menthol which are common in toothpaste. We could distinguish the teeth brushing habits of some volunteers not long before the samples were collected.

Sulfur-containing compounds, such as dimethylsulfide, hydrogen sulphide, and mercaptans (e.g., methylmercaptan and ethylmercaptan) are proposed as liver cancer biomarkers [104] but also appearing from poor oral hygiene. This can be a confounding factor mitigated by mouth washing thoroughly with water before breath sampling.

High levels of acetone in breath are related to smokers and lung cancer [110]. In addition to that, levels of acetone in breath are expected to increase during exercise [111]. Similar links could be established with nonanal. Nonanal in exhaled breath is associated with tobacco consumption, current or previous. Nonanal is a sub-product of the destruction of the cell membrane, and its finding may be indicative of cell damage in smokers [112]. As well as for acetone, high nonanal levels are related to exercise. When comparing the nonanal levels for our volunteers and their smoke habits, we could confirm those facts [Figure 36]. Three smokers took part in the study, for one of them we could collect only one sample in the morning. For the other two volunteers we collected breath samples in the morning and in the afternoon, obtaining high levels of nonanal for all the samples. Two volunteers had low levels of nonanal in the morning sample, but much higher in the afternoon after intense exercise.

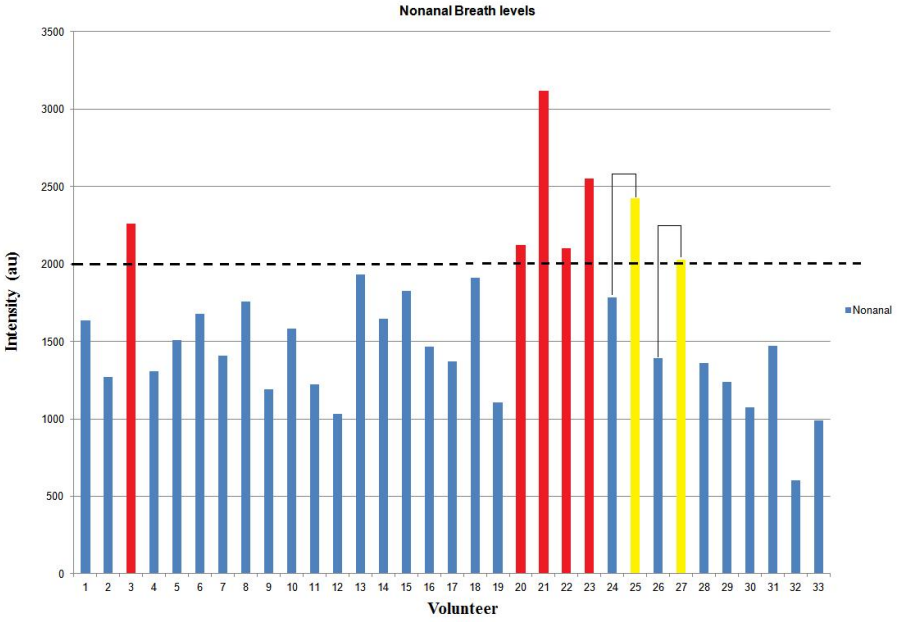


Figure 36: Nonanal relative level in the breath samples of different volunteers. Red color show smokers; yellow color show volunteers after exercise.

It is also worth pointing the presence of VOCs from food, especially the ones produced in thermally processed food. Pyridine and furan derivates are toxic compounds produced during the roasting, baking or frying food. We could observe relatively high levels of those compounds after coffee ingestion. Those markers could be interesting to monitor food poisoning.

Using our own self-developed VOCs collection system, we were able to collect the human breath samples of many volunteers. Collection volumes were optimised to allow the detection of low concentration VOCs. We could identify specific biomarkers, compounds that will show us the exposure to fragrances, outdoor contamination, the use of cosmetics or the ingestion of food and drinks. As well as smoking habits or exercise practises.

6. VOCs FROM FOOD

6.1. Possibilities for food type discrimination

6.1.1. Peanuts

In our study, the four most common types of peanuts from the United States (Runner, Valencia, Spain and Virginia) were provided to find the possible VOCs content difference. Because of a non-disclosure agreement, the exact type of peanut was not provided, therefore we labelled the four types as Peanut A, B, C and D. The main idea was to find the specific VOCs profile for each type and try to classify several blind samples of peanuts provided later.

6.1.1.1. Samples and methods

10 g of peanuts were ground and introduced into our conditioned self-constructed LDPE bags. Smaller 500 ml bags were used for this analysis. The bags were partially filled with nitrogen gas and placed inside the oven at 50 ^{o}C for 30 min.

We observed that 50 ^{o}C is the optimum temperature to release most of the VOCs from the samples and low enough to not modify, destroy their structure or create new compounds previously not present in the sample.

We studied the difference from holding the peanuts inside the oven for 5, 30 and 60 minutes [Figure 37].

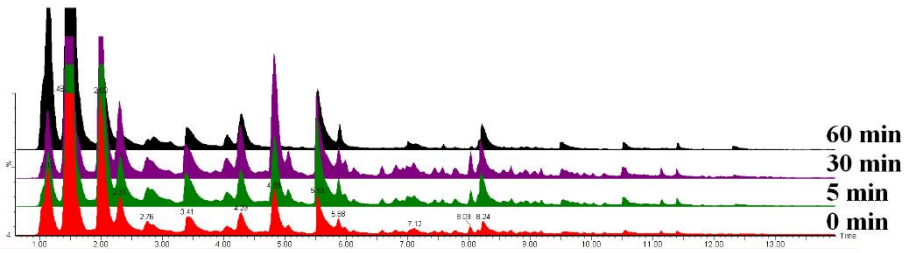


Figure 37: Chromatograms from peanut samples at different times inside the oven (50 ^{o}C)

We observed that the quantity of VOCs released from the peanuts samples was the highest after 30 minutes [Table 6]. Holding the sample for longer than 30 minutes will give us lower quantities. The reason for that

could be because the compounds started to leave the bag, or because the system has been saturated already.

Table 6: Compound areas at different times inside the oven

Ret Time (min)	Compound	Minutes inside the oven (at 50 °C)			
		0	5	30	60
01.48	2-Methylbutanal	103070	173927	160902	570779
03.34	2-Methylpyrazine	18606	33861	34641	22725
05.50	2,5-Dimethylpyrazine	35707	77834	74623	49496
08.03	Decane	2614	5021	8746	716

After the VOCs were released from the samples, a TD tube was connected to the bag and the compounds were trapped using a flow of 250 ml/min. Subsequently, the TD tube was placed into the TD/GC-MS system for its analysis. All four types of peanuts, together with eight different blind samples were analysed using the same methodology. All samples were analysed in duplicate.

6.1.1.2. TD/GC-MS conditions

The same conditions explained in section 3.2 and 3.3 [Table 3] were applied for this analysis, as the separation and identification of VOCs was satisfactory.

6.1.1.3. Results

Both duplicates for each sample gave exact results. A list of VOCs emitted from peanuts was identified using NIST Mass Spectral Library [Table 7]

Table 7: List of the most relevant VOCs emitted from peanuts and their relative concentrations

Ret. Time (min)	Compound	Peanut A	Peanut B	Peanut C	Peanut D
1.13	2-Butanone	4.3%	6.0%	6.9%	1.3%
1.48	2-Methylbutanal	22.2%	22.6%	40.7%	6.3%
1.99	1-Methylpyrrole	25.8%	13.8%	28.2%	89.2%
2.34	Toluene + Pentanol	5.1%	3.8%	0.0%	0.0%
2.84	Octane + Hexanal	35.1%	43.2%	4.8%	0.7%
3.34	2-Methylpyrazine	2.3%	2.7%	6.8%	0.9%
5.50	2,5-Dimethylpyrazine	4.5%	7.3%	11.7%	1.4%
6.96	Benzaldehyde	0.7%	0.5%	0.9%	0.1%

The four different types were compared [Figure 38] and statistical analysis was performed for all the samples provided. Principal component analysis (PCA) was studied for the samples analysed [Figure 39].

From these results we could see that three compounds are the key components for type discrimination: 2-methyl butanal / 1 – methyl pyrrole, and hexanal.

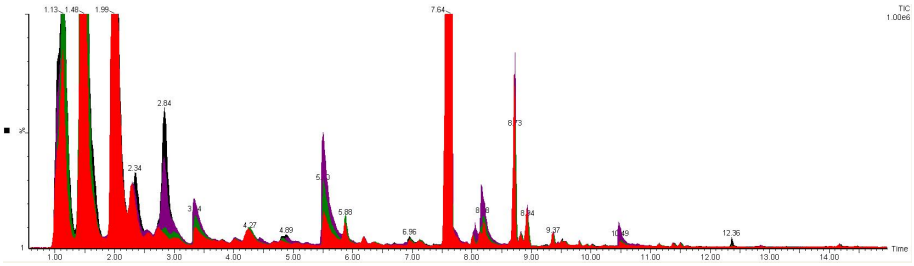


Figure 38: Chromatograms from peanuts samples. Peanut A (Black); Peanut B (Purple); Peanut C (Green); Peanut D (Red).

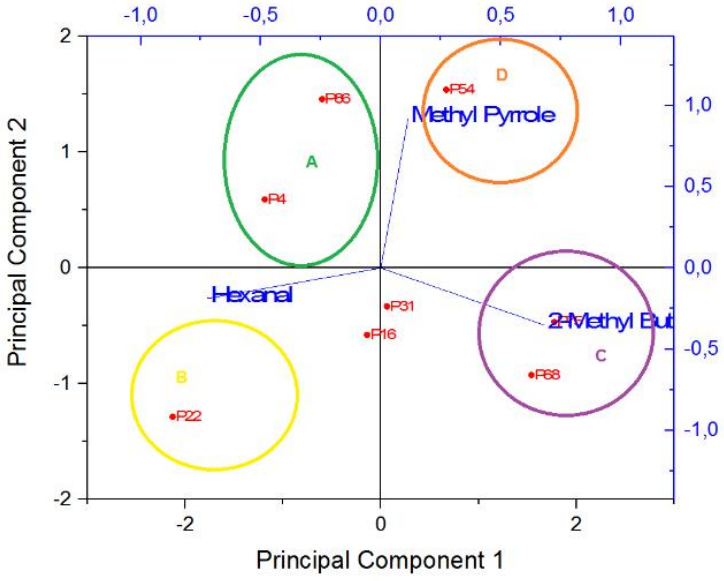


Figure 39: PCA analysis of peanut sample results

Only a small amount of samples were provided for the study. However, we could group them in clusters for the 4 different peanut types. Two samples (P31 and P16) were not included in any of the clusters. The sample providers confirmed that these two samples were type A peanuts with an off flavour. Therefore, our system could differentiate between the 4 types of peanuts, and even detect when one of the types had a change in quality.

6.1.2. Possibilities for coffee blend discrimination

6.1.2.1. Coffee samples and methods

Five different blends of the species *C. Arabica* and *C. Robusta* were provided. 100, 80, 50 and 20 % *C. Arabica*, and 100 % *C. Robusta*. Our research consisted of finding the VOCs profile that can discriminate between each different blend.

Similar to the methodology employed on peanuts, 0.5 g of ground coffee blend was introduced into our self-developed collection system (500 ml LDPE bags). As the coffee powder contains a higher amount of high volatile VOCs than peanuts, we reduce the time and temperature of the oven at 30 ºC for 5 minutes [Figure 40]. Because the conditions set for peanuts were saturating our system we continued with the new conditions, as we got much better intensity and separation of the compounds.

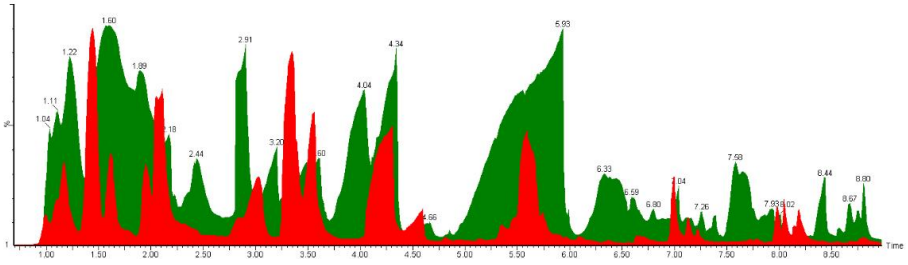


Figure 40: Coffee chromatogram at different oven conditions: Green – 50 ºC for 30 min; Red – 30 ºC for 5 min

Therefore, the bags were then partially filled with nitrogen gas and left to condition for 5 minutes inside the oven at 30 ºC. After all the VOCs were released into our collection system, they were trapped into TD tubes at a flow of 250 ml/min.

The same conditions explained for TD system in section 3.3 [Table 3] were applied for this analysis. Similar condition as the ones explained in section 3.2 for GC-MS analysis were employed. However, because most of the volatiles present in coffee elute within the first 10 minutes of analysis, we reduced the time of analysis by increasing the temperature ramps. We hold the column temperature at 40 °C for 3 min and then increased by 5 °C/min to 120 °C, followed by ramping at 25 °C/min to 220 °C. The total time of the analysis was 24 minutes. Identification of compounds was performed using NIST MS Search 2.0 (2005) mass spectrometer library [Figure 41]

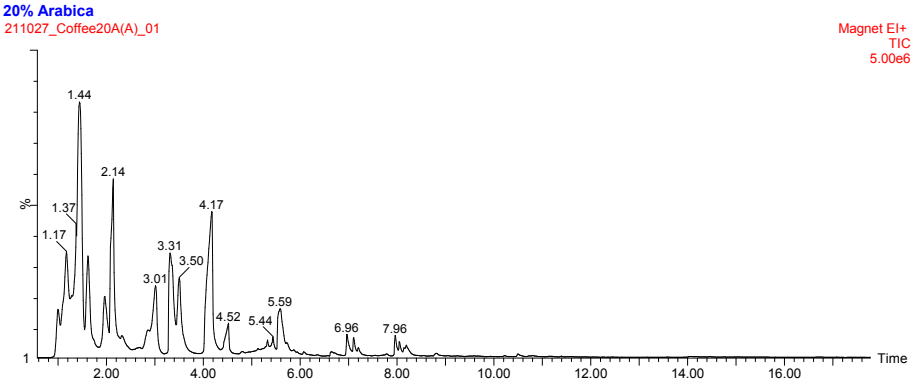


Figure 41: Chromatogram of a coffee bean sample (20 % C. Arabica*)*

6.1.2.2. Results

The average results from 5 different blend batches were obtained together with the standard deviation of the results [Table 8]. According to the literature [87], Arabica and Robusta coffee beans can be effectively classified according to their volatile profile despite the large variation found at individual coffee bean level. The most important volatiles in discriminating coffee species were mostly pyrazines. Using our measurements, we could distinguish the difference between 100 % C. Arabica and 100 % C. Robusta by applying PCA analysis [Figure 42]. As found by Nicola Caporaso [87], the important compounds for discrimination were pyrazines.

Table 8: VOCs detected in ground coffee and their average relative concentration with the standard deviation.

Ret. time (min)	Compound[2]	0% Arab. (Rel. %)	20% Arab. (Rel. %)	50% Arab. (Rel. %)	80% Arab. (Rel. %)	100% Arab. (Rel. %)
1.01	Acetone	0.8±0.2	1.66±1.08	1.7±1.1	1.3±0.4	1.3±0.6
1.09	2-Butanone	7.1±2.2	8.2±10.4	6.7±2.1	7.9±1.4	7.9±1.2
1.18	Methyl Furan					
1.32	Acetic Acid	12.4±1.5	13.5±6.5	10.6±1.5	10.5±2.6	9.9±2.2
1.43	Methyl Butanal + Methyl Acetate					
1.65	***2,3-Pentanedione**	**4±1**	**4.5±1.4**	**4.6±0.9**	**5.6±0.8**	**5.9±0.8**
1.80	Phenol	3.1±0.3	2.9±0.1	3.0±0.3	3.2±0.2	3.4±0.3
1.97	3-Methylpyrrole					
2.14-2.30	***Pyridine**	**9.5±1.2**	**10.5±1.1**	**11.2±1.1**	**13.3±0.5**	**13.8±0.5**
2.85	t-Butylacetamide	5.3±0.3	5.6±0.6	5.6±0.3	5.8±0.4	6.1±0.5
3.09	Dihydro-2-methyl-3-furanone					
3.39	Methylpyrimidine	14.5±1.9	12.9±2.6	12.9±1.1	12.0±1.9	12.0±2.1
3.60	3-Furaldehyde + 3,5-Dimethylpyrazole	6.5±0.4	6.2±0.6	6.5±0.3	6.9±0.5	6.9±0.2
4.42	Furfuryl alcohol	14.2±0.8	14.4±1.8	15.9±2.7	14.8±1.6	14.1±0.8
4.59	Acetoxyacetone	1.9±0.2	1.7±0.2	1.8±0.2	1.8±0.1	1.7±0.1
5.39	**Furfuryl formate**	**0.8±0.9**	**0.9±0.5**	**1.0±0.2**	**1.3±0.2**	**1.3±0.2**
5.51	*Acetylfuran	11.0±1.4	8.6±2.3	9.5±0.9	8.1±0.7	8.0±0.6
5.63	*4,5-Dimethylpyrimidine					
5.84	*2,3-Dimethylpyrazine					
6.70	1-Methoxy-2-methyl-3-butene+Furfuryl methyl ether	0.52±0.04	0.5±0.1	0.57±0.04	0.5±0.1	0.5±0.1
7.04	5-Methylfurfural	2.1±0.2	1.9±0.6	2.2±0.2	1.8±0.3	1.9±0.3
7.18	*2,3-Butanediol	1.1±0.4	1.0±0.2	1.0±0.2	0.7±0.2	0.8±0.2
7.27	1-Acetoxy-2-butanone	0.5±0.2	0.6±0.1	0.6±0.2	0.4±0.1	0.4±0.1
8.02	Furfuryl acetate	1.0±0.1	1.2±0.3	1.5±0.2	1.5±0.2	1.5±0.3
8.09	***2-Ethyl-6-methylpyrazine**	**1.2±0.3**	**1.0±0.3**	**0.9±0.2**	**0.8±0.2**	**0.7±0.2**
8.19	*2-Ethyl-3-methylpyrazine	1.9±0.8	1.7±0.5	1.5±0.3	1.3±0.3	1.2±0.3
8.24	*2-Ethyl-5-methylpyrazine					
8.30	*2,6-Dimethyl-5-aminopyridine					
8.84	1-Acetyl-1,4-dihydropyridine	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1
10.51	2-Ethyl-3,6-dimethylpyrazine	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.2±0.1

*: Compounds that can discriminate between 100 % *C. Arabica* and 100 % *C. Robusta*

[2] Compounds marked in bold letters suggest a possible tendency for blend discrimination

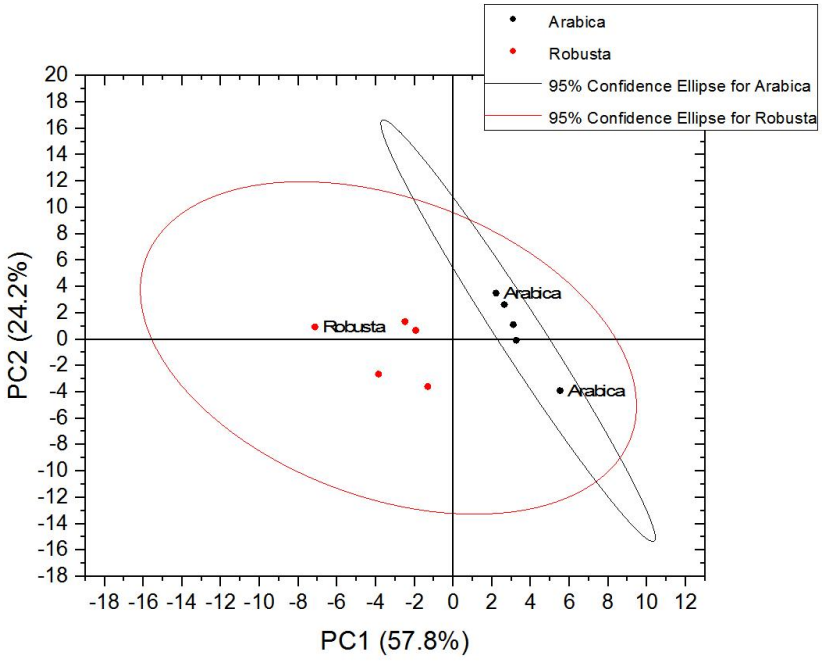


Figure 42: PCA for the discrimination between C. Arabica *and* C. Robusta

6.1.2.3. Different blends

Even though the standard deviation of the different batches is higher than 10 % in most of the compounds identified, we could observe several compounds that show a tendency to discriminate the different coffee bean blends.

Pyridine is one of those compounds. The representation of the relative percentage of pyridine in 5 different batches of coffee [Figure 43] showed a clear tendency in the results. The higher concentration of pyridine found, the higher content of *C. Arabica* was.

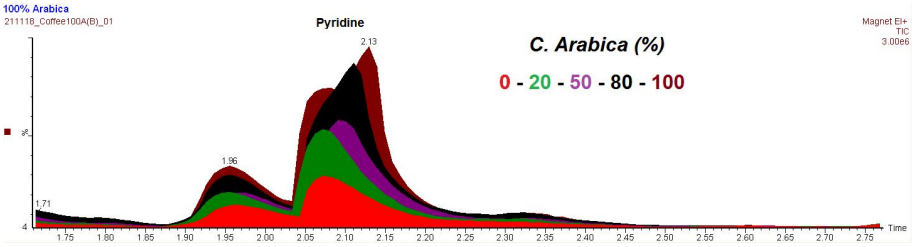


Figure 43: Difference in the pyridine signal for the different coffee blends

However, the deviation in the results obtained for the different batches make it difficult to discriminate blind samples [Figure 44]. This could be due to a difference during the roasting process for the different batches, or even from the mixtures of coffees from different locations.

