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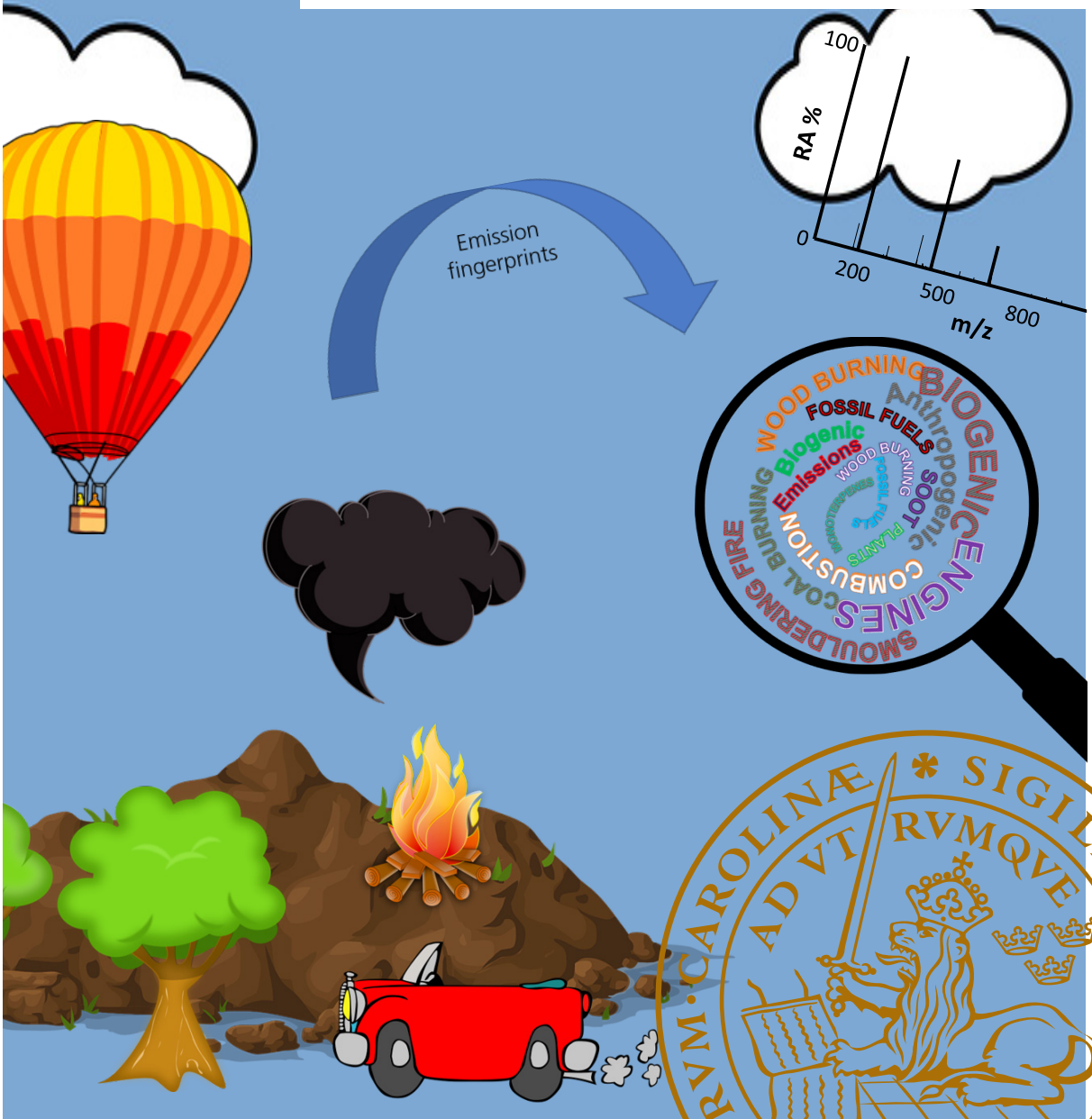