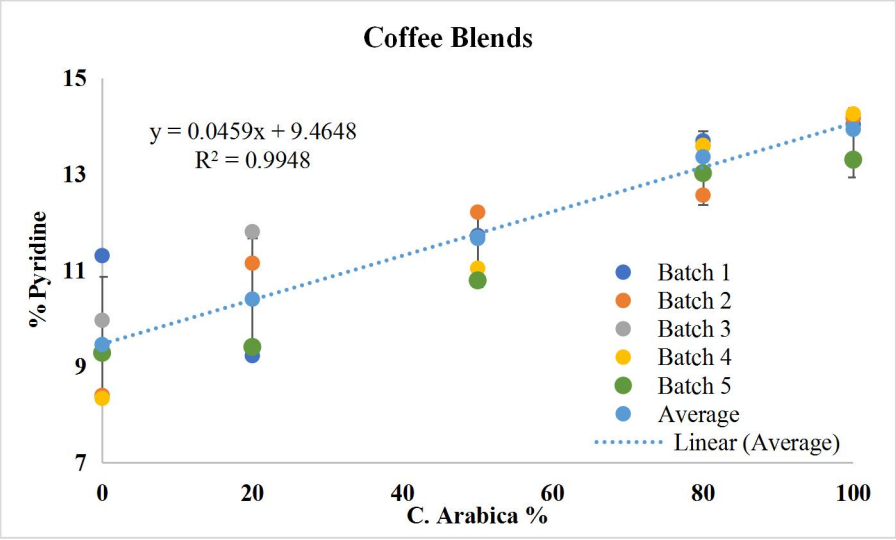


Figure 44: Dependence of relative concentration of pyridine on the content of C. Arabica

6.2. Potential toxic compounds quantification in coffee

During our study on breath samples, we realized a few compounds of high concentration coming from coffee [Figure 45]. That gave us an idea to analyse toxic compounds from food ingestion. We decided to focus on coffee samples, as the compounds detected in high concentrations can be considered as cancerogenic.

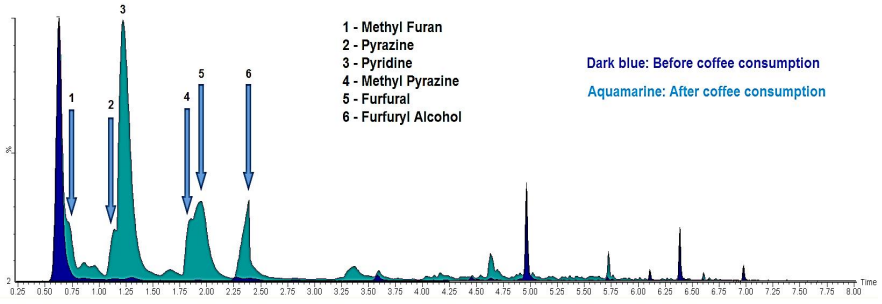


Figure 45: GC chromatogram of breath sample before (dark blue) and after (aquamarine) coffee consumption

Breath analysis focuses on the sampling and identification of emitted VOCs and could hence serve as a method for detection of toxic MRPs present in human breath. In this study we compared the quantity of both compounds in the coffee drink, with the actual levels found in breath after its consumption. We aimed to identify and quantify Py and FFA in human breath after coffee ingestion. Development of such a method could be widely adapted as a tool for detection of food intoxication. The graphical abstract of this method is shown in Figure 46.

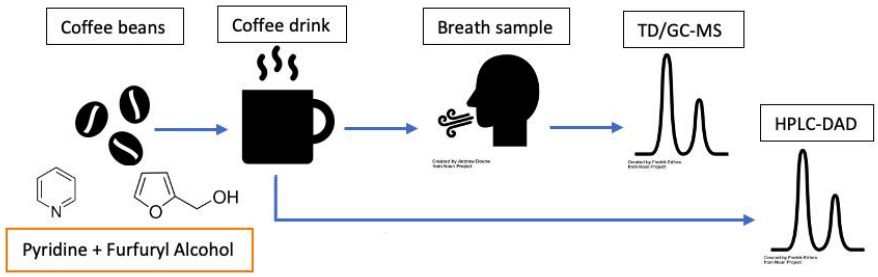


Figure 46: Graphical abstract of the analysis

6.2.1. Samples and methods

6.2.1.1. Coffee drink preparation

Coffee beans (dark roasted, 100 % Arabica) were purchased from a local store. Coffee drink samples were obtained using two different brewing methods, CafeRomatica (NIVONA) coffee machine and Italian moka pot. Two different parameters could be adjusted on the CafeRomatica coffee machine when the coffee beverages were prepared. The first parameter was the quantity of coffee beans used, and the second was the quantity of water used to brew the coffee. Light, normal and strong and coffee could be chosen and 3.50, 7.50 and 9.00 g of coffee beans were used respectively. The most common volumes of brewing water used by our volunteers were analysed (i.e., 30, 100 and 240 ml of water). For Italian moka pot coffee drinks, 12 grams of ground coffee and 200 ml of water were used.

6.2.1.2. HPLC analysis

Coffee drinks were prepared as described above. A sample of 2 ml was collected into a closed 2 ml plastic centrifuge vial to avoid evaporation of the VOCs and left to cool. The samples were then filtered using a 0.45 µm PTFE Captiva Econofilter (Agilent). An aliquot of 100 µl was spiked with Py and FFA standards and taken to a final volume of 1000 µl by adding deionized water with 0.1 % acetic acid. 50 µl of each sample were analysed using an Agilent 1100 series HPLC system with diode array detector and an Agilent Zorbax XDB-CN (3.0 x 150 mm, 3.5 µm) column.

Separation was achieved using a gradient mobile phase of 0.1 % Acetic acid in water (A) and Methanol (B) at 25 ºC. The gradient started at 95 % Phase A and increased to 20 % Phase B after 2.5 min, reaching 100 % phase B after 10 min and held until the end of the run (15 min). The flow rate used for the entire run was 0.5 ml/min. The absorption recorded for the detection of Py was 254 nm, and 217 nm for FFA. HPLC conditions were similar to the proposed by Albouchi [79] were furan derivates were analysed in coffee. As we wanted to analyse both Py and FFA in a single run using the same parameters, several changes were studied until the desired separation of both compounds was obtained [Figure 47].

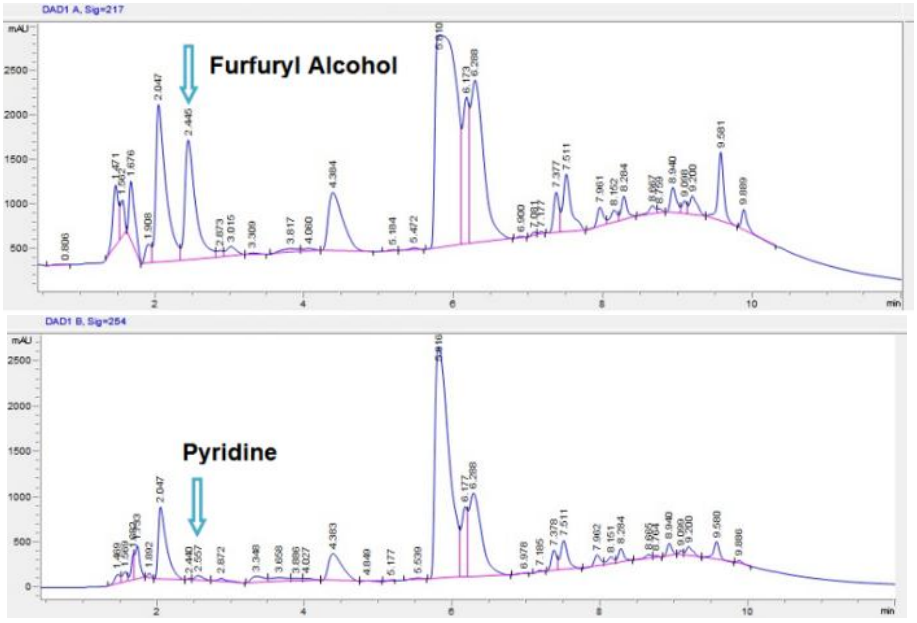


Figure 47: Chromatogram from spiked coffee sample. HPLC-DAD system. Wavelength 217 nm (up) and 254 nm (down).

6.2.1.3. TD/GC-MS analysis

Once the coffee drink was prepared and an aliquot of 2 ml was collected for HPLC analysis, the rest of the sample was consumed by the volunteer. Five volunteers participated in the study, providing samples during several days. None of the volunteers were smokers and could drink and eat any time before the study. Because of that, a breath sample was collected from the volunteer just before the coffee was consumed, using our self-modified 2-liter plastic bag. The background signal obtained was subtracted from the breath sample after coffee consumption. This helped to ensure the concentrations of MRPs obtained were coming only from the coffee ingested for the experiment. Immediately after finishing the drink, another breath sample was collected.

The breath sample was passed through a thermal desorption tube (Markes international Ltd. with Tenax® TA sorbent) at 250 ml/min (Air sampler Dupont Alpha-2). The same TD/GC-MS conditions as the ones indicated in section 3.2 and 3.3 were employed for the analysis of the samples. The magnetic scan was performed in the range *m/z* 50-100. For the quantification of the analytes, *m/z* 79 and *m/z* 98 were extracted for Py and FFA respectively.

6.2.1.4. Preparation of calibration solutions

For the HPLC analysis each coffee sample was spiked with a standard solution of 1, 2 and 4 ppm of Py and 10, 20 and 40 ppm of FFA and injected into the HPLC system. The linearity obtained for this method was satisfactory [Table 9]. Each coffee sample was prepared in triplicate to assess repeatability.

Table 9: r^2 values in standard addition calibration of coffee samples spiked with pyridine and furfuryl alcohol for 3 different aliquots.

Coffee Sample[a]		Pyridine			Furfuryl alcohol		
		1st	2nd	3rd	1st	2nd	3rd
L		0.9977	0.9972	0.9995	0.9832	0.9742	0.9760
N	30	0.9958	0.9963	0.9902	0.9830	0.9872	0.9892
S		0.9974	0.9982	0.9968	0.9636	0.9634	0.9643
L		0.9998	0.9997	0.9994	0.9923	0.9916	0.9916
N	100	0.9995	0.9998	1.0000	0.9854	0.9859	0.9793
S		0.9995	0.9994	0.9995	0.9924	0.9844	0.9836
L		0.9995	0.9980	0.9995	0.9846	0.9860	0.9840
N	240	0.9880	0.9913	n/a[b]	0.9978	0.9992	n/a[b]
S		0.9988	0.9984	0.9979	0.9862	0.9848	0.9874
Mk	200	0.9964	0.9999	n/a[b]	0.9852	0.9895	n/a[b]

[a] L (Light coffee) – 3.5 g; N (Normal coffee) – 7.5 g; S (Strong coffee) – 9 g; Mk (moka coffee) – 12 g and brewing water volume (ml). [b] Not enough sample to perform a third run of samples.

To calibrate the breath analysis, two water solutions of 5 and 50 ppm of both analytes were prepared. From each solution, 5, 10 and 20 µl were selected to obtain a 6-point calibration curve [Figure 48].

A self-made 2-liter plastic bag was partly filled with nitrogen gas and placed into the oven for a few minutes at 40 °C. Then, a volume from the standard solution was collected in a gas-tight syringe and quickly injected into the conditioned bag through the plastic valve and closed. The bag was then completely filled with nitrogen gas and a sample was collected for analysis using TD/GC-MS system. Before a set of breath samples was analysed, an injection of standard solution was performed to check the reliability of the calibration curve. When calculated concentration of the standard solution differed more than 10 %, a new calibration has been made.

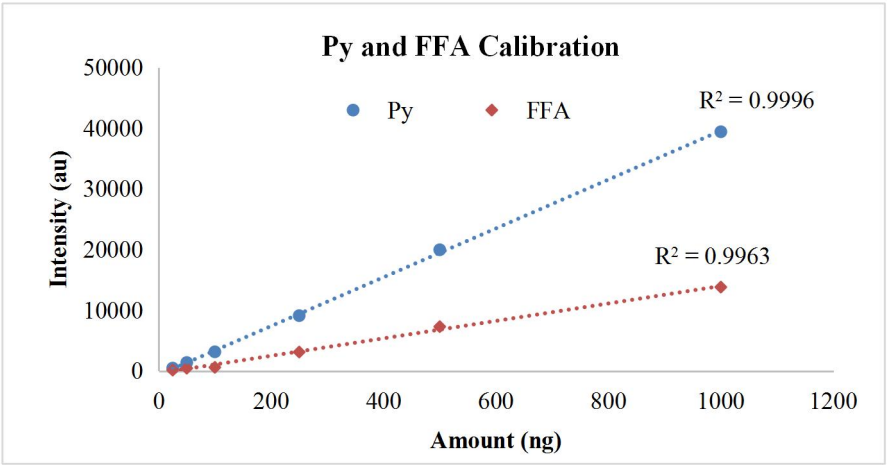


Figure 48: Pyridine (Py) and furfuryl alcohol (FFA) calibration curves obtained by TD/GC-MS.

6.2.2. Results and discussion

6.2.2.1. Dependence on the amount of ingested MRPs with their presence in breath

Regardless of the quantity of coffee beans used, levels of Py and FFA extracted per grams of coffee beans with the CafeRomatica coffee machine were similar [Table 10]. Higher quantities of both MRPs were obtained when preparing the coffee using an Italian moka pot due to higher temperature and pressure. used to brew the coffee. The quantities of toxic MRPs present in the coffee drinks, ranged from 0.2 to 3 mg for Py, and from 7 to 30 mg for FFA both depending on amount of water used for brewing the coffee.

Table 10: Quantities of pyridine (Py) and furfuryl alcohol (FFA) found in coffee drink.

Name[a]	Coffee[b] (g)	Water[c] (ml)	Sample[d] (µg/ml)		Cup[e] (mg/cup)		Beans[f] (mg/g)	
			FFA	Py	FFA	Py	FFA	Py
L30	3.5	30	236.7	7.5	7.1	0.23	2.0	0.07
N30	7.5		368.1	18.3	11.0	0.55	1.5	0.07
S30	9		427.9	20.2	12.8	0.61	1.4	0.07
L100	3.5	100	67.0	3.5	6.7	0.35	1.9	0.10
N100	7.5		126.2	8.7	12.6	0.87	1.7	0.12
S100	9		108.4	8.1	10.8	0.81	1.2	0.09
L240	3.5	240	40.1	3.1	9.6	0.75	2.7	0.21
N240	7.5		55.5	12.5	13.3	3.0	1.8	0.40
S240	9		67.8	5.1	16.3	1.2	1.8	0.14
Mk200	12	200	151.9	16.3	30.4	3.3	2.5	0.27

[a] L (Light coffee), N (Normal coffee), S (Strong coffee) Mk (moka coffee). [b] Grams of coffee used. [c] Millilitres of water used for brewing coffee. [d] Concentration of toxics in the sample, in micrograms per millilitre. [e] Milligrams of toxics in the full cup of coffee. [f] Milligrams of toxics per gram of coffee bean.
Note: standard deviation < 10 %

The levels of MRPs present per cup of coffee depended on the amount of coffee beans used to prepare the drink. Py increased from 0.2 mg using 3.5 g of coffee beans up to 1.2 mg when using 9 g of coffee; and FFA increased from 7 mg per coffee cup using 3.5 g of coffee beans, up to 16 mg using 9 g of coffee beans. Furthermore, using more water for brewing the coffee resulted in a higher amount of both MRPs.

6.2.2.2. Analysis of the breath samples

Similar levels of FFA in espresso coffee (1.6 mg/g) where found in other studies [113]. But also, lower levels were found in the literature, where maximum levels of 0.41 mg/g where detected [114]. Also, lower levels of Py (0.04 mg/g) were detected [115]. This difference in the concentrations of MRPs in coffee could be due to the different roasting procedures of the coffee beans [79], as well as the different bean sources might change the composition of the coffee [116].

Breath samples from all the volunteers showed concentrations of both toxic MRPs in a range from 1 to 1200 ng/l of Pyridine and to 760 ng/l of FFA [Figure 49]. We could observe a big dispersion of the results for each

sample replicate. According to the literature, values of Py in breath from 0.66 to 141 µg/l were found in patients with end-stage renal disease [117]; and up to 300 µg/l in active smokers [118]. Regarding FFA, we didn't succeed in finding concentration levels in breath

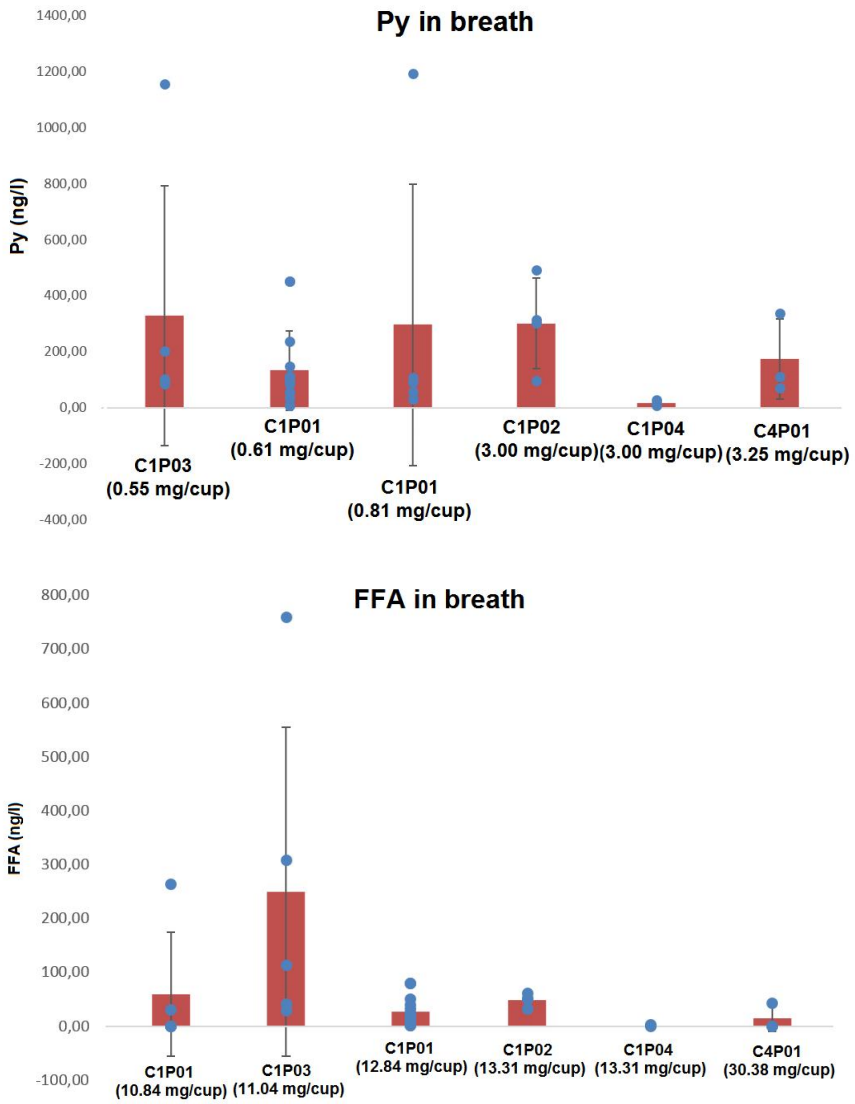


Figure 49: Concentration of Py and FFA (ng/l) found in breath for different volunteers with the quantities of toxics ingested by the volunteer in a cup of coffee (in parenthesis).

The concentrations of toxic MRPs in coffee drink were compared with the levels of toxics found in breath of each volunteer after drinking the

beverage [Figure 50] using the Pearson test. No correlation between levels of toxics ingested and their presence in breath was observed (*Pyridine = 0.00135* and *FFA = -0.168)*. Furthermore, we observed a big dispersion of the results for each volunteer ingesting the same amount of MRPs. Thus, we studied different factors that could influence the MRPs levels in breath when drinking coffee or collecting the sample.

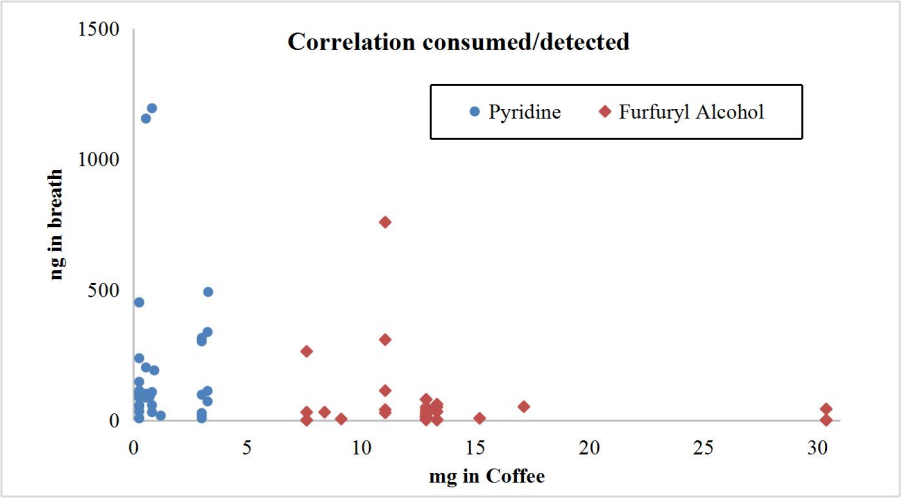


Figure 50: Correlation of toxics levels ingested with the levels detected in breath from volunteers

6.2.2.3. Factors affecting the results

The lack of correlation between ingested toxic MRPs in coffee and those present in breath, as well as the big dispersion in the results, indicates that there could be other factors influencing their concentration. We looked at 1) the time one takes to drink the coffee (or collection time after start of drinking coffee); and 2) the ingestion of water (or other liquids) after/while drinking coffee.

To check the possible influence of time, our volunteers, before collecting the breath sample, had small shot of coffee (N30) in a time range between 5 and 10 second. Just after drinking the coffee, a breath sample was collected. Subsequent breath samples were collected after 3, 6, 10, 15, 20, 30 and 45 minutes. We observed how the levels of both Py and FFA decrease with time obtaining the same results when repeating the experiment for

several days [Figure 51]. A power trendline was obtained for each data set that fit the results obtained the best.