# Extraction and chromatography of targeted emission markers in atmospheric aerosols

Hafiz Abdul Azeem

LUND
UNIVERSITY

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Abstract

Atmospheric aerosols are a highly complex and dynamic mixture of solid particles, liquid deplots and gases. They travel across the continents and affect global climate and human health in various ways, often negatively. One important aspect of research in atmospheric aerosols is the investigation of emissions to the atmosphere from various sources. Emission markers are compounds unique to their sources of emission, hence, they act as fingerprints and are extensively used in source apportionment studies. It is highly challenging to sample, extract and quantify the compounds of interest, e.g. emission markers, from a complex mixture of thousands of organic and inorganic compounds, minerals and metals ions. A common bottleneck is the stringent requirements on the analytical methods, demanding high selectivity and low limits of detection. The work presented here focuses on the development of various extraction and chromatography methods followed by mass spectrometry detection for the extraction, islation and quantitative anlaysis of targeted emission markers from complex aerosol samples. Development and optimization of various extraction and microextraction methods, optimization of different chromatography methods and mass spectrometry detection was motivated by the goals of higher selectivity, sensitivity, precision, accuracy, low limits of detection and low limits of quantification. Selection of greener solvents, reduced solvent use, shorter run times and eventually cheaper solutions were emphasised. The developed methods were compared with already existing methods in terms of 1) lower limits of detection, 2) reduced bias in analytical measurements and 3) greener alternatives. An interesting discovery on the formation of iron(III) complexes of 3-methyl-1,2,3-butane tricarboxylic acid, one of the emission markers for secondary biogenic emissions from monoterpenes, was also presented for the first time. The potential of the methods was demonstrated by their applications to aerosol samples as well as to solve societal problems like early detection of smouldering fire using a unique emission marker. Finally, detailed description of hollow-fibre liquid-phase microextraction method studied for the extraction of 3-methyl-1,2,3-butane tricarboxylic acid was presented along with the drawbacks of the method observed for the compound. It is expected that the research presented here will be a positive contribution in the estimation of emission markers and similar compounds from complex samples.



Key words: Atmospheric aerosols, emission markers, mass spectrometry, supercritical $CO_2$, trace analysis

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*Dedicated to my mother*

# Abstract

Atmospheric aerosols are a highly complex and dynamic mixture of solid particles, liquid deplots and gases. They travel across the continents and affect global climate and human health in various ways, often negatively. One important aspect of research in atmospheric aerosols is the investigation of emissions to the atmosphere from various sources. Emission markers are compounds unique to their sources of emission, hence, they act as fingerprints and are extensively used in source apportionment studies. It is highly challenging to sample, extract and quantify the compounds of interest, *e.g.* emission markers, from a complex mixture of thousands of organic and inorganic compounds, minerals and metals. A common bottleneck is the stringent requirements on the analytical methods, demanding high selectivity and low limits of detection. The work presented here focuses on the development of various extraction and chromatography methods followed by mass spectrometry detection for the extraction, islation and quantitative anlaysis of targeted emission markers from complex aerosol samples. Development and optimization of various extraction and microextraction methods, optimization of different chromatography methods and mass spectrometry detection was motivated by the goals of higher selectivity, sensitivity, precision, accuracy, low limits of detection and low limits of quantification. Selection of greener solvents, reduced solvent use, shorter run times and eventually cheaper solutions were emphasised. The developed methods were compared with already existing methods in terms of 1) lower limits of detection, 2) reduced bias in analytical measurements and 3) greener alternatives. An interesting discovery on the formation of iron(III) complexes of 3-methyl-1,2,3-butane tricarboxylic acid, one of the emission markers for secondary biogenic emissions from monoterpenes, was also presented for the first time. The potential of the methods was demonstrated by their applications to aerosol samples as well as to solve societal problems like early detection of smouldering fire using a unique emission marker. Finally, detailed description of hollow-fibre liquid-phase microextraction method studied for the extraction of 3-methyl-1,2,3-butane tricarboxylic acid was presented along with the drawbacks of the method observed for the compound. It is expected that the research presented here will be a positive contribution in the estimation of emission markers and similar compounds from complex samples.

# Popular scientific summary

How to identify the sources of air pollution? The smell of a BBQ meat steak charring on a grill can be easily recognized. The smell is distinguishable in the air containing pollens, fragrances of different flowers, smell of burning coal and numerous other gases and volatile compounds. Probably we need stronger sniffing noses to be able to identify, for example, if there is more wood burning in comparison to car emissions on a continental scale. Here comes the role of Analytical Chemistry, as a problem-solving science, which helps distinguish certain so-called "smells" or "fingerprints" of sources, here referred to as emission markers. Following their emission, these markers undergo numerous chemical reactions in the atmosphere. Travelling with the air masses across continents and oceans, their chemical structure is continuously changing. It is therefore very challenging to identify and quantify these markers out of the thousands of other chemical substances also present in the air. Such challenges have been dealt with in this thesis through the development and optimization of analytical methods. In analytical chemistry, the process of separating the emission markers from a complex sample matrix containing thousands of compounds is called extraction. Often extraction methods alone are not enough for the isolation of emission markers from complex aerosol samples. In modern analytical chemistry, instrumental methods such as chromatography are used, in conjunction to extraction methods, to separate the compounds of interest from other similar compounds using gases, liquids and supercritical fluids. These extraction and chromatographic methods require fine analytical chemistry skills to tune intricate details for the isolation and quantification of emission markers. The efficiency of the methods used for isolation and estimation of the compounds of interest can be represented as selectivity, sensitivity, precision, accuracy, limit of detection and limit of quantification. In this thesis, various extraction and microextraction methods – gas, liquid and supercritical fluid chromatography – and detection methods such as mass spectrometry were optimized and applied to aerosol samples with an overall goal to achieve better efficiency than already existing methods.

As mentioned above, limit of detection and quantification are important yardsticks used to compare efficiencies of analytical methods. Such parameters are also of high value when dealing with emission markers. One of the big challenges in such scientific studies is quantitative analysis of extremely small amounts of emission markers. Let's imagine the size of crawling insects in your garden. When measuring their lengths using a household measuring tape, the results are likely to be highly uncertain, especially if the size of the insects shrinks to microscopic lengths. How useful a household measuring tape would be for these organisms? Let's add further complexity to the situation by now if there are several organisms of microscopic lengths and similar appearances. In this scenario even, a household measuring tape – together with a microscope and everything available in a standard toolbox – cannot distinguish the organisms of interest. Then how could we measure their lengths? It is obvious that one needs advanced technological methods for the identification and measurement of the microorganisms on such a small scale. Similarly, emission markers are present in air in extremely small amounts and advanced analytical chemistry methods are needed to study them. Improving the limits of detection and quantification for selected emission markers was one of the focal points of the research presented here. Emphasis was given to greener analytical methods in terms of toxicity of the solvents and the quantities used.

The applications of the developed methods can be imagined by the societal impact of, for example, the case study presented on early detection of smouldering fires. Smouldering fire is a flameless form of fire that propagates at slow speed in fibrous combustible materials, for example, agricultural waste used for power production. In big piles of biomass, it can continue to grow for days and weeks unnoticed. Due to its flameless nature and relatively low temperature, it is hard to detect by standard methods until it transforms into flames causing devastating consequences. State of the art analytical chemistry methods of sampling, extraction, chromatography and mass spectrometry were used for the first time for early detection of smouldering fire by the analysis of emission marker in the aerosol particles released. Research in analytical chemistry can contribute to a large extent helping with today's environmental and societal challenges.

# List of publications

I. Extending the scope of dispersive liquid-liquid microextraction for trace analysis of 3-methyl-1,2,3-butanetricarboxylic acid in atmospheric aerosols leading to the discovery of iron(III) complexes
**Hafiz Abdul Azeem**, Teshome Tolcha, Petter Ekman Hyberg, Sofia Essén, Kristina Stenström, Erik Swietlicki and Margareta Sandahl
*Submitted*

II. Towards the isolation and estimation of elemental carbon in atmospheric aerosols using supercritical fluid extraction and thermo-optical analysis
**Hafiz Abdul Azeem**, Johan Martinsson, Kristina Eriksson Stenström, Erik Swietlicki and Margareta Sandahl.
*Anal. Bioanal. Chem. (2017) 409:4293–4300*