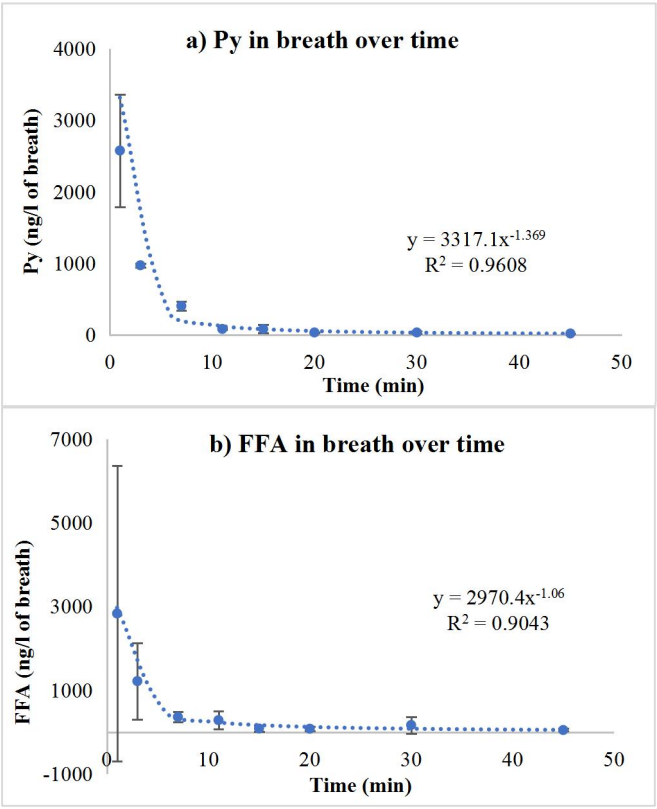


Figure 51: Concentrations of (a) pyridine (Py) and (b) furfuryl alcohol (FFA) detected in breath depending on the time of collecting the sample after coffee ingestion.

The results showed a consistent drop in the levels of both MRPs. A high deviation of the results (30 % for Py and more than 100 % for FFA) in the first seconds of breath sampling might be due to the fast drop in the concentration. The results could be different if the sampling is delayed just for a few seconds. The deviation of the results gets much lower with time. The most probable cause for the drop in the concentration is the high volatility of both compounds.

In the next experiment, our volunteers had the same amount of coffee (30 ml of Normal coffee) but consumed it more slowly, taking about 1 minute to finish the coffee. The breath sample was collected straight after finishing the drink. The second experiment indicates that levels of both toxic

MRPs are decreasing before the total amount of coffee is consumed [Figure 52]. This makes it difficult to relate the levels of toxic MRPs in a volunteer drinking a small espresso (30 ml) with other drinking a long coffee (240 ml), as they will need different times to finish the drink. Therefore, it is recommended to set the initial time when the person starts to drink the coffee, and not when the sample is collected after finishing the drink.

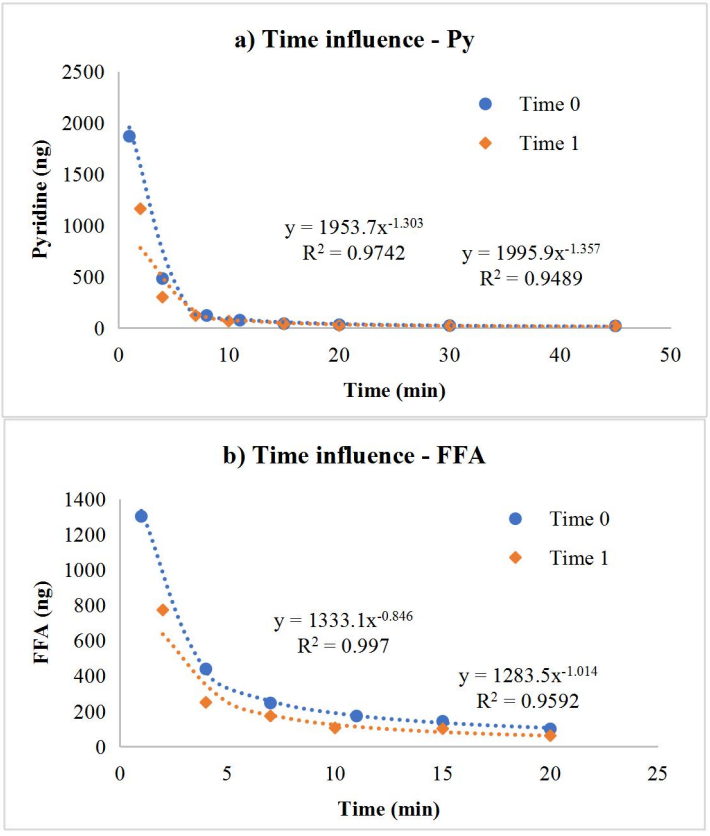


Figure 52: Quantity of Py (a) and FFA (b) detected in breath depending on the time of collecting the sample, finishing drinking the coffee within seconds (Time 0) and slowly for 1 minute (Time 1).

Another possible factor we studied was the ingestion of other liquids while drinking coffee as other liquids might dilute the toxic MRPs. We checked the influence of drinking the same coffee (30 ml, normal) with milk (100 ml), and the influence of drinking a glass of water after drinking the coffee. As the drink volume with milk is higher we counted the time from the start of ingesting the coffee, taking 2 minutes longer than drinking an espresso. There was no change on the concentration curve for Py when analysing the breath after drinking coffee with milk. However, we observed a drop in the signal for FFA [Figure 53]. A possible explanation for this behaviour could be from the casein in the milk diluting FFA.

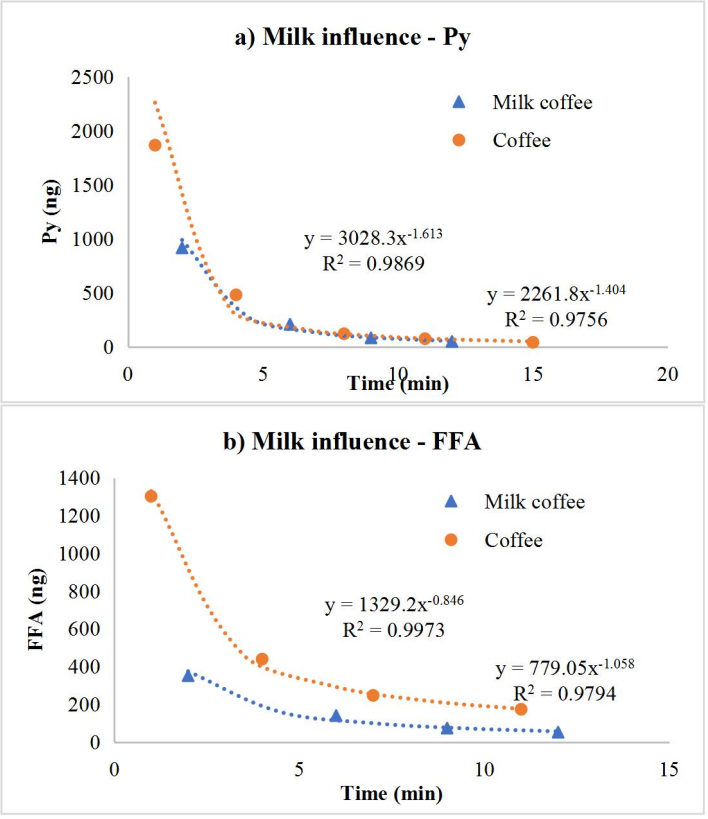


Figure 53: Quantity of Py (a) and FFA (b) detected in breath depending on the time of collecting the sample after coffee ingestion, comparing drinking Espresso 30 ml (Coffee) with Coffee 30 ml with milk 100 ml (Milk coffee).

To analyse the effect of drinking water after coffee, we collected the breath sample just after having the coffee, then the volunteer had a glass of water, and then we collected another the breath sample. We observed

[Figure 54] that drinking water after having a cup of coffee does not affect the levels of toxic MRPs in breath, and we detected the same drop in concentration as when drinking only coffee.

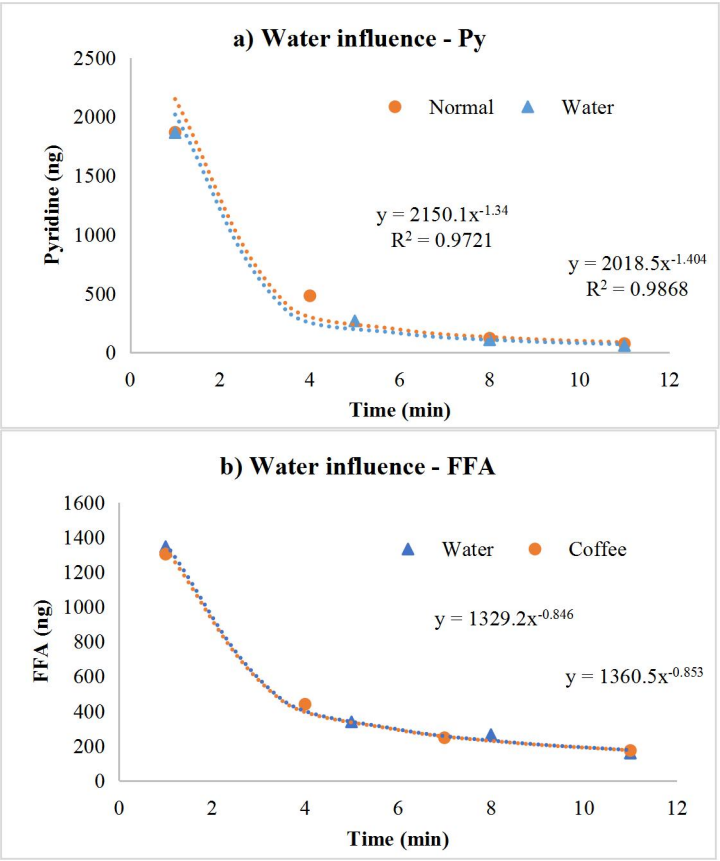


Figure 54: Quantity of Py (a) and FFA (b) detected in breath depending on the time of collecting the sample after coffee ingestion, drinking water after finishing the coffee (Water) compared with shot of coffee (Normal).

Our study showed that levels of both Py and FFA present in coffee can be detected from human breath emissions in concentrations as low as 7 ng/l of breath for Py and 1 ng/l of breath for FFA. Even though the levels of both compounds before coffee intake were below limit of detection (LOD), background signal was subtracted from the results. The intake of both compounds reached 3 and 30 mg per cup of coffee for Py and FFA respectively. Those levels are above the recommended levels established by JECFA (ADI of 0.002 mg/kg/day for Py and 0.5 mg/kg bw for FFA), and the

consumption of big amounts of coffee (as found in European Nordic countries) might be concerning for health outcomes. Because we did not find a direct correlation between the quantity of ingested coffee and levels of specific chemicals in a person's breath, we checked for possible factors affecting the results. The results show a very strong drop in the quantities of Py and FFA found in breath with time after finishing the coffee drink, probably due to fast evaporation of the compounds. Nevertheless, breath analysis has proven to be effective on detecting Py and FFA after their ingestion; even 45 minutes after the intake of 1 cup of coffee, levels of both MRPs could be still detected. The presence of very high levels of MRPs in breath might be due to food intoxication sources and could provide with proper medical help. This suggests the possible application of breath analysis for the detection of food intoxication by volatile compounds like MRPs.

All food products are characterised by their taste and smell. Those features come most of the time by their VOCs composition. Using our self-developed sampling device, we could obtain the food and drink VOCs profiles which can be used for their discrimination. We could differentiate between 4 types of peanuts and get an idea of the blends of coffee beans we are drinking. In addition to that, we could identify food poisoning in case of ingesting high amounts of possible toxics found in the food and drinks.

7. VOCs IN THE ENVIRONMENT

7.1. Indoor contamination

The use of fragrances

As we discussed previously, many exposures to VOCs are coming from the use of fragrances indoors. In order to understand the effect of this exposure in the human's breath composition, we analysed several air-fresheners compositions and selected the one with higher number of VOCs.

Three different air-fresheners were purchased from a local store. An air sample was collected from an empty 50 m^3 office room where the study was taking place. After getting the background signal of the room each air-freshener was applied in big amounts by pressing the nebulizer for 15 seconds. When the room was filled with the fragrances from the air-freshener an air sample was collected. The background signal was subtracted from the final sample [Figure 55].

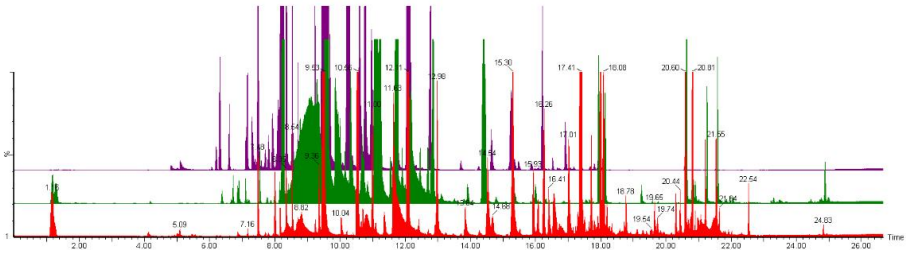


Figure 55: Chromatograms from air sample after three different air-freshener used.

As it was expected, the composition of air-freshener was composed mainly from terpenes, aldehydes, and synthetic musks.

After selecting the air-freshener, we used it to fill again the room. A volunteer, from which a breath sample was collected before exposure, stayed in the room for 10 minutes before another breath sample was collected. Looking at the results we could observe how several air-freshener VOCs are now present in the breath composition [Figure 56].

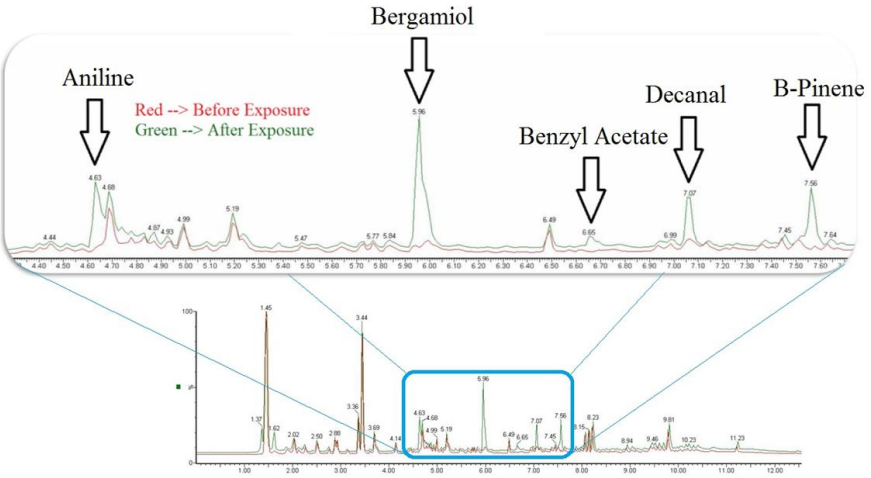


Figure 56: Comparison of chromatograms of breath sample before and after exposure to air-freshener

Being exposed to a high quantity of air-fresheners could modify the breath composition. Even though the air-freshener has more than 50 different VOCs, only a low number is present in the breath. This might be because most of the compounds could be absorbed by the body after exposure and be eliminated by other routes.

7.2. Outdoor contamination

Outdoor air samples from different locations within Lithuania were collected and compared to understand the different sources of VOCs and the variations of their levels.

We selected an industrial area in Klaipėda city, where several samples per day were collected from 3 different locations. The locations selected were close to 3 different production sites (Factory A, Factory B and Factory C). The main idea was to study how the industrial emissions change during the day. In addition to that, we compared the emissions between the 3 different locations.

The results [Figure 57] show very similar VOCs profiles at different times of the day. However, we could still observe some variations, especially for Factory C, where emissions of 2-phenoxyethanol (15.00 min) are dominant at 2 different times of the day. Also, an increase on camphene (9.52 min) peak for Factory A and toluene (3.82 min) peak for Factory B during the day. Those different levels could be due to the traffic in the area, rather than emissions from the factories.

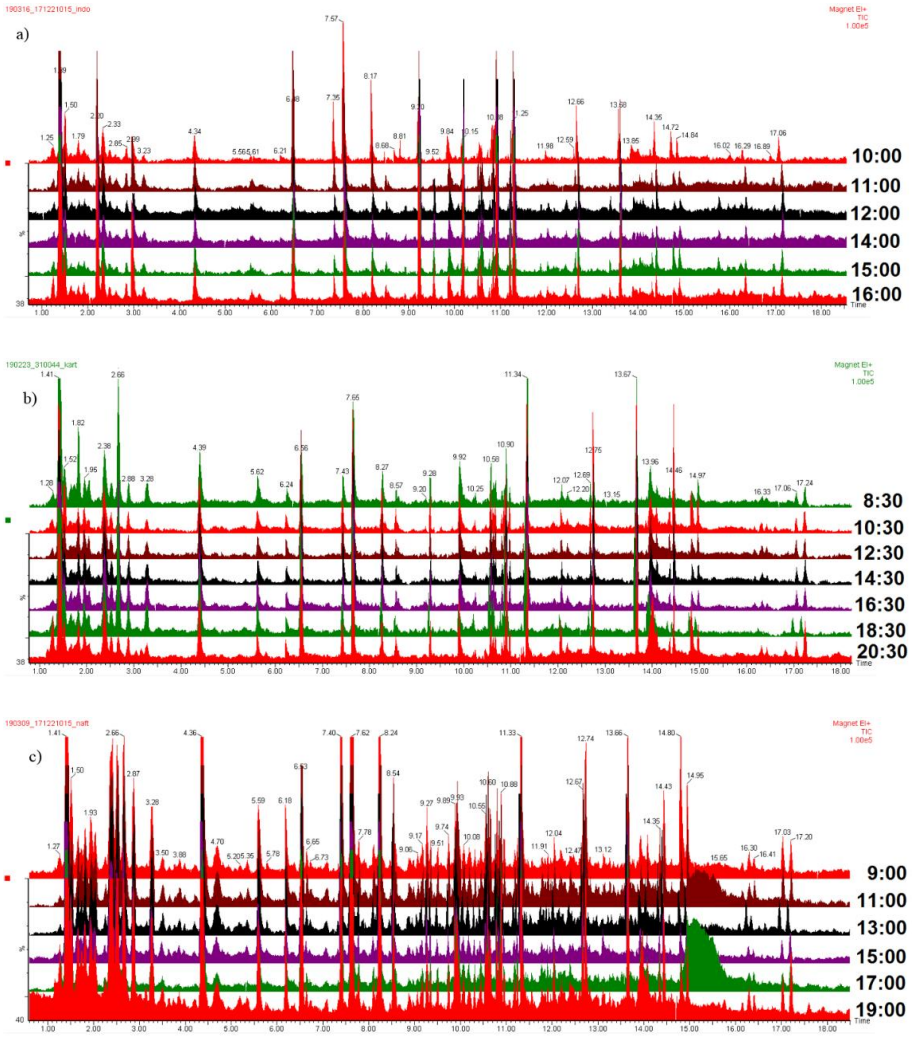


Figure 57: Air samples from a) Factory A, b) Factory B, c) Factory C, at different times a day.

Comparing the 3 locations [Figure 58] we also observe different VOCs profiles due to the different sources. Factory C has a higher amount of hydrocarbon emissions (from 1.00 to 3.00 min) and aromatic compounds like benzene derivates (from 9.00 to 13.00 min), probably from their production site; Factory A has many more terpene compounds (from 9.00 to 11.00 min) than the other two locations, maybe for the greenery area nearby.

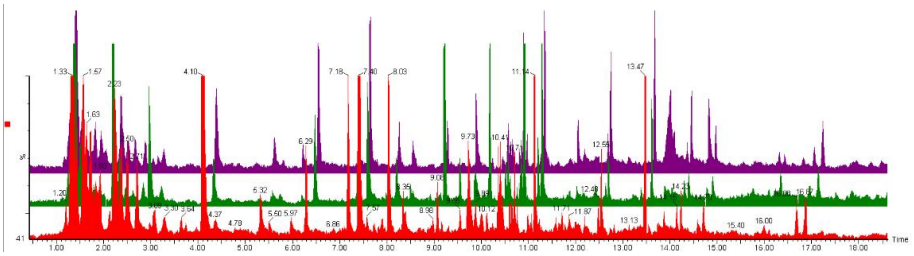


Figure 58: Comparison of 3 industrial locations. Red: Factory C; Green: Factory A; Purple: Factory B.

To continue the study of volatiles in the environment, we aimed to analyse the VOCs pattern from several locations in Vilnius centre in an area of 2 km^2 and identify the different emission sources influencing the air composition in Vilnius. We wanted to see if it was possible to differentiate the location in a relatively small area.

7.2.1. Locations selected in Vilnius city centre

We have checked the volatiles from 6 different locations in Vilnius, Lithuania. All the locations in a 2 km^2 area within Vilnius city centre [Figure 59]. The locations were chosen with the criteria to have different traffic and greenery conditions with a variety of VOCs sources.

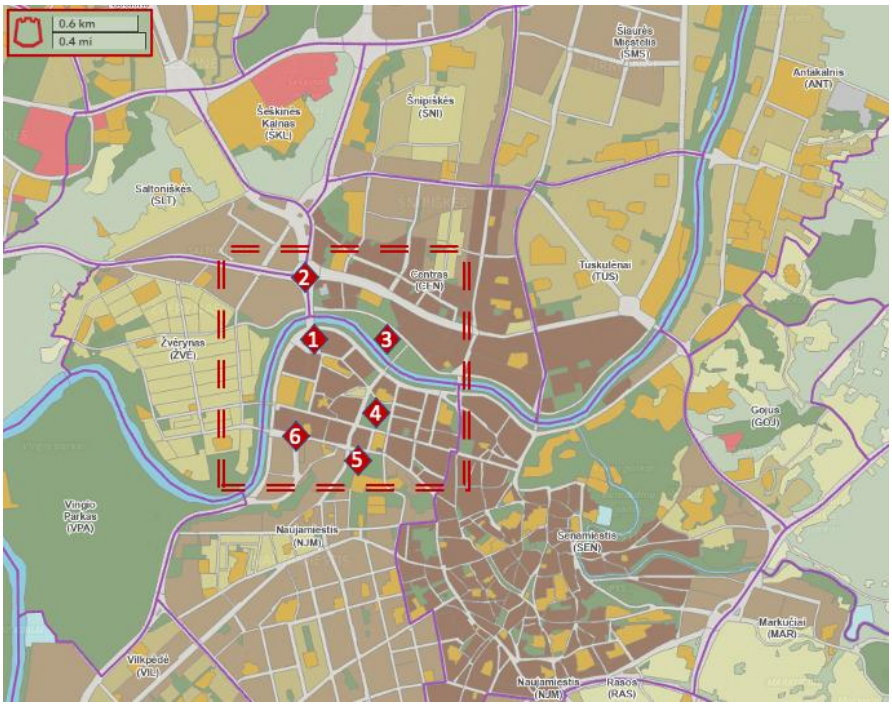


Figure 59: Vilnius map with the sampling locations [from www.maps.vilnius.lt]

Map legend:
1 – Petrol station close to heavy traffic road.
2 – Very heavy traffic road crossing (elevated pass).
3 – Large park area with low traffic road nearby.
4 – Small park area with low traffic road nearby.
5 – Small park area with heavy traffic road nearby.
6 – Very heavy traffic road (tunnel).

7.2.2. Equipment

TD tubes with Tenax® TA sorbent were employed for sample collection using the air sampler "Aircheck Sampler model 224-44XRM" from SKC Inc.

7.2.3. Sampling procedure

TD tubes were conditioned following manufacturers' instructions. Air samples were collected at the specific locations [Figure 59] by applying a flow of 1000 ml/min for 10 minutes. All the samples were collected the

same day, in a range of two hours to have similar weather conditions in all the locations [Table 11]. The samples were analysed within 3 hours from their collection time. Samples from the same exact locations were collected during 6 different days to compare the variations of the VOC profile.

7.2.4. Weather conditions

Weather conditions for the 6 days that samples were collected on are presented in Table 11.

Table 11: Weather conditions

Day 1 (3rd August 2021)						
Time	**Temp**	**Humidity**	**Wind**	**Wind Speed**	**Precip.**	**Condition**
10:20 AM	17 °C	77 %	WSW	17 km/h	0.0 mm	Mostly Cloudy
10:50 AM	16 °C	88 %	WSW	15 km/h	0.0 mm	LightRain Shower
11:20 AM	18 °C	83 %	WSW	19 km/h	0.0 mm	Mostly Cloudy
11:50 AM	18 °C	73 %	W	17 km/h	0.0 mm	Mostly Cloudy
Day 2 (20th August 2021)						
Time	**Temp.**	**Humidity**	**Wind**	**Wind Speed**	**Precip.**	**Condition**
10:20 AM	19 °C	60 %	WSW	17 km/h	0.0 mm	Fair
10:50 AM	20 °C	56 %	WSW	19 km/h	0.0 mm	Mostly Cloudy
11:20 AM	20 °C	60 %	SW	20 km/h	0.0 mm	Mostly Cloudy
11:50 AM	20 °C	60 %	WSW	19 km/h	0.0 mm	Mostly Cloudy
Day 3 (23rd August 2021)						
Time	**Temp.**	**Humidity**	**Wind**	**Wind Speed**	**Precip.**	**Condition**
10:20 AM	19 °C	56 %	SSW	7 km/h	0.0 mm	Fair
10:50 AM	20 °C	52 %	VAR	7 km/h	0.0 mm	Partly Cloudy
11:20 AM	18 °C	56 %	W	6 km/h	0.0 mm	Mostly Cloudy
11:50 AM	19 °C	56 %	VAR	6 km/h	0.0 mm	Mostly Cloudy
Day 4 (24th August 2021)						
Time	**Temp.**	**Humidity**	**Wind**	**Wind Speed**	**Precip.**	**Condition**
10:20 AM	15 °C	63 %	NNE	17 km/h	0.0 mm	Partly Cloudy
10:50 AM	15 °C	63 %	NNE	19 km/h	0.0 mm	Mostly Cloudy
11:20 AM	15 °C	59 %	N	22 km/h	0.0 mm	Partly Cloudy
11:50 AM	16 °C	55 %	NNE	20 km/h	0.0 mm	Partly Cloudy

Day 5 (25th August 2021)						
Time	**Temp**	**Humidity**	**Wind**	**Wind Speed**	**Precip.**	**Condition**
2:50 PM	17 °C	52 %	W	17 km/h	0.0 mm	Mostly Cloudy
3:20 PM	17 °C	52 %	SW	19 km/h	0.0 mm	Mostly Cloudy
3:50 PM	16 °C	55 %	WSW	15 km/h	0.0 mm	Mostly Cloudy
4:20 PM	17 °C	52 %	WSW	13 km/h	0.0 mm	Mostly Cloudy
Day 6 (7th September 2021)						
Time	**Temp.**	**Humidity**	**Wind**	**Wind Speed**	**Precip.**	**Condition**
10:20 AM	15 °C	55 %	SW	13 km/h	0.0 mm	Fair
10:50 AM	15 °C	55 %	SSW	15 km/h	0.0 mm	Fair
11:20 AM	16 °C	48 %	SW	15 km/h	0.0 mm	Fair
11:50 AM	16 °C	45 %	WSW	15 km/h	0.0 mm	Fair

Source: www.wunderground.com. The Weather Company, an IBM business

7.2.5. Analysis of the sample

TD tubes were loaded into the TD system (ATD 400; Perkin Elmer) where the tubes were heated, releasing the trapped compounds into the system using the parameters from Section 3.3 Table 3. Compounds were then separated and identified using a GC-MS system (Agilent 6890N coupled to mass spectrometer Waters/Micromass AutoSpec Premier). The GC-MS analysis parameters are the same as the specified in the Section 3.2. Compound identification was performed by library match using NIST MS Search 2.0 (2005) mass spectral library. Data statistical analysis was performed using R Core Team, The "jamovi" project and OriginLab software [119, 120].

7.2.6. Results and discussion

The compounds from all the locations were identified and their relative concentration was obtained by collecting the areas from the chromatograms [Table 12].

Table 12: Main compounds identified in the locations studied and their average relative concentration for each location

Ret time	**Compound**	**Pos. sources**	**Loc 1**	**Loc 2**	**Loc 3**	**Loc 4**	**Loc 5**	**Loc 6**

			Rel. %	**Rel. %**	**Rel. %**	**Rel. %**	**Rel. %**	**Rel. %**
1.00	Acetone	P / C / H	6.2±4.2	8.1±7.1	10.0±10.3	6.7±1.4	5.6±2.2	5.8±1.6
1.13	tert-Butyl methyl ether	F	5.7±3.2	2.4±0.6	2.8±1.1	2.3±0.7	2.5±0.2	2.7±0.7
1.40	*Benzene	P / C	4.7±1.2	5.8±1.3	3.8±1.4	4.8±1.2	4.9±1.4	5.2±1.9
1.59	Heptane	F	1.9±0.3	2.5±0.8	2.9±2.2	3.1±2.0	3.0±1.4	3.1±1.9
1.81	Butyl Octanol	P / C	0.5±0.4	0.9±0.6	0.7±0.5	0.9±0.7	0.8±0.5	0.8±0.6
1.98	Hexone	O	0.5±0.4	0.6±0.2	0.6±0.3	0.8±0.4	0.6±0.3	0.6±0.3
2.24	*Toluene	P / C	20.7±6.7	18.1±5.5	12.7±5.4	15.0±3.7	17.8±4.8	17.4±2.3
2.72	Octane	P / C	0.9±0.3	0.5±0.1	0.7±0.3	0.5±0.2	0.8±0.3	3.3±3.8
3.05	Butyl Acetate	F	1.0±0.6	2.9±2.3	3.0±2.1	1.7±0.5	1.6±0.6	1.3±0.5
3.97	*Ethyl Benzene	P / C	3.8±1.4	3.9±1.6	2.9±0.7	3.1±1.0	3.7±0.8	4.1±0.8
4.22	*m,p xylene	P / C	13.0±4.5	11.5±2.5	8.9±3.1	9.6±3.5	12.7±2.5	13.1±2.3
4.77	*o xylene	P / C	4.6±1.4	5.2±1.1	3.6±1.1	4.3±1.2	5.0±0.6	5.4±1.0
5.84	#a-pinene	V / F	2.9±1.8	4.5±3.0	6.8±4.1	5.4±3.6	3.0±1.6	3.0±0.8
6.31	#Camphene	V / F	1.1±1.0	0.7	1.1±0.4	0.6	0.8	<LOD
6.41	Dimethylolpropane	O	8.5±2.3	<LOD	<LOD	<LOD	<LOD	<LOD
6.54	*Propyl Benzene	P / C	1.1±0.7	1.1±0.3	1.2±1.3	0.6±0.1	0.9±0.2	1.0±0.1
6.74	*1,2,4-Trimethylbenzene	P / C	2.4±1.2	1.9±0.5	2.7±2.8	1.3±0.7	2.4±1.0	2.3±0.2
6.85	*m-Ethyltoluene	O	1.3±0.5	0.8±0.2	2.6	1.0	0.9±0.7	1.0±0.1
6.90	Benzaldehyde	V / F	2.6±1.7	6.0±1.9	6.7±5.5	9.4±5.8	5.1±2.5	4.0±1.3
7.14	#4-Carene	V / F	0.6	0.9	2.2±1.4	3.4	<LOD	<LOD
7.23	*p-Ethyltoluene	P / C	0.8±0.3	1.0±0.3	2.1	0.6	0.9±0.3	1.1±0.2
7.72	*1,2,3-Trimethylbenzene	P / C	3.8±1.4	3.5±0.8	2.9±2.3	3.2±2.3	3.9±1.5	3.6±1.2
8.02	4-Ethyloctane	P / C	0.8±0.5	1.1±0.4	0.9±0.2	1.2±0.4	1.1±0.6	1.3±0.5
8.14	#Terpinen	V / F	1.3±1.1	3.2±1.9	9.3±8.5	7.2±8.1	2.9±3.3	1.6±0.3
8.22	Octanal	P / C	0.7±0.7	0.5±0.2	1.4±0.2	1.7±0.9	1.0±0.3	0.8±0.2
8.53	*1,3,5-Trimethylbenzene	P / C	0.7±0.4	0.9±0.1	1.3±1.4	0.7±0.1	0.9±0.2	1.1±0.3
8.85	#D-Limonene	V / F	0.6±0.3	0.7±0.2	2.3±1.8	1.5±1.5	0.9±0.7	0.5±0.1
8.90	p-Methylstyrene	O	0.6±0.2	0.73±0.04	0.5	0.9±0.1	0.6±0.1	0.7±0.2
9.03	2-Ethyl-1-decanol	O	0.6±0.3	0.6±0.2	1.0±0.3	1.2±0.4	1.0±0.6	1.0±0.4
9.42	*1,3-Diethylbenzene	P / C	0.1±0.1	0.4	<LOD	<LOD	0.3	<LOD
9.51	*1-Methyl-3-propylbenzene	P / C	0.6±0.2	0.8±0.2	<LOD	<LOD	0.6±0.1	0.8±0.2
9.68	*1-Methyl-2-propylbenzene	P / C	0.4±0.3	1.1±0.6	<LOD	<LOD	0.7	1.1±0.7
9.72	*2-Ethyl-1,4-dimethylbenzene	P / C	0.8±0.4	1.0±0.5	<LOD	<LOD	1.5±0.2	1.1±0.4
9.90	2-indanol	O	0.5±0.3	0.43±0.03	0.7	<LOD	0.4±0.1	0.3
10.11	Acetophenone	V / F	1.9±1.0	3.8±1.8	4.4±2.6	4.6±2.5	2.6±1.6	2.3±0.5
10.28	*1,2-Dimethyl-4-ethylbenzene	P / C	0.4±0.2	1.9±1.6	<LOD	<LOD	1.1±0.5	1.5±0.9
10.36	*1-Ethyl-2,4-dimethylbenzene	P / C	0.7±0.5	0.8±0.5	<LOD	1.2	0.7±0.1	0.6
10.53	*1,2,4,5-Tetramethylbenzene	P / C	0.6±0.2	1.3±0.6	<LOD	0.7±0.4	0.9±0.3	0.8±0.1
11.13	Undecane	O	1.3±0.7	1.4±0.4	1.1±0.5	1.2±0.3	1.3±0.5	1.9±0.4

11.38	Nonanal	F / B	5.8±5.5	2.5±1.4	4.8±4.2	3.3±1.1	4.7±3.1	3.1±1.5
11.52	*1,2,3,4-Tetramethylbenzene	P / C	0.2±0.1	0.7	<LOD	<LOD	0.2	0.6±0.1
11.59	*1,2,3,5-Tetramethylbenzene	P / C	0.5±0.3	0.7±0.2	<LOD	<LOD	0.7±0.2	0.7±0.2
12.42	4-Methylindane	O	0.5±0.7	0.4±0.1	<LOD	<LOD	<LOD	<LOD
12.51	*1,2-Dimethyl-3-ethylbenzene	P / C	0.3±0.2	0,8	<LOD	<LOD	0.3	0.5
13.53	1-Methylene-1H-indene	O	0.7±0.6	1.4±0.5	1.2±0.2	1.1±0.1	1.0±0.4	1.5±0.5
14.18	6-Methyltridecane	O	1.0±0.9	2.0±1.5	1.2±0.6	1.8±0.8	1.2±0.5	1.8±0.4
14.44	Decanal	V / F	1.0±1.0	1.9±1.3	2.7±1.0	3.3±3.2	3.0±1.1	1.4±0.3
14.89	Benzothiazole	F	0.6±0.1	1.5	0.8	1.2	1.3±1.5	1.4
15.03	Cyclohexyl isothiocyanate	O	0.7±0.5	1.2±0.3	1.4±0.4	1.4±0.6	1.9±1.2	1.1±0.4
16.58	#Isobornyl acetate	V	1.0	0.8±0.1	3.8±3.6	1.3	0.3	0.4
17.06	Tridecane	P / C	1.1±0.9	1.3±0.2	1.2±0.5	1.3±0.5	1.2±0.5	1.5±0.4
*Total Benzene derivates			58.9±16.7	56.0±7.7	37.1±15.2	41.9±9.0	54.8±13.3	57.4±2.4
#Total Terpenes			4.7±3.7	8.1±6.0	22.1±18.5	14.4±13.2	6.8±5.4	5.0±1.3

<LOD: below limit of detection

Sources: P – Petrol / C – Car exhaust / FA – Fuel additive / F – Fragrances / V – Vegetation / B – Human breath / O – Other

In the area of two square kilometres, we found different sources of VOCs; each area gave a distinctive pattern of compounds [Figure 60].

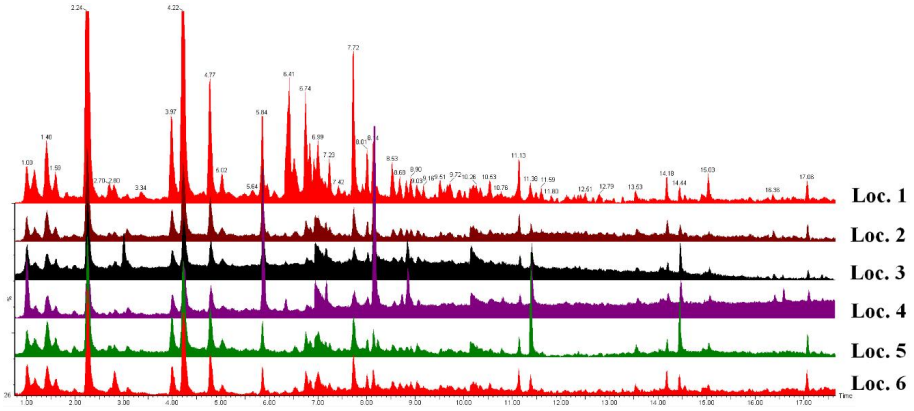


Figure 60: GC chromatogram of the 6 locations (same day)

As we collected the volatiles from those location during several days, we could identify punctual events happening in each location. Re-fuelling the petrol station [Figure 61], cutting the grass, installation of new benches (newly painted).

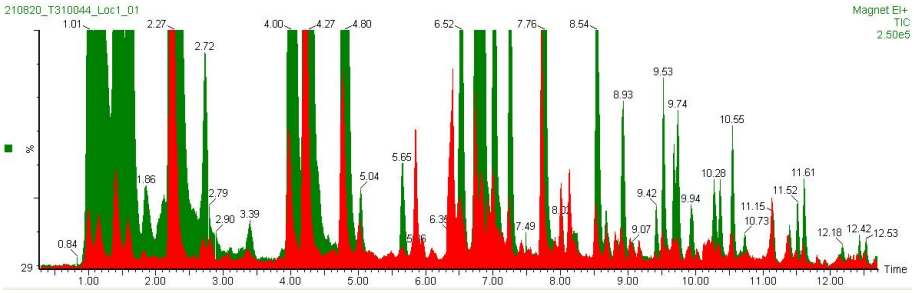


Figure 61: Increase of VOC levels when re-fuelling petrol. Green: Re-fuelling event Location 1 (Day 2); Red: Usual day Location 1 (Day 6)

Meteorological events also affected the results; during a windy day, the composition of VOCs was diluted, obtaining a decrease in the levels of compounds [Figure 62].

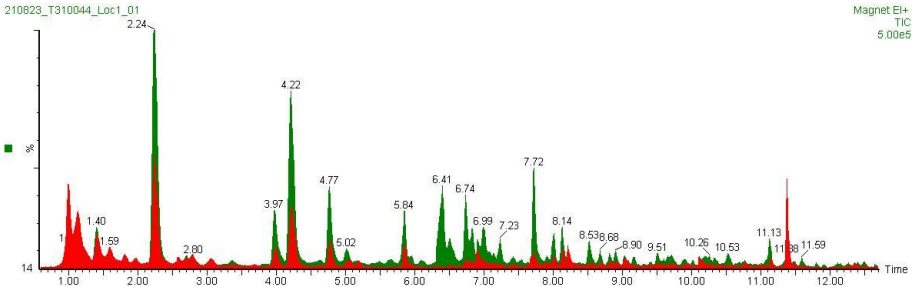


Figure 62: Decrease of VOC levels on a windy day. Green: wind speed 7 km/h Location 1 (Day 3); Red: wind speed 17 km/h Location 1 (Day 1)

Even though the levels of VOCs might differ depending on various factors and weather conditions, we studied the full profile for each location. We calculated the average relative concentration of VOCs for the 6 different locations during all the sampling days [Table 12]. Principal Component Analysis (PCA) was performed to decrease the number of variables [Figure 63].

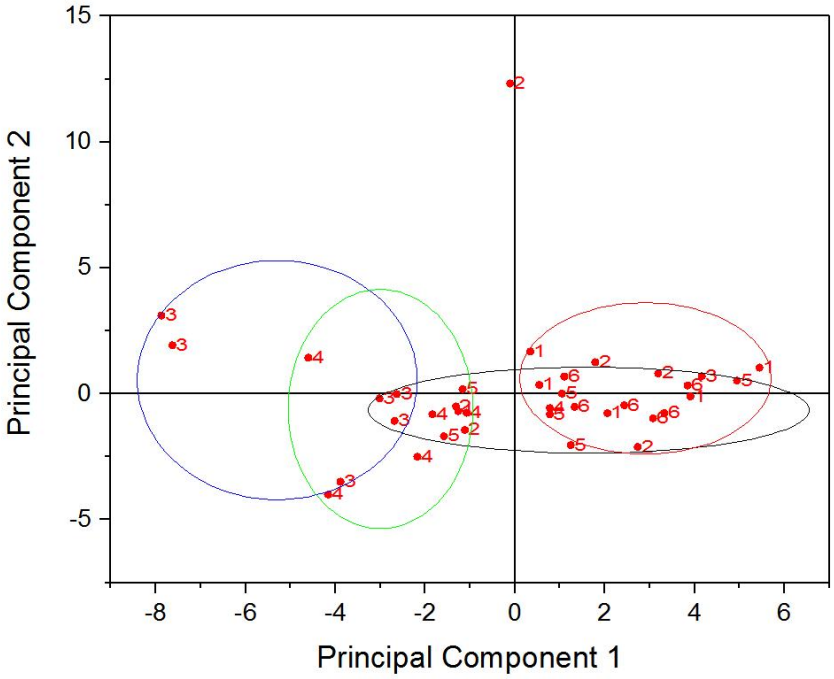


Figure 63: PCA analysis for location results

The data showed that the main difference across the locations were the presence of benzene derivates, and terpene compounds. Therefore, we decided to group those variables, benzene derivates come mainly from fuel sources and vehicle exhaust, while terpenes come mainly from vegetation sources [Figure 64].

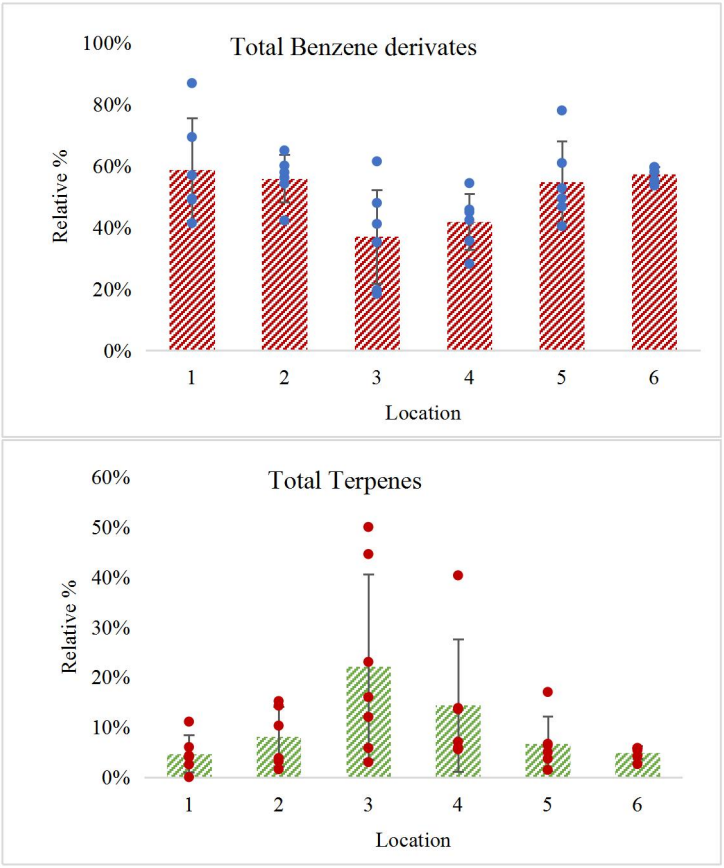


Figure 64: Relative concentrations of total benzene derivates (top) and total terpenes (bottom) for each location. Column graph - Total average; scatter graph - Daily value.