III. Supercritical fluid chromatography–diode array detection–tandem quadrupole mass spectrometry for the analysis of non-polar to polar classes of organic emission markers in atmospheric aerosols
**Hafiz Abdul Azeem**, Daniel Molins-Delgado and Margareta Sandahl
*Manuscript*

IV. Carbonaceous aerosol source apportionment using the Aethalometer model – evaluation by radiocarbon and levoglucosan analysis at a rural background site in southern Sweden
Johan Martinsson, **Hafiz Abdul Azeem**, Moa K. Sporre, Robert Bergström, Erik Ahlberg, Emilie Öström, Adam Kristensson, Erik Swietlicki and Kristina Eriksson Stenström
*Atmos. Chem. Phys. (2017) 17:4265–4281*

V. Levoglucosan as a tracer for smouldering fire
Dan Madsen, **Hafiz Abdul Azeem**, Margareta Sandahl, Bjarne Husted, Patrick van Hees
*Fire Tech. (2018) 54:1871–1885*

*Dan Madsen and Hafiz Abdul Azeem participated equally and share the first authorship of the manuscript*

# Author's contribution

I. HAA: Synthesized the research idea, performed all the planning and design of the experiments, performed most of the experiments and wrote the whole manuscript
TT: Supported in DLLME experiments
PEH: Supported in preliminary experiments
SE: Supported with identification of MBTCA-iron(III) complexes
ES: Revised the manuscript
KS: Revised the manuscript
MS: Involved in the planning and revised the manuscript

II. HAA: Involved in the planning of the project, planned the experimental strategy, performed supercritical fluid experiments and wrote the whole manuscript
JM: Supported with TOA analysis
KS: Involved in the planning and revised the manuscript
ES: Involved in the planning and revised the manuscript
MS: Involved in the planning and revised the manuscript

III. HAA: Synthesized the initial research idea, planned the project, performed the experiments and wrote the whole manuscript.
DMD: Supported with MS method development
MS: Involved in the planning and revised the manuscript

IV. HAA: Involved in the planning, performed chemical analysis of levoglucosan and wrote parts of the manuscript
JM: Designed the study, analysed all data and wrote the manuscript
MKS: Generated the HYSPLIT results
RB: Assisted in the writing process
EA: Involved in aerosol sampling
EÖ: Involved in aerosol sampling
AK: Involved in the planning and revised the manuscript
ES: Involved in the planning and revised the manuscript
KS: Involved in the planning and revised the manuscript

V. HAA: Involved in the planning of the work, designing the experimental strategy including sampling, performed all the chemical analysis and participated in writing of the manuscript.
DM: Synthesized the initial research idea, performed smouldering fire experiments and wrote parts of the manuscript
MS: Involved in the planning and revised the manuscript
BH: Involved in the planning and revised the manuscript
PVH: Revised the manuscript

# Publications not included in this thesis

I. Paul H. Gamache (Ed.): Charged aerosol detection for liquid chromatography and related separation techniques
**Hafiz Abdul Azeem**
*Anal. Bioanal. Chem. (2018) 410: 2663*

II. A targeted metabolomic protocol for quantitative analysis of volatile organic compounds in urine of children with celiac disease
Natalia Drabinska, **Hafiz Abdul Azeem** and Urszula Krupa-Kozak
*RSC Adv. (2018) 8: 36534–36541*

III. Evaluation of a process cascade for extraction of lipid, starch, and protein from wheat bran
Roya Sardari, Samuel Sutiono, **Hafiz Abdul Azeem**, Mats Larsson, Charlotta Turner and Eva Nordberg Karlsson
*Manuscript*

IV. Relationship and impact of biogenic secondary organic aerosol on the aethalometer model residual carbon
Johan Martinsson, **Hafiz Abdul Azeem** and Moa Sporre
*Manuscript*