However, the big variance of the results might be a problem to get statistically significant differences. We firstly performed Levene's test [Table 13] for the analysis of variance homogeneity; and Shapiro-Wilk test [Table 14] for the study of the normal distribution of the results. Looking at the test results we cannot assume a normal distribution of the results for the total terpene concentration nor assume equal variances. Consequently, we performed a Welch's One-way ANOVA for total terpenes, and Fisher's One-Way ANOVA for total benzene derivates [Table 15]

Table 13: Homogeneity of Variances Test (Levene's)

	F	df1	df2	p
Terpenes	4.72	5	31	0.003
Benzene Derivates	2.18	5	31	0.082

Table 14: Normality Test (Shapiro-Wilk)

	W	p
Terpenes	0.868	< .001
Benzene Derivates	0.949	0.087

Note. A low p-value suggests a violation of the assumption of normality

Table 15: One-Way ANOVA

		F	df1	df2	p
Terpenes	Welch's	1.81	5	13.0	0.180
Benzene Derivates	Fisher's	3.82	5	31	0.008

The analysis showed a non-statistically significant result for total terpenes concentration for the six locations (*p=0.180*), but a statistically significant result for total benzene derivates concentration (*p=0.008)*. We finally performed a Tukey (equal variances) Post-Hoc Test [Table 16] to identify in which locations the results were statistically significantly different. Only the locations No. 4 and No. 6 showed a statistically different result.

Table 16: Games-Howell Post-Hoc Test – Benzene Derivates

		1	2	3	4	5	6	
1	Mean difference	—	2.91	21.8	16.95	4.12	1.47	
	p-value	—	0.998	0.224	0.338	0.996	1.000	
2	Mean difference		—	18.9	14.04	1.21	-1.44	
	p-value		—	0.127	0.120	1.000	0.997	
3	Mean difference			—	-4.89	-17.72	-20.36	
	p-value			—	0.976	0.293	0.080	
4	Mean difference				—	-12.83	-15.48	*
	p-value				—	0.431	0.050	
5	Mean difference					—	-2.65	
	p-value					—	0.995	
6	Mean difference						—	
	p-value						—	

Note. * p < .05

We analysed the VOC content and their relative concentrations in 6 different locations in Vilnius within an area of 2 km^2. The results show a tendency in which areas with higher traffic load (Locations 1, 2, and 6) have a higher concentration of anthropogenic VOCs. In addition to that, the levels of biogenic VOCs were higher in locations dominated by greenery (Locations 3 and 4). Location 5 (a park with heavy traffic) have a mix of both conditions; therefore, the weight of anthropogenic and biogenic VOCs is similar. We observe a very disperse variation of the results for each location. This is due to a number of parameters that could affect the results: punctual events like re-fuelling in a petrol station, cutting the grass, fumigating plants, traffic conditions, installation or renewal of urban furniture, as well as weather conditions like air temperature, wind speed, wind direction or rain. Due to these variations and data that contain many outliers, we could not establish statistically significant results. We could not establish that the differences in VOCs compositions across locations were statistically significant. However, we still observed differences in VOCs compositions across the six locations. This shows that even though the area of sampling was rather small, the different sources of VOCs have a strong influence in the air composition. This difference in the air composition allowed us to discriminate different environments in the city.

We have studied the VOCs composition from different environments around Lithuania. Our study shows how many VOCs sources have a different weight depending on the location, as well as the time of the day. Locations close to forest will have a much different VOCs profile than an industrial area due to the variation in their sources. Terpenoids will have a major presence in a forestry environment, and fossil fuels in industrial or urban areas. The VOCs profile for every location is continuously responding to the changes in local events, traffic conditions, greenery in the surroundings, weather conditions and seasonal changes.

8. DISCUSSION ON THE RESULTS

After our research on VOCs, we could get a better understanding of the volatile composition of our environment. We can identify specific marker compounds from their different sources and might be able to relate them.

Understanding all different VOCs and their sources could give us a relation between the three main topics we studied in our research [Figure 65].

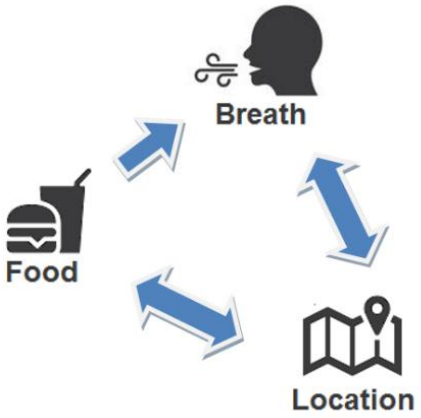


Figure 65: The links between the three studied VOCs sources

8.1. Markers in breath from ingested food

When analysing the human breath, we can identify VOCs from specific types of food. The presence of compounds like alkylpyrazines or furanic compounds are commonly coming from roasted or baked food products, especially in great quantities are found in roasted coffee. Limonene is found in high concentrations in fruits or sweets. Analysing the human breath can give us an idea of the food it was ingested in a short period of time. This could be useful to identify a possible food poisoning situation.

8.2. Markers in breath from specific locations and exposures

When studying the human breath VOCs profile, we could identify some compounds coming from specific locations. The presence of a high quantity of terpenes could be due to the person living or staying close to a forest or a park. In the other hand, when the presence of hydrocarbons and benzene derivate is higher, we could stablish that the person was stuck in a traffic jam, is living in a polluted environment or close to a busy road. In addition to that, the presence of compounds like menthol and derivates are specific from toothpaste products. Other terpenes can appear from the exposure to cosmetic, fragrances or cleaning products. This could be useful to know if the person is being exposed to a contaminated environment.

8.3. Locations markers from people's presence

The VOCs composition of the air in a specific location is determined by the different sources that are nearby. Terpene compounds are typical from greenery and hydrocarbon or benzene derivates are coming from car exhaust and fossil fuels. Furthermore, the presence of compounds typically produced by humans could show the presence of people in the area. High amounts of isoprene, acetone or nonanal can indicate that the area is frequented by people, like sports areas, theatres, or markets. However, further studies need to be performed in those locations to confirm those facts.

8.4. Markers in the location from food presence

Gastronomic events, like food markets, food halls or just a simple home kitchen, fill the air with specific VOCs. By analysing the VOCs composition of different locations, we could identify where food is present. However, further studies need to be performed in those locations to confirm those facts.

8.5. Markers in food depending on the locations

When food is growing or is produced in a specific location, all the chemical compounds in their environment could influence their own composition. The study of the VOCs composition of foods from the same type might be able to discriminate between, ecological, PDO or PGI products.

9. CONCLUSIONS

1. We have successfully developed a cheap and easy to assemble VOCs collection system which is suitable for the study of VOCs from human breath. And we have performed a complete parameter optimization.

2. Our developed VOCs collection system is also suitable for the study of VOCs from solid samples. Specially food samples like peanuts and ground coffee gave us very good results

3. VOCs found in human breath could be used as markers for different sources. Food ingestion, smoking, exercise or use of toothpaste are clearly identified during our research.

4. Human breath VOCs profile can be influenced by the environment. Exposure to high amount of fragrances, to be stuck on a traffic jam, or living close to a forestry area could be detected in breath.

5. The VOCs composition can be a tool for discrimination of factors like origin, exposure, or disease. Food products grown in different locations or being exposed to different chemical composition will show different VOCs profiles. When talking about people, different VOCs profile could be a key element for disease diagnosis.

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Annex A. Breath sampling questionnaire (English and Lithuanian versions)

QUESTIONNAIRE

Dear Sir / Madam, we are researchers of the FTMC Metrology Division. We are currently investigating exhaled air to understand its volatile organic compound (VOC) profile, and how it could be used for disease diagnose and monitoring. We would highly appreciate if you could answer the questions below. The confidentiality of the answers is fully guaranteed and the data you provide will only be used for statistical analysis purposes.

Date _ _ / _ _ / _ _ _ _ (Day / Month / Year)

Bag number _ _ _ _ _ _

A. PERSONAL INFORMATION

1. Sex: ☐ Male ☐ Female
2. Age:
   - ☐ <20 years old (y.o.)
   - ☐ 20 to 29 y.o.
   - ☐ 30 to 39 y.o.
   - ☐ 40 to 49 y.o.
   - ☐ 50 to 65 y.o.
   - ☐ >65 y.o.

B. CURRENT OCCUPATION

1. Please, indicate the sector of industry/workplace you work in.

☐ Cosmetics / Beauty industry
☐ Food industry
☐ Office workplace
☐ Pharmaceuticals / Chemical industry
☐ Education
☐ Metalurgic / Automotion / Construction
☐ Petrol
☐ Design / Art / Fashion / clothing industry
☐ Other, specify:

2. How long have you been doing this job? Specify years or months, if less than one year

|__|__| years |__|__| months

3. Mark the materials you are working with.

☐ Oil, gasoline, or diesel (fuels) ☐ Bitumen, bitumen products
☐ Combustion products, including gasoline/diesel exhausts, ash
☐ Metals ☐ Pharmaceuticals ☐ Paints / coatings ☐ Printing inks
☐ Dyes ☐ Solvents ☐ Pesticides, biocides or disinfection products

☐ Cosmetics or hair treatment products (hair dyes etc.)
☐ Other hazardous materials, hazardous waste or other chemicals
☐ Other compound Specify.....

4. How long are you in contact with those materials?

|__||__| Hours/day

5. Do you use Personal Protective Equipment (PPE)?

☐ Yes ☐ No ☐ Don't know

If yes, please specify:

☐ Gloves ☐ Mask ☐ Glasses ☐ Labcoat ☐ Other: .....

C. WORK ENVIRONMENT

1. In which area is your work located?

☐ City centre ☐ Close to the city centre ☐ Suburb/metropolitan area
☐ Industrial ☐ Rural/Village ☐ Other areas Specify:.....

2. Are you located in a shopping center?

☐ Yes ☐ No

3. Mark sources of contamination within 300 m of your workplace.

☐ A waste incineration plant
☐ A petrol station
☐ A scrap yard
☐ A site where solvents are used (e.g. painting business)
☐ A farmland
☐ A printing business
☐ A dry cleaning service
☐ A car repair plant
☐ A carpentry
☐ A construction site
☐ Other industrial facilities Specify facility.....

4. According to the vehicular traffic, how do you classify the road in which your work is located?

☐ Highway ☐ Heavy traffic road ☐ Frequent traffic road
☐ Light traffic road ☐ Pedestrian road ☐ Don't know

5. Does your work place have at least one window facing a street with constant traffic?

☐ Yes ☐ No

6. How often do heavy vehicles (buses, trucks...) circulate near your work?

☐ Never/Rarely ☐ Medium frequency ☐ Continuously ☐ Don't know

D. LIFESTYLE

1. Do you smoke?

☐ Yes ☐ No, but I am passive smoker ☐ No ☐ Don't know

2. Which type of smoking?

☐ Ordinary tobacco cigarettes ☐ Electronic cigarettes ☐ IQOS

3. How often do you usually smoke?

☐ Regularly ☐ Occasionally ☐ Don't know

4. Do you use cosmetics and/ or fragrances?

☐ Yes ☐ No ☐ Don't know

5. Do you practise any sport?
☐ Regularly ☐ Occasionally ☐ No
6. When do you do sports?
☐ Before work ☐ During working hour ☐ After work ☐ Weekends

E. HEALTH
1. Did you recently have a cold / flu? (less than 1 week ago)
☐ Yes ☐ No ☐ Don't know
2. Please select the diseases or conditions that your doctor has diagnosed.
☐ Asthma (allergic asthma included)
☐ Diabetes (type 1) ☐ Diabetes (type 2)
☐ Cancer (malignant tumour, also including leukaemia and lymphoma)
☐ Other diseases or conditions. Specify: .....
3. If you have marked cancer, please specify what kind of cancer.
☐ Bladder ☐ Breast ☐ Cervix(cervical) ☐ Colon
☐ Kidney ☐ Melanoma ☐ Leukaemia ☐ Liver
☐ Other Specify.....

4. Which medicines prescribed to you, have you used in the past two weeks? Please, indicate the commercial name of the medicine, dose and frequency of use.

Commercial name	Dose	Frequency of use

Thank you for your responses!

FIZINIŲ IR TECHNOLOGIJOS MOKSLŲ CENTRAS

KLAUSIMYNAS

Gerbiamasis (-oji), mes esame FTMC metrologijos skyriaus darbuotojai. Šiuo metu vykdome projektą, kurio metu tiriame iškvepamąjį orą ir jo sudėtį. Tiriant junginius, esančiu iškvėptame ore, galėsime sužinoti daugiau apie ligų diagnozę ir stebėseną. Būtume labai dėkingi, jeigu Jūs atsakytumėte į apklausoje pateiktus klausimus. Atsakymų konfidencialumas visiškai užtikrinamas, duomenys bus naudojami tik apibendrintos statistinės analizės tikslais.

Data _ _ / _ _ / _ _ _ _ (Diena / Mėnesis / Metai)

Maišelio Nr. _ _ _ _ _ _

A. ASMENINĖ INFORMACIJA

1. Lytis: ☐ Vyras ☐ Moteris
2. Amžius:
   - ☐ mažiau nei 20 metų
   - ☐ nuo 20 iki 29 metų
   - ☐ nuo 30 iki 39 metų
   - ☐ nuo 40 iki 49 metų
   - ☐ nuo 50 iki 65 metų
   - ☐ daugiau nei 65 metai

B. DARBO POVEIKIS

1. Nurodykite pramonės sektorių (darbo vietą), kuriame dirbate.

☐ Kosmetikos / Grožio industrija
☐ Maisto industrija
☐ Biuras
☐ Farmacijos / Chemijos industrija
☐ Švietimas
☐ Metalurgija / Transportas / Statybos
☐ Degalų pramonė
☐ Dizaino / Meno / Mados / Rūbų industrija
☐ Kita (įrašykite savo):

2. Kiek laiko dirbate šiame darbe? Nurodykite metus arba mėnesius, jei mažiau nei vienerius metus

| __ | __ | metų | __ | __ | mėnesių

3. Pažymėkite tas medžiagas, su kuriomis Jūs dirbate.

☐ Nafta, benzinas ar dyzelinas ☐ Bitumas, bitumo produktai
☐ Degimo produktai, įskaitant benzino/ dyzelino išmetimus, pelenus ar suodžius ☐ Metalo dulkės/Metalai ☐ Vaistai ☐ Dažai/ dangos
☐ Spaudos dažai ☐ Dažai ☐ Tirpikliai ☐ Pesticidai, biocidai arba dezinfekavimo produktai
☐ Kosmetika ar plaukų priežiūros priemonės (plaukų dažai ir kt.)
☐ Kitos pavojingos medžiagos, pavojingos atliekos ar kitos cheminės medžiagos
☐ Kiti junginiai Nurodykite.....

4. Kiek valandų per dieną turite kontaktą su šiomis medžiagomis?
   |__||__| val./per dieną

5. Ar naudojate asmenines apsaugos priemones (AAP)?
☐ Taip ☐ Ne ☐ Nežinau

Jei taip, kokias:

☐ Pirštinės ☐ Kaukė ☐ Akiniai ☐ Laboratorinis chalatas
☐ Kita: .....

C. DARBOVIETĖS APLINKA

1. Kur yra įsikūrusi jūsų darbovietė?
☐ Miesto centre ☐ Netoli miesto centro ☐ Priemiestyje / didmiestyje
☐ Pramoninis rajonas ☐ Kaimas
☐ Kitos sritys. Nurodykite sritį .....

2. Ar darbovietė prekybos centre?
☐ Taip ☐ Ne
3. Pažymėkite tuos taršos šaltinius, kurie yra ne daugiau nei 300m atstumu nuo jūsų darbo vietos.
☐ Atliekų deginimo įmonė
☐ Degalinė
☐ Laužavietė
☐ Įmonė, kurioje naudojami tirpikliai (pvz., Dažymo įmonė)
☐ Žemės ūkio naudmenos
☐ Spausdinimo verslas
☐ Valymo paslaugos
☐ Automobilių remonto įmonė
☐ Medienos apdirbimo įmonė
☐ Statybvietė
☐ Kiti pramoninė tarša. Nurodyti taršos šaltinį .....

4. Kaip klasifikuojate kelią, pagal transporto priemonių eismą, prie kurio įsikūrusi Jūsų darbo vieta?
☐ Greitkelis ☐ S*unkiojo* transporto kelias ☐ Intensyvaus eismo kelias
☐ Mažo intensyvumo eismo kelias ☐ Pėsčiųjų takas ☐ Nežinau

5. Ar jūsų darbo vietoje yra bent vienas langas nukreiptas į gatvę, kurioje vyksta nuolatinis eismas?
☐ Taip ☐ Ne ☐ Nežinau

6. Ar dažnai šalia jūsų darbo važinėja sunkiosios transporto priemonės (autobusai, sunkvežimiai...)?
☐ Niekada/ retai ☐ Vidutiniu dažniu ☐ Nuolat ☐ Nežinau

D. GYVENIMO BŪDAS

1. Ar Jūs rūkote?
☐ Taip ☐ Ne, bet esu pasyvus rūkytojas ☐ Ne ☐ Nežinau
2. Jei atsakėte taip, pažymėkite ką Jus rūkote.
☐ Įprastas tabako cigaretes ☐ Elektronines cigaretes ☐ IQOS
3. Kaip dažnai jūs rūkote?
☐ Reguliariai ☐ Retkarčiais ☐ Nežinau

4. Ar Jus naudojate kosmetikos priemones ir (arba) kvepalus?
☐ Taip ☐ Ne ☐ Nežinau
5. Kaip dažnai sportuojate?
☐ Dažnai ☐ Kartais ☐ Nesportuoju
6. Kokiu metu renkatės sportuoti?
☐ Prieš darbą ☐ Darbo metu ☐ Po darbo ☐ Savaitgaliais

E. SVEIKATA
1. Ar neseniai buvote paršalę/sirgote gripu? (mažiau nei prieš savaitę)
☐ Taip ☐ Ne ☐ Nežinau
2. Pasirinkite šias ligas ar būkles, kurias Jums diagnozavo gydytojas.
☐ Astma (įskaitant alerginę astmą)
☐ Cukrinis diabetas (1 tipas) ☐ Cukrinis diabetas (2 tipas)
☐ Vėžys (piktybinis navikas, taip pat apimantis leukemiją ir limfomą)
☐ Kitos ligos ar būklės. Nurodykite: .....

3. Jei pažymėjote, kad Jums diagnozuotas vėžys, nurodykite, koks vėžys.
☐ Šlapimo pūslės ☐ Krūties ☐ Gimdos kaklelio ☐ Žarnyno
☐ Inkstų ☐ Melanoma ☐ Leukemija ☐ Kepenų
☐ Kita Nurodykite.....

4. Kuriuos vaistus Jus vartojote per pastarąsias dvi savaites? Nurodykite vaisto komercinį pavadinimą, dozę ir vartojimo dažnumą.

Komercinis pavadinimas	Dozė	Vartojimo dažnis

Dėkojame už Jūsų atsakymus!

Annex B. Sorbents used in thermal desorption tubes

Sorbent		Volatility range		Suitable analytes	Max temp (°C)
		Hydrocarbon range	Bolling point (°C)		
Porous polymer	Tenax TA	C6~C30	100 ~ 450	Aromatics, apolars, polar compounds bp >150ºC, and semi-volatiles.	350
	Tenax GR	C6~C30	100 ~ 450	Aromatics, apolars, polar compounds >150ºC, and semi-volatiles.	350
	HayeSep D	C5 ~ C12	50 ~ 200	Low molecular weight compounds, acetylene, halogens, and sulfur groups. GB/GE derivative of VX (CWA).	290
	PoraPak N	C5 ~ C10	50 ~ 200	Polar VOCs, acrylonitrile, acetonitrile, propionitrile, pyridine, volatile alcohols, ethanol, methyl ethyl ketone.	190
	PoraPak Q	C5 ~ C12	50 ~ 200	Oxygenated compounds	250
Graphitised carbon black	Carbograph 2TD Carbopack C Carbotrap C	C8 ~ C20	130 ~ 340	Alkyl benzenes and large aliphatics. Heavy organics: PCBs, PNAs.	400
	Carbograph 1TD Carbopack B Carbotrap B	C5 ~ C14	50 ~ 250	A wide range from medium to high volatility: Ketones, alcohols, and aldehydes (but not formaldehyde). Non-polars within volatility range. Perfluorocarbon tracer gases. BTX.	400
	Carbograph 5TD Carbopack X	C3 ~ C8	50 ~ 150	Especially good for 1,3-butadiene and light hydrocarbons.	400
	Carbopack Y Carbotrap Y	C12 ~ C20	50 ~ 340	Less volatile hydrocarbons. Pesticides e.g. alachlor atrazine, isoprene, and formothyon.	400
Carbonised molecular sieve	Sulficarb	C3 ~ C8	-30 ~ 150	Thiols. VVOCs (vinyl chloride, CS2, methanol, ethanol, and acetone). Used for sterically large VVOCs (SF6).	400
	Carbosieve S-III	C2 ~ C5	-90 ~ 80	Permanent gases, and ethene to n-C5, e.g.	400

				chloromethane. Also ethylene from small volumes.	
	Carboxen 1000	C2 ~ C5	-60 ~ 80	Permanent gases, and ultra-volatile hydrocarbons, e.g. vinyl chloride.	400
	Carboxen 1003	C2 ~ C5	-60 ~ 80	Permanent gases, and ultra-volatile hydrocarbons, e.g. ethane.	400
Zeolite molecular sieve	Molecular sieve 5A	C2 ~ C4	-90 ~ 80	Permanent gases, and nitrous oxide	400
Other	Silica gel	N/A	N/A	Low-boiling polar compounds, especially useful for separating chlorinated or sulfur compounds from matrices with hydrocarbon interferences.	200
	Quartz wool	N/A	N/A	SVOCs	400

SUMMARY

Darbo tikslas ir uždaviniai

Šios disertacijos tikslas - surinkti, ištirti ir suprasti lakiuosius organinius junginius (LOJ) iš įvairios kilmės šaltinių, naudojant dujų chromatografijos su termine desorbcija (TD) techniką, ir galimus jų panaudojimo būdus. Siekdami šio tikslo, daugiausia dėmesio skyrėme jau egzistuojančios LOJ surinkimo sistemoms ir bandėme sukurti savo sistemą. Įvykdę šį etapą ir kartu su turimomis analizės priemonėmis (TD/GC-MS sistemomis) tęsėme LOJ rinkimą ir jų tyrimą. Pagrindinės tyrimo užduotys:

- Sukurti savo LOJ iš iškvėpto oro mėginių ėmimo sistemą;
- Optimizuoti lakiųjų mėginių ėmimo sąlygas, naudojant terminę desorbciją, ir jų analizę, naudojant dujų chromatografiją kartu su masių spektrometrija;
- Paimti kvėpavimo mėginius ir nustatyti galimus ligos, maisto nurijimo ar aplinkos poveikio žymenis;
- Surinkti LOJ iš maisto emisijų ir identifikuoti kilmės nustatymo žymenis;
- Rinkti mėginius iš lauko aplinkos ir nustatyti vietos identifikavimo žymenis;
- Išanalizuoti rezultatus, ieškant sąsajų tarp trijų skirtingų sričių (žmogaus kvėpavimas – maistas – aplinka).

Darbo naujumas

Šio tyrimo metu sukūrėme LOJ mėginių ėmimo iš iškvėpto oro sistemą. Nors ši sistema yra panaši į rinkoje esančią, mums pavyko sukurti savo sistemą iš pigių ir lengvai įsigyjamų medžiagų bei nesudėtingo gamybos proceso. Kadangi mes tvarkėme žmonių kvėpavimo mėginius, buvo svarbu vengti kryžminio užteršimo. Dėl to, mūsų sistema yra tinkama vienkartinei analizei. Sistemą lengva sukurti, o gaunami rezultatai - patikimi. Taip pat, patvirtinome kietų mėginių analizės sistemą su sėkmingais rezultatais.
Pirmą kartą bandėme išanalizuoti aplinkos, žmogaus kvėpavimo ir maisto LOJ sąsajas.

Sukūrėme specifinį kalibravimo metodą LOJ kiekybiniam matavimui dujinėje matricoje, kuris gali būti naudojamas ir kietiems mėginiams.
Pirmą kartą mes panaudojome LOJ profiliavimą iš iškvėpto oro, toksiškų junginių maiste aptikimui.

Ginamieji teiginiai

1. Mūsų sukurta kvėpavimo surinkimo metodika yra tinkama tirti LOJ žmogaus iškvėptame ore.
2. Ta pati mėginių ėmimo sistema galėtų būti naudojama LOJ iš kietųjų mėginių surinkimui.
3. Specifiniai LOJ gali būti naudojami kaip žymenys įvairiems tikslams.
4. Aplinkoje esantys LOJ įtakoja žmogaus iškvėpto oro profilį, taip pat LOJ sudėtį maiste.
5. LOJ sudėtis gali būti priemonė, leidžianti atskirti tokius aspektus kaip kilmė, poveikis ar liga.

1. ĮVADAS

Lakieji organiniai junginiai (LOJ) yra cheminiai junginiai, nuolat supantys mus kasdienėje aplinkoje. Didelės šių junginių koncentracijos poveikis gali sukelti sveikatos ir aplinkos problemų. Jų tyrimas ir nuolatinis stebėjimas yra svarbūs oro kokybės kontrolei tiek patalpose, tiek lauke. Be to, jų emisijos iš maisto tyrimas gali būti naudingas nustatant galimą maisto produktų klastojimą ar užteršimą. Iš žmogaus kvėpavimo išsiskiriančių LOJ tyrimas galėtų būti naudojamas kaip ligų diagnozavimo priemonė.

Tyrimo metu ištyrėme šiuo metu egzistuojančius LOJ surinkimo įrenginius ir sukūrėme savo pigią, lengvai naudojamą ir patikimą sistemą. Savaime modifikuoti LDPE maišeliai buvo puikiai pritaikyti kaip prietaisas žmogaus kvėpavimo LOJ surinkimui.

Surinktų LOJ analizei ir identifikavimui buvo naudojama terminė desorbcija, sujungta su dujų chromatografijos masės spektrometrijos (TD/GC-MS) sistema.

Buvo renkami ir analizuojami žmogaus kvėpavimo mėginiai, siekiant suprasti LOJ profilį ir jo svyravimus, priklausomai nuo kelių veiksnių, tokių kaip rūkymas ar mitybos įpročiai, kvapų poveikis ar eismo užterštumas, sporto praktika ar net ligos.

Sukurtas novatoriškas kalibravimo metodas, leidžiantis kiekybiškai įvertinti toksiškus junginius, esančių iškvėptame ore suvalgius maistą.

Ta pati sukurta sistema buvo naudojama LOJ iš maisto emisijoms surinkti. Maisto LOJ tyrimas buvo sėkmingai atliktas atskiriant skirtingas žemės riešutų ir kavos mišinių rūšis.

Galutiniame mūsų tyrime buvo analizuojami oro mėginiai iš kelių vietovių, siekiant išanizuoti įvairius LOJ profilius. Įvairūs LOJ šaltiniai, ypač iškastinio kuro, augmenijos ir kvapiųjų medžiagų, gali suteikti mėginiams išskirtinį LOJ modelį, leidžiantį atpažinti konkrečias vietas. Be to, žmonės, apsilankę šiose vietovėse, gali paveikti savo iškvėpto oro LOJ profilį.

1.1. LOJ surinkimo įrenginio sukūrimas

Pagrindinis mūsų tikslas buvo sukurti prietaisą, galintį surinkti LOJ iš žmogaus iškvėpto oro. Šis prietaisas turėjo būti pagamintas iš įperkamų ir lengvai prieinamų medžiagų, pakankamai jautrus, kad būtų galima nustatyti kuo daugiau junginių, ir pakankamai tvirtas, kad būtų gauti patikimi rezultatai ir geras pakartojamumas.

1.2. Lakiosios medžiagos iš žmogaus iškvėpto oro

Pagrindinis šio skyriaus tikslas buvo suprasti žmogaus iškvėptame ore esančius LOJ, nustatyti kuo daugiau junginių, jų kilmę ir variantus. Tokia analizė padėtų rasti specifinius biologinius žymenis atspindinčius skirtingas situacijas (teršalų poveikį, mitybos įpročius ar net ligų diagnozę).

Kai mūsų iškvėpto oro surinkimo sistema buvo visiškai sukurta, pradėjome rinkti mėginius. Iškvėpto oro mėginiai buvo paimti iš savanorių iš skirtingų darbo sektorių, esančių biuruose skirtinguose miesto vietose. Kai kuriais atvejais iš kiekvieno savanorio buvo paimti du mėginiai: vienas ryte ir antrasis po pietų. Kiekvienas savanoris užpildė trumpą anketą [A Priedas]. Mes taip pat rinkome kvėpavimo mėginius, kad nustatytume kvapų ir maisto toksinų poveikį.

1.3. Lakiosios medžiagos iš maisto

Pagrindinis šio skyriaus tikslas buvo maisto išskiriamų LOJ analizė. Norėdami surinkti iš maisto išsiskiriančius junginius, naudojome tą pačią sistemą, kurią sukūrėme žmogaus kvėpavimo mėginiams rinkti. Susitelkėme į konkrečių junginių identifikavimą, kad būtų galima nustatyti maisto autentiškumą. Taip pat nustatėme ir kiekybiškai įvertinome toksiškus LOJ, susidarančius termiškai apdorotame maiste.

1.4. Lakiosios medžiagos iš aplinkos

1.4.1. Mėginiai patalpose

Daugelis LOJ šaltinių yra patalpose - nuo maisto produktų ir gaminimo procesų iki valymo priemonių ir kvapų. Kvepalų buvimas namuose ir biuruose yra labai paplitusi praktika kaip švaros, gaivumo ir komforto samprata. Tačiau ypatingai didelis cheminių medžiagų, esančių šiuose kvapuose, poveikis gali sukelti sveikatos problemų. Šioje tyrimo dalyje išanalizavome keletą oro gaiviklių, kad sužinotume jų sudėtį ir poveikį žmonių iškvėptam orui. Iš savanorių, praleidusių tam tikrą laiką vietose, kuriose yra gausesnė oro gaiviklio koncentracija, surinkome iškvėpto oro mėginius, kad surastume konkrečius kvapų junginius.

1.4.2. Lauko mėginiai

Šiame skyriuje surinkome LOJ iš kelių Lietuvos taškų. Klaipėdoje pramoninėje teritorijoje buvo paimti ir palyginti keli mėginiai skirtingu paros metu per kelias dienas. Be to, oro mėginiai buvo paimti iš Vilniaus miesto centro, palyginti nedideliame plote, kad būtų galima rasti konkretų žymenį kiekvienam skirtingam taškui. Norėjome parodyti, kad net mažame plote galima rasti skirtingų junginių, kurie yra būdingi skirtingoms vietoms.

1.5. Žmogaus iškvėptame ore, maiste ir aplinkoje esančių LOJ sąsajos

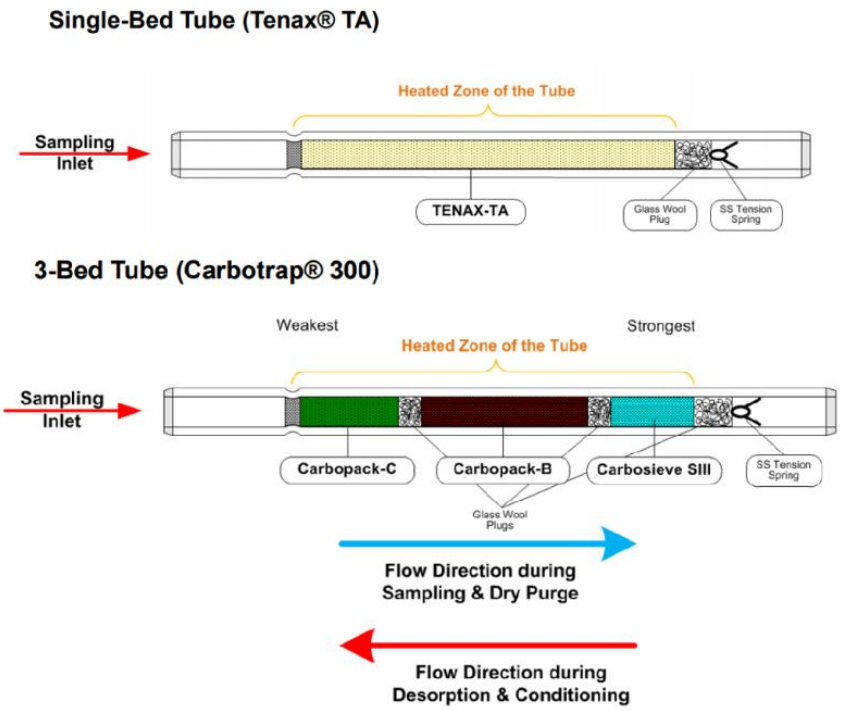
Norėdami užbaigti savo tyrimą, surinkę visus duomenis, analizavome galimą ryšį tarp LOJ šaltinių. Kai kurių specifinių junginių buvimas iškvėpime gali mums pasakyti, ar sergame, kokius produktus naudojame (kosmetika) ar vartojame (maistą ar gėrimus), kokioje aplinkoje gyvename (ar praleidžiame didžiąją laiko dalį) ir ar užsiimame tam tikromis sporto šakomis. Nustatydami LOJ kiekį, galime nustatyti maisto produktų kilmę, taip pat galimas juose esančias toksines medžiagas. Galiausiai, dėl specifinių iš maisto gaunamų LOJ arba mūsų kūne gaminamų antropogeninių LOJ, galime atskirti vietoves, kuriose dažnai lankosi žmonės arba kuriose yra maisto produktų.

2. ĮRANGA

2.1. TD vamzdeliai ir sorbentai

Pagrindinis mūsų TD sistemos elementas yra nerūdijančio plieno sorbento vamzdelis (8,9 cm ilgio, 0,635 cm išorinio skersmens), užpildytas sorbentu. Rinkoje yra daug įvairių sorbentų. Jų skirtumai priklauso nuo gimininguмo skirtingiems komponentams ir gali būti pasirenkami atsižvelgiant į konkrečius poreikius [B Priedas].

Sorbentai dedami į nerūdijančio plieno vamzdelį, kuris bus naudojamas sulaikytiems LOJ surinkti ir analizuoti [Pav. 1]. Į tą patį mėgintuvėlį galima įkelti kelis sorbentus, kad, priklausomai nuo tyrimo, jis būtų dar konkretesnis,.



Pav.1: terminės desorbcijos vamzdeliai

Savo tyrimams nusprendėme naudoti tik Tenax® TA sorbento TD vamzdelius. Daugiausia dėl didelio LOJ suderinamumo diapazono (C6 iki C30) ir jo stabilumo.

Mėginiai pompuojami per TD vamzdelius naudojant oro siurblį (SKC Inc. Aircheck Sampler 224-44XRM modelis) [Pav. 2]. Kai mėginiai praeina per sorbentą, pagrindiniai komponentai lieka viduje, kol įkaista.

Pav. 2: SKC Inc. Aircheck Sampler 224-44XRM modelis

Terminės desorbcijos (TD) sistemą sudaro mėginio šildymo taškas ir aušinama gaudyklė, skirta junginio fokusavimui. TD vamzdeliai pakraunami į TD sistemą (ATD 400; Perkin Elmer), kur vamzdeliai pašildomi, išleidžiant sulaikytus junginius į GC sistemą.

Kai junginiai palieka TD, jie atskiriami ir identifikuojami naudojant GC-MS sistemą (Agilent 6890N, sujungta su Mass Spectrometer Waters/Micromass AutoSpec Premier) [16 pav.].

Pav. 3: Dujų chromatografijos didelės skiriamosios gebos masės spektrometrijos sistema

Sistemoje naudota kapiliarinė kolonėlė DB-5MS, 30 m, 0,25 mmID, 0,25 mm (Agilent Technologies). Atliekant daugumą šio tyrimo metu atliktų analizių, geras junginių atskyrimas buvo pasiektas 3 minutes palaikant kolonėlės temperatūrą 40 °C laipsnių, o po to padidinant 5 °C/min iki 120 °C, o po to padidinant temperatūrą 10 °C/min iki 220 °C

MS analizė buvo atlikta viso nuskaitymo režimu, naudojant skenavimo diapazoną *m/z* 50–200. Jonų šaltinis buvo palaikomas 250 °C temperatūroje, o kiekvienam matavimui buvo naudojama 70 eV jonizacijos energija (EI+). Junginys buvo identifikuotas pagal bibliotekos atitiktį, naudojant NIST MS Search 2.0 (2005) masės spektrinę biblioteką.

3. MĖGINIŲ ĖMIMO SISTEMOS KŪRIMAS

Kaip buvo aptarta įvade, yra keli LOJ surinkimo būdai. Tačiau LOJ surinkimas iš žmogaus iškvėpto oro yra šiek tiek sudėtingesnis. Manome, kad iškvėpto oro LOJ analizė puikiai tinka diagnozuojant ligas. Todėl pirmiausia norėjome sutelkti dėmesį į prietaiso, skirto kvėpavimo mėginiams rinkti, kūrimą. Mūsų tikslas buvo sukurti panašų dizainą kaip ir ReCIVA® kvėpavimo analizatorius (Owlstone Medical, JK). Tačiau stengėmės, kad sistema būtų lengvesnė ir prieinamesnė. Kai kurias kaukes, maišelius ir jungtis įsigijome iš UAB „Intersurgical“ įmonės [Pav. 4] ir sukonstravome kelis prototipus.

Pav. 4: Veido kaukė su 4 mėginių ėmimo vamzdeliais (kairėje); savadarbė TD vamzdžio jungtis (vidurinė); T formos kryptinis vožtuvas (dešinėje)

Mūsų sukurta sistema yra panaši į Tedlar® maišelius [Pav. 5], kurie dėl savo paprastumo, atkuriamumo, lengvo transportavimo ir santykinai pigumo (apie 10 € / 3 l maišelis) plačiai naudojami oro surinkimui. Tie vienkartiniai maišeliai pagaminti iš polimero polivinilfluorido, kurį patentavo Dupont™.

Pav. 5: Tedlar® maišelis (kairėje) ir sukurta Minigrip LDPE plastikinių maišelių surinkimo sistema (dešinėje).

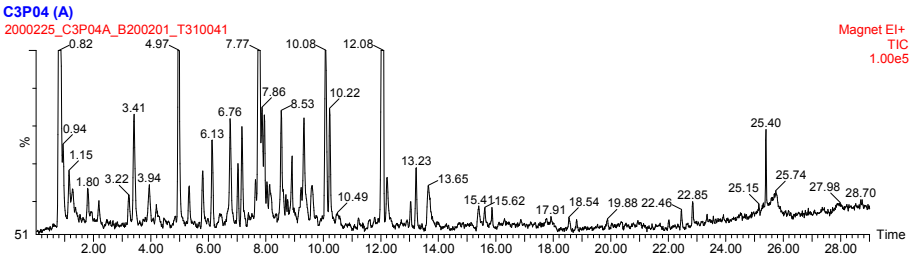
Sėkmingai sukūrėme įrankį LOJ rinkimui iš kelių šaltinių. Sistema yra jau turimų polimerinių mėginių ėmimo maišų supaprastinimas. Kadangi dėl šios modifikacijos maišelio kaina yra daug pigesnė, galėtume eksperimentuoti su skirtingų dydžių maišeliais įvairioms reikmėms. Maišelį labai lengva ir greita sukonstruoti bei kondicionuoti, jį labai patogu naudoti kvėpavimo mėginiams paimti ir jį lengva utilizuoti. Maišelį galėtume užpildyti ne tik dujų mėginiais (iškvėpimo mėginiais), bet ir kietais mėginiais.

Be to, naudojant terminės desorbcijos sistemą, sujungtą su dujų chromatografijos masės spektrometru (TD/GC-MS), sėkmingai gavome geriausius LOJ surinkimo, analizės ir identifikavimo parametrus. Mėginių ėmimo laikas buvo optimizuotas, kad LOJ surinkimas būtų kuo greitesnis, išvengiant junginių proveržio, taip pat surinktų LOJ pėdsakų. Desorbcijos temperatūros ir laikai buvo parinkti taip, kad visi įstrigę junginiai būtų išleisti greitai, naudojant kuo mažiau išteklių. Chromatografiniai parametrai leido teisingai atskirti daugumą junginių per greičiausią laiką, kad būtų galima tinkamai identifikuoti surinktus LOJ.

4. LOJ ŽMOGAUS IŠKVĖPIME ORE

Kai mūsų mėginių ėmimo sistema buvo paruošta, paėmeme iš savanorių kvėpavimo mėginius. Darbuotojai iš keturių skirtingų biurų sutiko savanoriauti mūsų tyrime. Iš daugumos mūsų savanorių buvo paimti du kvėpavimo mėginiai, vienas mėginys ryte, vos atvykus į savo darbo vietą, o antras mėginys prieš jiems paliekant darbą po pietų. Visiems savanoriams buvo pateiktas klausimynas [A Priedas], siekiant gauti svarbius duomenų analizės parametrus. Pagrindinė tyrimo idėja buvo nustatyti įtaką, galinčią pakeisti jų kvėpavimo sudėtį, pradedant nuo maisto ir rūkymo įpročių, sporto, vaistų ar ligų iki biuro patalpų aplinkos įtakos. Norint ištirti galimą darbovietės poveikį, be kvėpavimo mėginių buvo paimtas ir biuro oro mėginys.

Savanorių buvo paprašyta du kartus užpildyti 3 l LDPE plastikinius maišelius (jau kondicionuotus) iškvėptu oru, kad būtų surinkta iš viso 6 l iškvėpto oro mėginio. Surinkti kvėpavimo mėginiai pumpuojami per terminės desorbcijos vamzdelį 1000 ml/min srautu. Surinkus ir išanalizavus kvėpavimo mėginius iš 23 skirtingų savanorių skirtingu paros metu [Pav.6], buvo aptikti mažiausiai 123 skirtingi LOJ ir dauguma jų identifikuoti [Lentelė 1]. Junginiams identifikuoti buvo naudojama NIST MS Search 2.0 (2005) masės spektrinė biblioteka.