# Abbreviations

| | |
|---|---|
| **BC** | Black Carbon |
| **BSOA** | Biogenic Secondary Organic Aerosols |
| **BVOC** | Biogenic Volatile Organic Compounds |
| **CCF** | Face-centred Central Composite Design |
| **DAD** | Diode Array Detector |
| **DLLME** | Dispersive Liquid-Liquid Microextraction |
| **EC** | Elemental Carbon |
| **$EC_{FF}$** | Elemental Carbon from fossil fuel combustion |
| **HFLPME** | Hollow-Fibre Liquid-Phase Microextraction |
| **EMV** | Electron Multiplier Voltage |
| **ESI** | Electrospray Ionization |
| **QToF** | Tandem Quadrupole Time of Flight Mass Spectrometry |
| **GC** | Gas Chromatography |
| **LC** | Liquid Chromatography |
| **LLE** | Liquid-Liquid Extraction |
| **MS** | Mass Spectrometry |
| **MS/MS** | Tandem Mass Spectrometry |
| **MBTCA** | 3-methy-1,2,3-butane tricarboxylic acid |
| **OC** | Organic Carbon |
| **$OC_{Bio}$** | Organic Carbon from biogenic emissions |
| **$OC_{BM}$** | Organic Carbon from biomass burning |
| **PAHs** | Polycyclic Aromatic Hydrocarbons |
| **SFC** | Supercritical Fluid Chromatography |
| **SFE** | Supercritical Fluid Extraction |
| **SOA** | Secondary Organic Aerosols |
| **TC** | Total Carbon |
| **TD** | Thermal Desorption |
| **QqQ** | Tandem Triple Quadrupole Mass Spectrometry |
| **TOPO** | tri-n-octyl-phosphineoxide |
| **UHPLC** | Ultra-High Performance Liquid Chromatography |

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# Prologue

Often analytical chemists fall in love with certain instrumental techniques and tend to jump into the laboratory the very moment they encounter an interesting idea or a scientific problem. That is why this is a common impression for general scientific community that all the scientific problems in analytical chemistry can be solved with fancy machine(s). The science of analytical chemistry starts with the most powerful machine in the world, "the brain". My mentors did an excellent job helping me train this machine to investigate gaps in knowledge based on available scientific information, formulate explicit research questions and find out the most suitable and simplest analytical methods to answer the research questions.

For the last five years I worked on various sample preparation, chromatography and mass spectrometry methods in an effort to push the limits of detection of targeted analytes from complex aerosol samples and looking for green alternatives in terms of reducing amount of solvents and type of solvents used, reducing analysis time and overall costs. Unlike so-called chemical analysis where the goal is to use analytical methods to provide qualitative and/or quantitative information from the given samples, environmental analytical chemistry requires 1) a good understanding of chemical transformations in the environment that lead to identification of gaps in knowledge, 2) information on various sampling methods and associated challenges, 3) an up-to-date knowledge of a range of extraction and chromatographic methods, their advantages and limitations, and ability to select the right analytical approach to solve the challenges and 4) ability to identify important variables of extraction, chromatography and mass spectrometry methods for further optimising and fine-tuning. Efforts were made to 1) improve analytical methods, 2) provide information on important variables in analytical methods and 3) discover new scientific information both related to the properties of analytes as well as their role in the atmosphere.

The following chapters reflect the author's research journey to identify gaps in knowledge, formulate explicit research questions, synthesize comprehensive plan for the experimental work and integrate coherent scientific arguments with experimental results to answer the research questions. However, the sole intention of the overall effort was education and training in analytical reasoning to be able to contribute to the progress of science and humanity. I hope that together, we, the whole scientific community, will contribute to humanity and a better world for clean

air and water, sufficient food for everyone, cure for diseases, intercultural harmony leading to tolerance, . . . , and most of all peace.

# 1 Emission markers

*"The most alarming of all man's assaults upon the environment is the contamination of air, earth, rivers, and sea with dangerous and even lethal materials" – Rachel Carson*[1]

Atmospheric aerosols are mixtures of solid and liquid particles suspended in air. The aerosol dynamics, chemical composition and concentration differ significantly in different parts of the atmosphere. The aerosols are usually divided into primary and secondary aerosols. The former represents aerosols emitted directly into atmosphere at or close to the source whereas the later indicates aerosols produced in the atmosphere by chemical transformations of volatile precursors into less volatile and highly oxidized species[2-4]. The chemical composition of atmospheric aerosols is highly complex, including a wide range of different classes of small organic molecules and large graphite-like structures (soot). Inorganic compounds include various salts and metals. The organic molecules and soot, also known as carbonaceous aerosols, present in the atmosphere constitute the largest fraction of overall aerosol emissions[5].