Pav. 5: Savanorio kvėpavimo mėginio chromatograma, paimta ryte

Lentelė 17: Žmogaus iškvėptame ore aptiktų ir identifikuotų LOJ sąrašas

Nr	L_R (min)	Junginys	Galimi šaltiniai
1	0.86	Acetonas	Antropogeninis
2	0.86	Izoprenas	Antropogeninis
3	0.94	Hekanas	Benzinas / Autom. išmetimosios dujos
4	1.11	Oktanas	Benzinas / Autom. išmetimosios dujos
5	1.21	Izopropilo acetatas	Organinis tirpiklis
6	1.13	Benzenas	Benzinas / Autom. išmetimosios dujos
7	1.30	Alilo metilo sulfidas	Antropogeninis
8	1.38	Metilo propilo sulfidas	Antropogeninis
9	1.44	Anglies rūgštis	Kita
10	1.52	1-(metiltio)- 1-propenas	Antropogeninis
11	1.59	Nežinoma	Nežinoma
12	1.73	Piridinas	Maisto MRP
13	1.80	Toluenas	Benzinas / Autom. išmetimosios dujos
14	2.16	Heksanalas	Antropogeninis
15	2.46	Oktanalas	Antropogeninis
16	2.27	Tetrachloroetilenas	Organinis tirpiklis / Sausas valymas
17	2.35	Dihidro-2-metil-3-furanonas	Maisto MRP
18	2.41	Butilo acetatas	Organinis tirpiklis / Kvepalai
19	2.47	Metilformatas	Maisto MRP
20	2.65	Metilpirazinas	Maisto MRP
21	2.66	C_nH_n	Benzinas / Autom. išmetimosios dujos
22	2.78	Furfurolas	Maisto MRP
23	2.93	Propano rūgštis	Antropogeninis
24	3.30	Furfurilo alkoholis	Maisto MRP
25	3.20		
26	3.38	Ksilenas (o,m,p)	Benzinas / Autom. išmetimosios dujos
27	3.90		
28	3.77	Heksano rūgštis	Antropogeninis
29	4.15	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
30	4.26	Aldehidas	Antropogeninis
31	4.45	Butil glikolis	Organinis tirpiklis
32	4.52	Metilformatas	Kita
33	4.56	Acetilfuranas	Maisto MRP
34	4.71	Dimetilpirimidinas	Maisto MRP
35	4.82	Nežinoma	Nežinoma
36	4.91	α-pinenas	Augalija / Kvepalai
37	5.05	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
38	5.20	Heksadecanolis	Kosmetika / Kvepalai
39	5.23	Heksileno glikolis	Kosmetika / Kvepalai
40	5.26	Kamfenas	Augalija / Kvepalai
41	5.52	Propil benzenas	Benzinas / Autom. išmetimosios dujos
42	5.76	m-Etilo metilbenzenas	Benzinas / Autom. išmetimosios dujos
43	5.97	psi-kumenas	Augalija / Kvepalai

44	6.06	β-pinenas	Augalija / Kvepalai
45	6.26	Etilo toluenas	Benzinas / Autom. išmetimosios dujos
46	6.39	Nežinoma	Nežinoma
47	6.58	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
48	6.69	Mesitilenas	Augalija / Kvepalai
49	6.72	β-mircenas	Augalija / Kvepalai
50	6.83	Vinilfuranas	Kita
51	6.98	Dekanas	Benzinas / Autom. išmetimosios dujos
52	7.11	3-karenas	Augalija / Kvepalai
53	7.37	Dipropilenglicolio monometilo eteris	Kita
54	7.47	Nežinoma	Nežinoma
55	7.49	Dipropilenglicolio monometilo eteris	Kita
56	7.59	m-cimenas	Augalija / Kvepalai
57	7.73	Limonenas	Augalija / Kvepalai
58	7.87	Eukaliptolis	Augalija / Kvepalai
59	7.91	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
60	7.94	Kaprilo rūgštis	Kita
61	7.98	Dekanalas	Augalija / Kvepalai
62	8.01	Etilheksanolis	Augalija / Kvepalai
63	8.09	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
64	8.13	1-fenil-1,2-butandiolis	Kita
65	8.50	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
66	8.64	4-karenas	Augalija / Kvepalai
67	8.71	(Metiltridekanas) C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
68	8.87	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
69	8.93	Etil metil benzenas	Benzinas / Autom. išmetimosios dujos
70	9.10	(Metil dekanas) C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
71	9.23	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
72	9.31	Dihidromircenolis	Augalija / Kvepalai
73	9.41	Heksahidronerolidolis	Augalija / Kvepalai
74	9.51	4-karanolis	Augalija / Kvepalai
75	9.59	α-kumil alkoholis	Augalija / Kvepalai
76	9.90	Dihidrokarveolis	Augalija / Kvepalai
77	9.99	2-butil-1-oktanolis	Antropogeninis
78	10.12	Linalil antranilatas	Augalija / Kvepalai
79	10.08	Undekanas	Benzinas / Autom. išmetimosios dujos
80	10.15	Nonanalas	Antropogeninis
81	10.16	Bergamiolis	Augalija / Kvepalai
82	10.46	Mentadienolis	Augalija / Kvepalai
83	10.62	Nežinoma	Nežinoma
84	10.83	Nežinoma	Nežinoma
85	11.18	Kamforas	Augalija / Kvepalai
86	11.52	Izomentonas	Augalija / Kvepalai
87	11.71	Nežinoma	Nežinoma

88	11.86	Izomentolas	Augalija / Kvepalai
89	11.96	Nežinoma	Nežinoma
90	12.16	Mentolas	Augalija / Kvepalai
91	12.20	Nežinoma	Nežinoma
92	12.18	Naftalenas	Benzinas / anglis
93	12.52	Nežinoma	Nežinoma
94	12.68	Benzoine rūgštis	Augalija / Kvepalai
95	12.87	Nežinoma	Nežinoma
96	13.00	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
97	13.16	Dekanalas	Augalija / Kvepalai
98	13.64	2-fenoksi etanolis	Kita
99	14.25	D-karvonas	Augalija / Kvepalai
100	14.63	Linalil izobutiratas	Augalija / Kvepalai
101	14.66	Oksalo rūgštis, bis(trimetilsilil) esteris	Kita
102	15.35	Izobornil acetatas	Augalija / Kvepalai
103	15.60	4-tert-butilcicloheksil acetatas	Kosmetika / Kvepalai
104	15.86	Tridekanas	Benzinas / Autom. išmetimosios dujos
105	16.25	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
106	16.85	Oksalo rūgštis	Kita
107	17.12	α-terpinil butiratas	Augalija / Kvepalai
108	17.68	β-vinilnaftalenas	Benzinas / Anglis
109	17.89	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
110	18.48	C_nH_n	Benzinas / Autom. išmetimosios dujos
111	18.74	Kariofilenas	Augalija / Kvepalai
112	19.12	Verdilo acetatas	Augalija / Kvepalai
113	19.27	Oksalio rūgštis	Kita
114	20.33	α-cetonas	Kosmetika / Kvepalai
115	20.76	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
116	20.87	3-bifenilolis	Kita
117	22.32	Oksalio rūgštis	Kita
118	22.43	Ftalio rūgštis	Plastifikatorius
119	22.78	Benzofenonas	Kita
120	23.03	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
121	23.16	C_nH_{2n+2}	Benzinas / Autom. išmetimosios dujos
122	23.23	Nežinoma	Nežinoma
123	25.36	Izopropilio miristatas	Augalija / Kvepalai

Naudodami savo pačių sukurtą LOJ surinkimo sistemą, galėjome surinkti kvėpavimo mėginius iš daug savanorių. Surinkimo tūris buvo optimizuotas, kad būtų galima aptikti mažos koncentracijos LOJ. Galėjome nustatyti konkrečius biologinius žymenis, junginius, kurie parodys kvapų poveikį, lauko taršą, kosmetikos naudojimą ar maisto ir gėrimų suvartojimą. Taip pat matėme rūkymo įpročius ar mankštos praktikas.

5. LOJ IŠ MAISTO

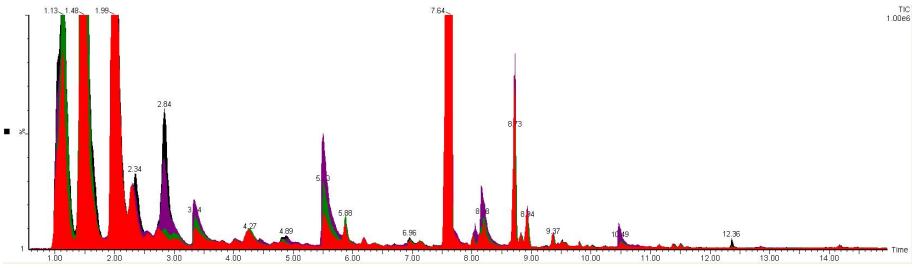
5.1. Maisto rūšies identfikavimo galimybės

Mūsų tyrime buvo analizuoti keturi dažniausiai pasitaikantys žemės riešutų tipai iš Jungtinių Valstijų (Runeris, Valencija, Ispanija ir Virdžinija) [Pav. 6], siekiant nustatyti galimą LOJ kiekio skirtumą. Dėl neatskleidimo sutarties nebuvo pateikta tiksli žemės riešutų rūšis, todėl keturias rūšis pažymėjome žemės riešutais A, B, C ir D. Pagrindinis tikslas buvo surasti konkretų kiekvienos rūšies LOJ profilį ir pabandyti juos klasifikuoti.

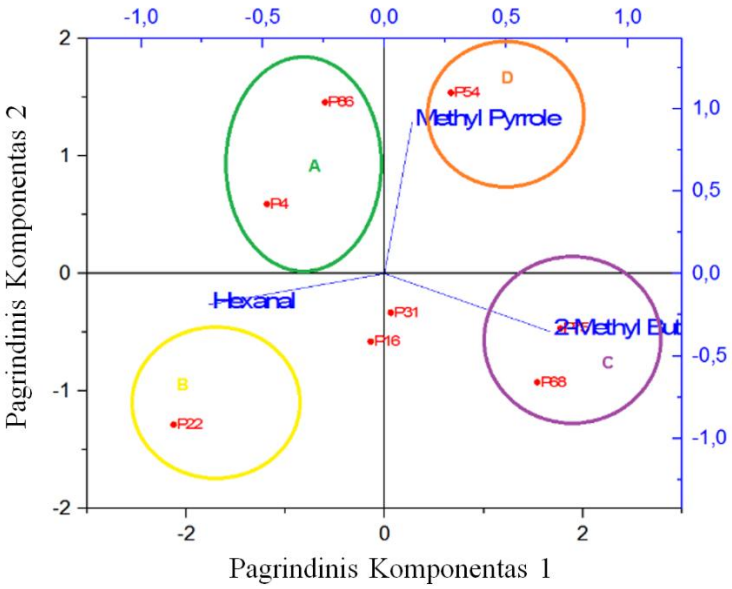
Pav. 6: Žemės riešutų rūšys. Iš Whitley's Peanut Factory [www.whitleyspeanut.com/funfacts]

Buvo lyginami keturi skirtingi tipai [Pav. 7] ir atlikta visų pateiktų imčių statistinė analizė. Ištirta analizuotų mėginių pagrindinių komponentų analizė (PCA) [Pav. 8].

Iš šių rezultatų matome, kad trys junginiai yra pagrindiniai tipo identifikacijos komponentai: 2-metilbutanalis / 1 – metilpirolis ir heksanalas.



Pav 7: Žemės riešutų mėginių chromatogramos. Žemės riešutas A (juodas); Žemės riešutas B (violetinė); Žemės riešutas C (žalias); Žemės riešutas D (raudonas).



Pav 8: Žemės riešutų mėginių rezultatų PCA analizė

Tyrimui buvo pateiktas tik nedidelis mėginių kiekis. Tačiau galėtume juos suskirstyti į grupes pagal 4 skirtingus žemės riešutų tipus. Du mėginiai (P31 ir P16) nebuvo įtraukti į jokią grupę. Mėginių teikėjai patvirtino, kad šie du mėginiai buvo A tipo žemės riešutai, turintys pašalinį skonį. Todėl mūsų sistema galėjo atskirti 4 žemės riešutų tipus ir netgi aptikti, kada pasikeitė vieno iš rūšių kokybė.

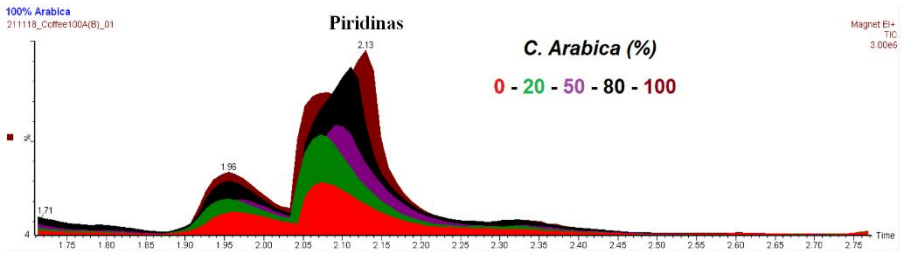
5.2. Kavos mišinio identifikavimo galimybės

Buvo analizuoti penki skirtingi *C. Arabica* ir *C. Robusta* rūšių mišiniai. 100, 80, 50 ir 20 % *C. Arabica* ir 100 % *C. Robusta*. Mūsų tyrimas apėmė LOJ profilį, kuris gali atskirti kiekvieną skirtingą mišinį.

Panašiai kaip ir žemės riešutams, į mūsų pačių sukurtą surinkimo sistemą (500 ml LDPE maišelius) buvo įvestas 0.5 g maltos kavos mišinio.

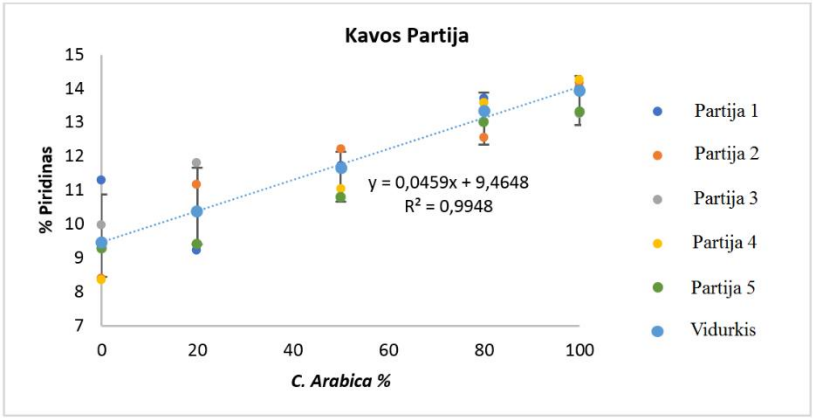
Nors daugumoje nustatytų junginių skirtingų kavos partijų standartinis nuokrypis yra didesnis nei 10 %, galėjome pastebėti keletą junginių, kurie rodo tendenciją identifikuoti skirtingus kavos pupelių mišinius.

Piridinas yra vienas iš tų junginių. Santykinis piridino procentas 5 skirtinguose kavos partijose [Pav. 9] parodė aiškią rezultatų tendenciją. Kuo didesnė piridino koncentracija, tuo didesnis *C. Arabica* kiekis.



Pav. 9: Skirtingų kavos mišinių piridino signalo skirtumas

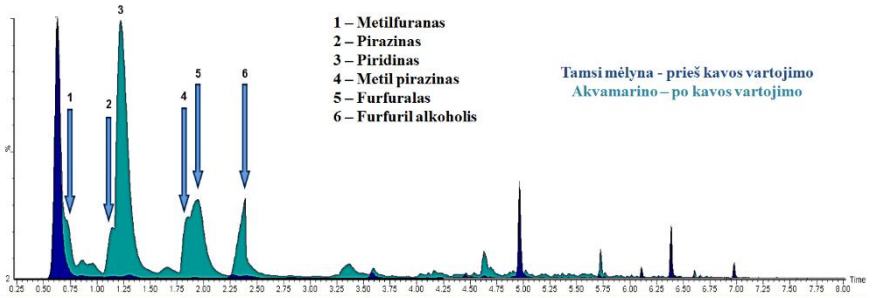
Tačiau dėl skirtingų gautų rezultatų nuokrypio tarp skirtingų kavos partijų, sunku atskirti užmaskuotus mėginius [Pav. 10]. Taip gali nutikti dėl skirtingų ženklelių skrudinimo proceso skirtumų arba net dėl kavos mišinių iš skirtingų vietų.



Pav 10: Santykinės piridino koncentracijos priklausomybė nuo C. Arabica *kiekio*

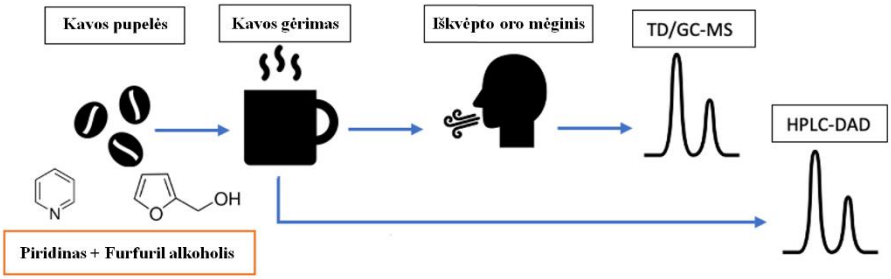
5.3. Galimų toksiškų junginių kiekybinis nustatymas kavoje

Iškvėpto oro mėginių tyrimo metu pastebėjome keletą didelės koncentracijos junginių, gaunamų iš kavos [Pav. 11]. Tai davė mums idėją išanalizuoti toksiškus junginius, atsirandančius su maistu. Nusprendėme sutelkti dėmesį į kavos mėginius, nes didelės koncentracijos junginiai gali būti laikomi kancerogeniniais.



Pav 11: Iškvėpto oro mėginio GC chromatograma prieš (tamsiai mėlyna) ir po (akvamarino) kavos vartojimo

Mes siekėme nustatyti ir kiekybiškai įvertinti Py ir FFA žmogaus iškvėpime po kavos nurijimo. Tokio metodo sukūrimas galėtų būti plačiai pritaikytas kaip priemonė apsinuodijimui maistu nustatyti. Šio metodo grafinė santrauka parodyta Pav. 12.



Pav 12: Grafinė analizės santrauka

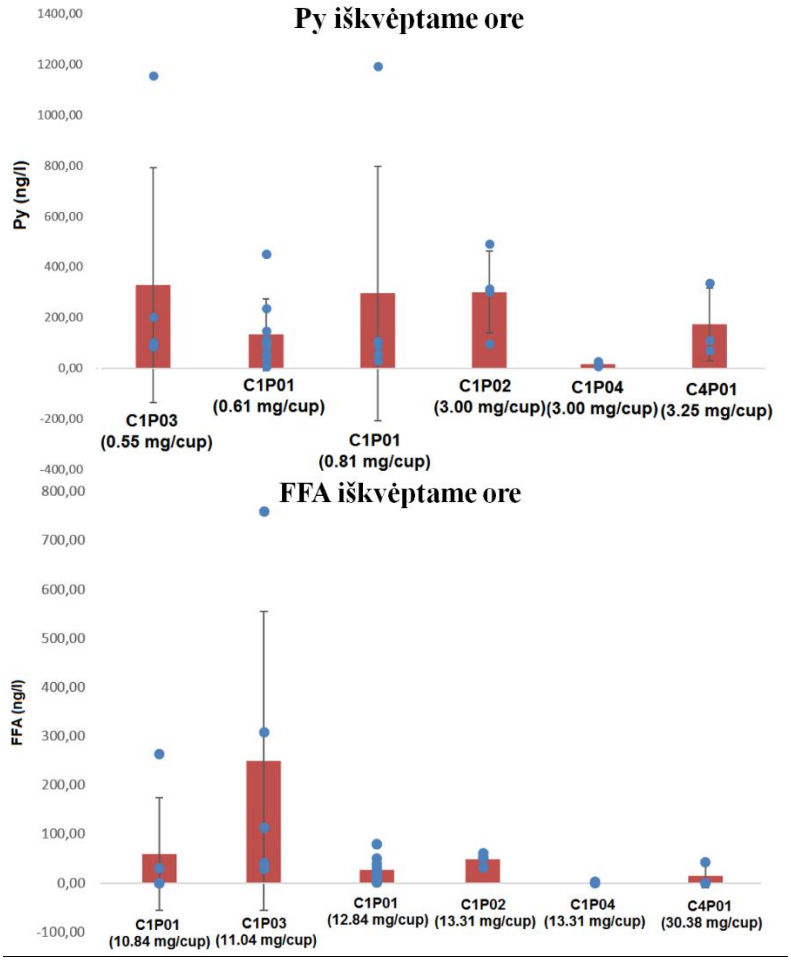
Nepriklausomai nuo sunaudotų kavos pupelių kiekio, Py ir FFA kiekis, ekstrahuotas viename grame kavos pupelių su CafeRomatica kavos aparatu, buvo panašus [Lentelė 2]. Didesni abiejų MRP kiekiai buvo gauti ruošiant kavą naudojant itališką moka kavinuką dėl aukštesnės temperatūros ir slėgio, naudojamą virti kavai. Toksiškų MRP kiekiai kavos gėrimuose svyravo nuo 0,2 iki 3 mg Py ir nuo 7 iki 30 mg FFA priklausomai nuo vandens kiekio, sunaudoto kavai plikyti.

Lentelė 2: Piridino (Py) ir furfurilo alkoholio (FFA) kiekis kavos gėrime.

Pavad.[a]	Kava[b] (g)	Vanduo[c] (ml)	Mėginis[d] (µg/ml)		Puodele[e] (mg/cup)		Pupelės[f] (mg/g)	
			FFA	Py	FFA	Py	FFA	Py
Si30	3.5	30	236.7	7.5	7.1	0.23	2.0	0.07
N30	7.5		368.1	18.3	11.0	0.55	1.5	0.07
St30	9		427.9	20.2	12.8	0.61	1.4	0.07
Si100	3.5	100	67.0	3.5	6.7	0.35	1.9	0.10
N100	7.5		126.2	8.7	12.6	0.87	1.7	0.12
St100	9		108.4	8.1	10.8	0.81	1.2	0.09
Si240	3.5	240	40.1	3.1	9.6	0.75	2.7	0.21
N240	7.5		55.5	12.5	13.3	3.0	1.8	0.40
St240	9		67.8	5.1	16.3	1.2	1.8	0.14
Mk200	12	200	151.9	16.3	30.4	3.3	2.5	0.27

[a] Si (Silpna kava), N (Normali kava), St (Stipri kava) Mk (moka kava). [b] Sunaudoti kavos gramai. [c] Mililitrai vandens, sunaudoto kavos ruošimui. [d] Toksiškų medžiagų koncentracija mėginyje, mikrogramais mililitre. [e] Miligramai toksinių medžiagų pilname kavos puodelyje. [f] Miligramai toksinių medžiagų grame kavos pupelių.
Pastaba: standartinis nuokrypis < 10 %

Visų savanorių kvėpavimo mėginiuose abiejų toksiškų MRP koncentracijos buvo nuo 1 iki 1200 ng/l piridino ir iki 760 ng/l FFA [Pav. 13]. Galėjome stebėti didelę kiekvieno mėginio pakartojimo rezultatų sklaidą. Literatūros duomenimis, pacientams, sergantiems paskutinės stadijos inkstų liga, Py vertės iškvėptame ore buvo nuo 0,66 iki 141 µg/l [117]; ir iki 300 µg/l aktyviai rūkantiems [118]. Kalbant apie FFA, mums nepavyko rasti koncentracijos lygio iškvėptame ore.



Pav 13: Py ir FFA koncentracija (ng/l), nustatyta skirtingų savanorių iškvėptame ore, nurodant toksinių medžiagų kiekius, kuriuos savanoris išgėrė kavos puodelyje (skliausteliuose).