Increasing awareness of the effects of aerosols on human health and climate motivates the study of different sources of emissions, their relative contributions in the total emission budget and the resulting atmospheric aerosol loadings. Both anthropogenic and natural emissions are important in this context. Although the carbonaceous fraction of aerosol alone is highly complex and dynamic, quantitative analysis of certain compounds unique to singular emission sources can be used to determine the origin. Such compounds serve as fingerprints of emission sources and known as emission markers or tracers. Ideally, an emission marker should be present as a stable aerosol with stoichiometrically constant amounts relative to other components[6]. The present thesis addresses analytical challenges in the determination of various emission markers in carbonaceous fraction of atmospheric aerosols.

## 1.1 Anthropogenic emissions

Excluding wild forest fires, combustion of biomass and fossil fuels are the two biggest anthropogenic sources of carbonaceous aerosols today. There are several

emission markers that provide interesting information regarding the types of fuels and process of combustion. However, the most widely known are sugars, polycyclic aromatic hydrocarbons (PAHs) and soot.
All biomass contains cellulose as part of its skeleton. The building blocks of cellulose are sugar molecules that are released to the atmosphere when cellulose is pyrolysed during burning. Levoglucosan (1,6-anhydro-beta-D-glucose) and its isomers are produced at high temperature pyrolysis (>300 °C) of cellulose as a unique signature of biomass burning[7, 8]. Levoglucosan is fairly stable in the atmosphere and can be used as emission marker[8, 9]. The information from quantitative analysis of levoglucosan was utilized for source apportionment of carbonaceous aerosols in southern Sweden (Paper IV) and for early detection of smouldering fire (Paper V) using systematic sampling and gas chromatography coupled to mass spectrometry (GC–MS) analysis.

PAHs are a class of organic compounds with two or more aromatic rings fused together. PAHs are known for their carcinogenic and mutagenic nature[10, 11]. They are released to the atmosphere from various sources. Generally, PAHs are divided into 1) petrogenic, originating from petroleum product exposed to environment for example oil spills; 2) pyrogenic, produced during high temperature combustion of biomass and fossil fuels; and 3) biogenic, from plants and microorganisms[12]. Based on emission profiles, certain PAHs have been identified as emission markers for their respective sources. For example, picene is used as emission marker for coal combustion[13]. Similarly a group of PAHs representing engine emissions has been reported[14]. Paper III presents a supercritical fluid chromatography coupled to mass spectrometry (SFC–MS) method for the analysis of selected PAHs together with other emission markers in atmospheric aerosols constituting various classes of organic compounds ranging from non-polar to polar ones.

Another important fraction of carbonaceous aerosols is soot. Several aliases including black carbon (BC) and elemental carbon (EC) are customary, and typically refer to the measurement technique used to derive the mass concentration in question[15]. The different quantification techniques include light absorption methods, *e.g.* aethalometer, and thermo-optical methods, respectively. Where each method provides important information on one or more of the properties of soot, none is able to establish a complete characterization and quantification of soot that can be directly compared and confirmed by other experimental methods. Hence the amount of soot measured by various methods may not be the same in a given sample. Pöschl *et al*[16] illustrated optical and thermal classification of soot measured in terms of BC, EC and corresponding chemical structures (a modified illustration is shown in Figure 1).