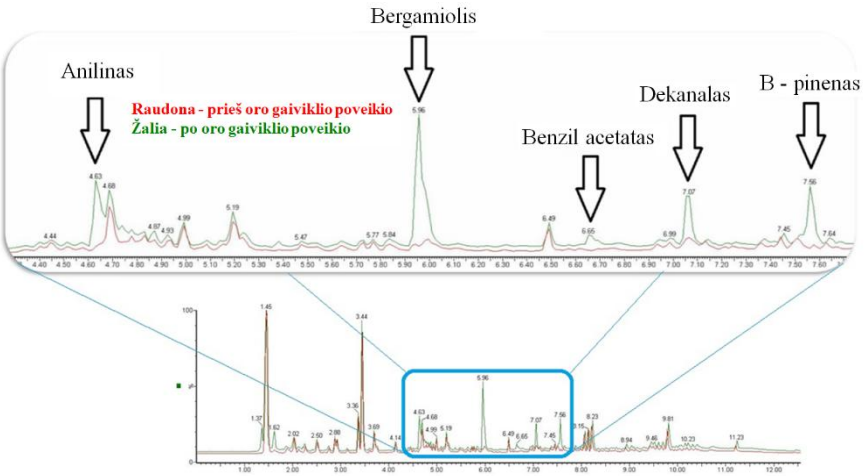
Visi maisto produktai pasižymi skoniu ir kvapu. Šios savybės dažniausiai atsiranda dėl jų LOJ sudėties. Naudodami mūsų pačių sukurtą mėginių ėmimo įrenginį, gavome maisto ir gėrimų LOJ profilius, kurie gali būti naudojami jų identifikacijai. Atskyrėme 4 žemės riešutų rūšis ir identifikavome, kokius kavos pupelių mišinius geriame. Taip pat, galėtume nustatyti apsinuodijimą maistu, jei būtų nurytas didels kiekis galimai toksinių medžiagų, esančių maiste ir gėrimuose.

6. LOJ esantys aplinkoje

6.1. Vidaus patalpų užterštumas

Kaip jau aptarėme anksčiau, daug LOJ patiriama naudojant kvapiąsias medžiagas patalpose. Siekdami suprasti jų poveikį žmogaus iškvėpto oro sudėčiai, išanalizavome keletą oro gaiviklių kompozicijų ir pasirinkome tą, kurioje yra didesnis LOJ skaičius.

Savanoris, iš kurio prieš buvimą oro gaivikliu išpurkštoje patalpoje buvo paimtas iškvėpto oro mėginys, išbuvo patalpoje 10 minučių. Po to buvo paimtas kitas iškvėpimo mėginys. Rezultatai parodė, kad iškvėpimo sudėtyje yra keletas orą gaivinančių LOJ [Pav. 14].



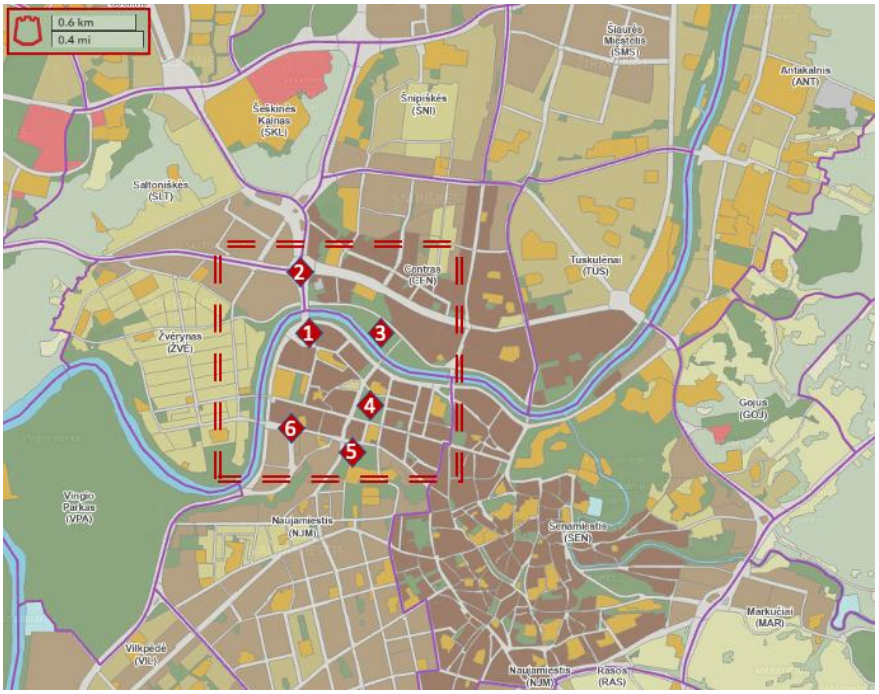
Pav. 14: Iškvėpto oro mėginio chromatogramų palyginimas prieš ir po oro gaiviklio poveikio.

Žmogui patekus į patalpą, kurioje yra didelis oro gaiviklių kiekis, gali pasikeisti jo ikvėpto oro sudėtis. Nors oro gaiviklis turi daugiau nei 50 skirtingų LOJ, iškvėptame ore yra nedaug. Taip gali būti dėl to, kad daugumą junginių organizmas gali absorbuoti ir pašalinti kitais būdais.

6.2. Lauko užterštumas

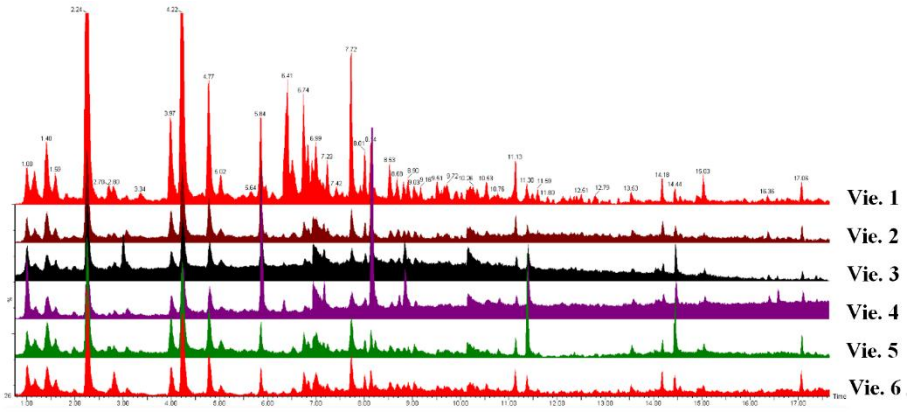
Siekėme išanalizuoti LOJ modelį iš kelių Vilniaus centro vietų 2 km^2 plote ir nustatyti skirtingus emisijos šaltinius, turinčius įtakos oro sudėčiai Vilniuje. Norėjome pažiūrėti, ar galima atskirti tam tikrą vietą palyginti mažame plote.

Mes patikrinome lakiuosius junginius iš 6 skirtingų vietų Vilniuje, Lietuvoje. Visos vietovės buvo 2 km^2 plote Vilniaus miesto centre [Pav. 15]. Vietovės buvo pasirinktos atsižvelgiant į kriterijus, kad būtų skirtingos eismo sąlygos ir želdinių kiekiai su įvairiais LOJ šaltiniais.



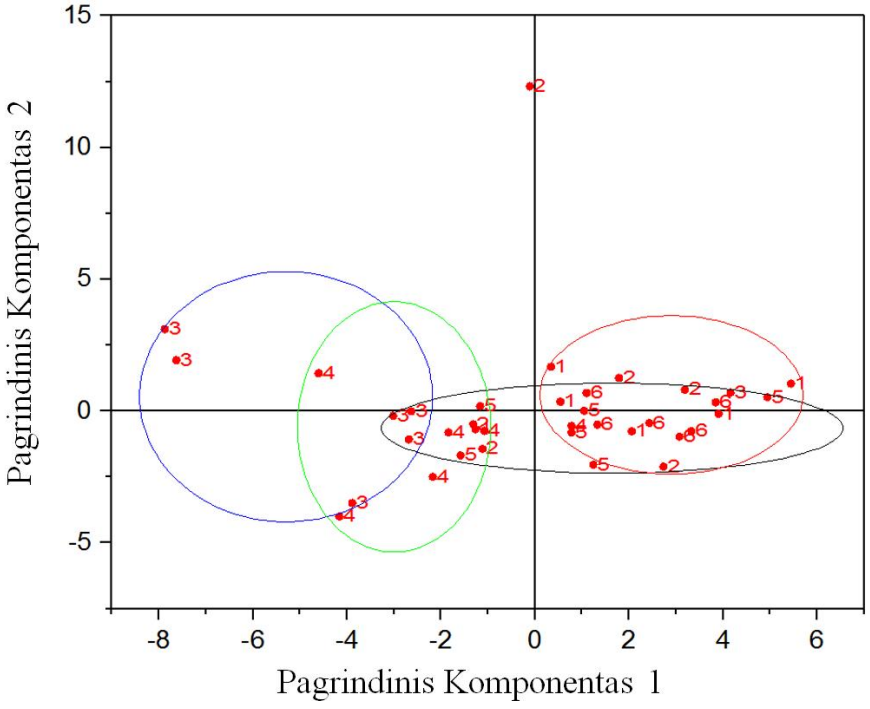
Pav. 15: Vilniaus žemėlapis su mėginių ėmimo vietomis [from www.maps.vilnius.lt]

Dviejų kvadratinių kilometrų plote radome skirtingų LOJ šaltinių; kiekviena sritis davė savitą junginių modelį [Pav. 16].



Pav. 16: 6 vietų GC chromatograma (tą pačią dieną)

Nors LOJ lygiai gali skirtis priklausomai nuo įvairių veiksnių ir oro sąlygų, mes ištyrėme visą kiekvienos vietos profilį. Apskaičiavome vidutinę santykinę LOJ koncentraciją 6 skirtingose vietose per visas mėginių ėmimo dienas. Siekiant sumažinti kintamųjų skaičių, buvo atlikta pagrindinių komponentų analizė (PCA) [Pav. 17].



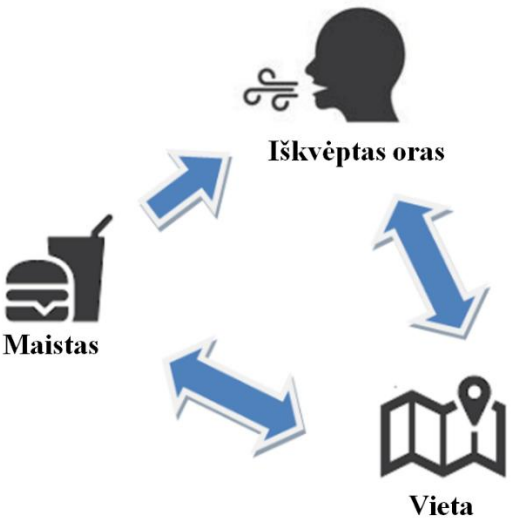
Pav. 17: PCA analizė vietovės rezultatams gauti

Ištyrėme LOJ sudėtį įvairiose Lietuvos aplinkose. Mūsų tyrimas rodo, kiek LOJ šaltinių turi skirtingą svorį, priklausomai nuo vietos ir paros laiko. Vietovės, esančios netoli miško, turės kitokį LOJ profilį nei pramoninės zonos dėl jų šaltinių skirtumų. Terpenoidai bus plačiai naudojami miškininkystės aplinkoje, o iškastinis kuras – pramoninėse ar miesto zonose. Kiekvienos vietos LOJ profilis nuolat reaguoja į vietos įvykių pokyčius, eismo sąlygas, aplinkos želdinius, oro sąlygas ir sezoninius pokyčius.

7. REZULTATŲ APTARIMAS

Atlikę LOJ tyrimus, galėjome geriau suprasti lakią mūsų aplinkos sudėtį. Galime nustatyti konkrečius žymenų junginius iš skirtingų jų šaltinių ir galime juos susieti.

Suprasdami visus skirtingus LOJ ir jų šaltinius, galime nustatyti ryšį tarp trijų pagrindinių temų, kurias analizavome savo tyrime [Pav. 18].



Pav. 18: Sąsajos tarp trijų tirtų LOJ šaltinių

7.1. Žymenys iškvėptame ore po suvartoto maisto

Analizuodami žmogaus iškvėptą orą, galime nustatyti LOJ iš tam tikrų maisto rūšių. Tokių junginių kaip alkilpirazinai ar furano junginiai dažniausiai gaunami iš skrudintų ar keptų maisto produktų, ypač dideli kiekiai skrudintoje kavoje. Didelė limoneno koncentracija randama vaisiuose ar saldumynuose. Analizuodami žmogaus iškvėptą orą, galime susidaryti supratimą apie maistą, kurį jis valgė per trumpą laiką. Tai gali būti naudinga nustatant galimą apsinuodijimo maistu situaciją.

7.2. Iškvėpto oro žymenys iš konkrečių vietų ir jų poveikis

Tirdami žmogaus iškvėpavimo LOJ profilį, galėjome nustatyti kai kuriuos junginius, kilusius iš konkrečių vietovių. Didelis terpenų kiekis gali atsirasti dėl to, kad žmogus gyvena ar būna šalia miško ar parko. Kita vertus, kai angliavandenilių ir benzeno darinių yra daugiau, galime nustatyti, kad žmogus įstrigo spūstyje, gyvena užterštoje aplinkoje ar netoli judraus kelio. Be to, tokių junginių kaip mentolis ir dariniai yra būdingi dantų pastos gaminiams. Kiti terpenai gali atsirasti dėl kosmetikos, kvapiųjų medžiagų ar valymo priemonių poveikio. Tai gali būti naudinga norint sužinoti, ar asmuo yra paveikiamas užterštos aplinkos.

7.3. Vietovių žymenys, parodantys žmonių buvimą

Oro LOJ sudėtį konkrečioje vietoje lemia įvairūs netoliese esantys šaltiniai. Terpeno junginiai būdingi želdiniams, o angliavandenilių arba benzeno dariniai – iš automobilių išmetamųjų dujų ir iškastinio kuro. Be to, junginių, kuriuos paprastai gamina žmonės, buvimas gali rodyti žmonių buvimą vietovėje. Didelis izopreno, acetono ar nonanalio kiekis gali parodyti, kad vietovėje lankosi daug žmonės, pavyzdžiui, sporto zonos, teatrai ar turgūs. Tačiau norint patvirtinti šiuos faktus, tose vietose reikia atlikti tolesnius tyrimus.

7.4. Žymenys, parodantys maisto produktus

Gastronominiai renginiai, tokie kaip maisto turgūs, maisto salės ar tiesiog paprasta namų virtuvė, užpildo orą specifiniais LOJ. Analizuodami skirtingų vietovių LOJ sudėtį, galėtume nustatyti, kur yra maisto produktų. Tačiau norint patvirtinti šiuos faktus, tose vietose reikia atlikti tolesnius tyrimus.

7.5. Žymenys maiste, priklausomai nuo vietovės

Kai maistas yra gaminamas tam tikroje vietoje, visi jo aplinkoje esantys cheminiai junginiai gali turėti įtakos jų pačių sudėčiai. Tiriant tos pačios rūšies maisto produktų LOJ sudėtį, gali būti įmanoma atskirti ekologiškus, SKVN ar SGN produktus.

8. IŠVADOS

1. Sėkmingai sukūrėme pigią ir lengvai sumontuojamą LOJ surinkimo sistemą, tinkančią tirti LOJ iš žmogaus iškvėpavimo. Taip pat atlikome pilną parametrų optimizavimą.

2. Mūsų sukurta LOJ surinkimo sistema taip pat tinka tirti LOJ iš kietųjų mėginių. Ypač gerų rezultatų davė maisto mėginiai, tokie kaip žemės riešutai ir malta kava.

3. Žmogaus iškvėptame ore randami LOJ gali būti naudojami kaip skirtingų šaltinių žymenys. Maisto nurijimas, rūkymas, mankšta ar dantų pastos naudojimas aiškiai nustatyti mūsų tyrimo metu.

4. Žmogaus kvėpavimo LOJ profilį gali paveikti aplinka. Kvėpuojant gali būti aptiktas didelis kvapiųjų medžiagų kiekis, įstrigimas eismo spūstyje arba gyvenimas šalia žalio ploto.

5. LOJ sudėtis gali būti priemonė, leidžianti atskirti tokius veiksnius kaip kilmė, poveikis ar liga. Maisto produktai, užauginti skirtingose vietose arba veikiami skirtingos cheminės sudėties, turės skirtingus LOJ profilius. Kalbant apie žmones, skirtingas LOJ profilis gali būti pagrindinis ligos diagnozavimo elementas.

CURRICULUM VITAE

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PROFILE

Analytical Chemistry graduate with work experience in multicultural environments, always eager to learn something new and trying to improve constantly. Passionate about Chemistry and my job. Comfortable in team working, active and not afraid to take on the responsibility.

WORK EXPERIENCE

February 2015 – Present; **Junior Researcher Associate** at Center for Physical Sciences and Technology, Metrology Department, Laboratory for metrology in Chemistry (Lithuania). Development of new methodologies for the analysis of contaminants in several matrixes. GC-HRMS and TD-GC-FID.

February 2015 – February 2016; **Chemical Technologist** at Innovative Pharma Baltics (Lithuania). Development and improvement of new products; preparation of the necessary documentation for its registration and commercialization.

August 2013 – August 2014; **Chemist trainee** at Novartis Pharma, NIBR – Center for Proteomic Chemistry, Basel (Switzerland). Chemical compound registration and classification due to structural information. Quality control using UPLC-MS.

January 2013 — July 2013; **Research Assistant** at Jožef Stefan Institute, Ljubljana (Slovenia). Extraction and analysis of fatty acids in dairy products and their relation to geographical origin, animal origin and adulteration. Lipid extraction from archaeological samples to identify the presence of dairy products using GC-FID and GC-C-IRMS.

April 2012 — June 2012; **Laboratory Technician** at AIDIMA Furniture, Wood and Packaging Technology Institute, Valencia (Spain). Quality control of the NOx reducing agent AUS32 for diesel engines at the environmental department using ICP-MS and FT-IR.

October 2010 — June 2011; **Laboratory Technician** at the Faculty of Chemistry and Chemical Engineering at University of Ljubljana (Slovenia). Doing my Bachelor's final project about synthesis of inorganic compounds with organic ligands using FT-IR.

June 2008 — September 2008; **Laboratory Technician** at University of Valencia (Spain). Study of time retention of phenols within different columns and mobile phases. HPLC-UV/VIS.

EDUCATION

September 2017 — Present; **PhD Student** at Vilnius University, Lithuania. Topic: Study on volatile organic compounds (VOC's) as potential biomarkers for disease diagnosis and monitoring.

September 2011 — July 2012; **Master's Degree in Analytical and Experimental Chemistry** at University of Valencia, Spain. Master Thesis: Direct mercury analysis of hair samples and its relation with fish consumption. Direct Mercury Analyser (DMA).

September 2005 — June 2011; **Bachelor's Degree in Chemistry** at University of Valencia, Spain.

SKILLS

Language skills
Spanish and Catalan — Mother tongues
English — Proficient User
Lithuanian — Independent User

Other Skills
Proficient in using liquid and gas chromatography (**GC-HRMS, GC-FID**, **UPLC-MS**, **HPLC-UV/vis**, **GC-C-IRMS**), Fourier transform infrared (**FT-IR**), X-ray microscopy and Direct Mercury Analyser (**DMA**)

Team working skills acquired through group projects and laboratory practicals during university studies.

Leadership skills developed while training and delegating tasks to new interns at Novartis , Jožef Stefan Institute, Innovative Pharma Blatics and Center for Physical Sciences and Technology.

Ability to adapt to **multicultural** and **new working environments** gained while living abroad and working in different laboratories.

Strong **organisational skills** acquired while arranging football training sessions and matches for two local football teams in Valencia and short excursions around Europe.

Excellent knowledge of **Microsoft OfficeTM.** Windows and OS X user.

PUBLICATIONS

1 **Adrian Vicent-Claramunt**, Evaldas Naujalis. *Cheap and easy human breath collection system for trace volatile organic compounds screening using thermal desorption – gas chromatography mass spectrometry*. MethodsX Volume 8, 2021, 101386. doi: 10.1016/j.mex.2021.101386

2 **Adrian Vicent Claramunt**, Audrius Sadaunykas, Simonas Balciunas, Birute Knasiene, Audrius Zolumskis, Evaldas Naujalis. *Profiling of Volatile Organic Compounds for Environment Discrimination in Vilnius City*. Chemija Volume 33, No 1, 2022. doi:10.6001/chemija.v33i1.4661

CONFERENCES ATTENDED

1 **A. Vicent Claramunt**, E. Naujalis. Study on background profile of volatile organic compounds (VOCs) from human breath. 8-oji Doktorantų ir jaunųjų mokslininkų konferencija FizTech 2018. Presentation at local conference (Vilnius)

2 **A. Vicent Claramunt**, E. Naujalis. Influence of exposure to air-fresheners on VOCs profile in human breath. Doktorantų ir jaunųjų mokslininkų konferencija FizTech 2019. Presentation at local conference (Vilnius)

3 **A. Vicent Claramunt**, E. Naujalis. Breath volatile organic compounds profile after short exposure to high concentrations of indoor fragrance chemicals. International Societies of Exposure Science (ISES) and Indoor Air Quality and Climate (ISIAQ) joint annual meeting 2019: The built, natural, and social environments: impacts on exposures, health and well-being". Poster presentation at international conference (Kaunas)

4 **A. Vicent Claramunt**, E. Naujalis. Influence of Exposure to Fragrances on VOCs Profile in Human Breath. Open readings 2020 (63rd Edition). Poster presentation at international conference (Vilnius)

5 **A. Vicent Claramunt**, E. Naujalis. Influence of exposure to fragrances on VOCs profile in human breath. Doktorantų ir jaunųjų mokslininkų konferencija FizTech 2020. Presentation at local conference (Vilnius)

6 **A. Vicent Claramunt**, E. Naujalis. Correlation between pyridine and furfuryl alcohol ingestion and their presence in breath. Open readings 2021 (64th Edition). Poster presentation at international conference (Online)

7 **A. Vicent Claramunt**, E. Naujalis. Exhaled breath analysis for the determination of food contaminants. Breath Biopsy Conference 2020 - Owlstone Medical. Poster presentation at international conference (Online)

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NOTES

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