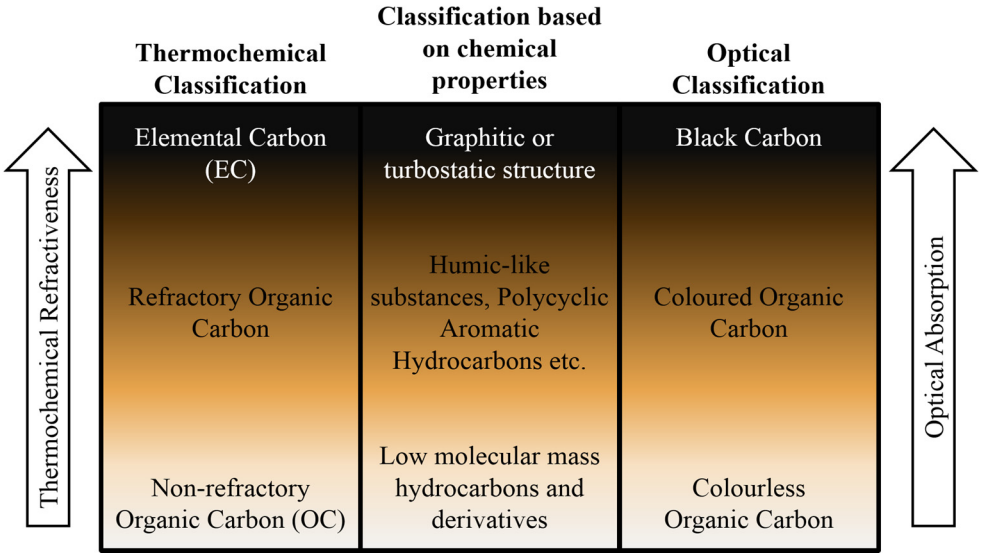



**Figure 1.** Classification of carbonaceous fraction of atmospheric aerosol particles according to thermochemical, chemical and optical properties, adapted and modified from Pöschl *et al*[16].

EC originates from oxygen-starved combustion of biomass and fossil fuels. It is also linked to severe morbidity and mortality[17, 18]. On the other hand, it can be used as an emission marker for low oxygen, high temperature combustion of biomass and fossil fuels. The available methods for estimation of EC – such as thermal methods, Raman spectroscopy and insolubility methods – work on different principles and the information provided by different techniques is not comparable due to the fact that each method accounts for EC mass concentration based on different properties, as described earlier[8, 9, 19]. In addition, none of the available method is completely free from interferences and bias (both positive and negative). These shortcomings were addresses in Paper II using supercritical $CO_2$ method followed by thermal optical analysis.

## 1.2 Biogenic emissions

Emissions from plants and trees did not receive as much attention as anthropogenic emissions until the recent decades. Study of biogenic emissions is important for various reasons including the fact that the chemistry of atmosphere and aerosol is impossible to understand without taking biogenic emissions into account. Often biogenic emissions are dependent on temperature, photosynthetically available radiations, water availability and other disturbances (for instance from insects, cutting and other structural damages, high ozone uptake) and vary over seasons. Emissions of monoterpenes from plants are among the largest of biogenic

emissions[4]. Gas phase alpha-pinene undergoes transformations and produces, among other compounds, cis-pinonic acid as biogenic secondary organic aerosol (BSOA). After a series of complex photo-oxidation reactions and transformations, cis-pinonic acid produces 3-methyl-1,2,3-butane tricarboxylic acid (MBTCA) as a multi-generation oxidation product[20]. Both cis-pinonic acid and MBTCA have been used as secondary biogenic monoterpene emission markers, in order to estimate and quantify the impact of natural biogenic sources to the secondary organic aerosol (SOA) loading. Development of a microextraction method to deal with the challenges in the trace analysis of MBTCA and discovery of its interesting complex formation behaviour has been described in Paper I. Furthermore, primary biogenic emission markers like arabitol for fungal spores, sucrose for pollens and fructose representing mixed biogenic emissions with predominately pollen origin have been studied in Paper III.

## 1.3 Sampling

Aerosol sampling is an extensive field of study and different sampling strategies are adopted based on scientific needs in question as well as available resources. In this research two different types of samples were used. Aerosol particles were collected on quartz-fibre filters using low-volume air sampler (Paper I, III, IV) and two different high-volume air samplers (Paper II and V). The classification, *i.e.* low and high volume sampling, is based on the amount of air passed through a filter in a given sampling time[2, 21]. Naturally low volume-sampling results in the collection of smaller amounts of particulate matter and sometimes the analyte(s) of interest being available in extremely small amounts makes the detection and quantification challenging. On the other hand high volume sampling also have some disadvantages as described by Kristensen, K.[22]. The description of various sampling techniques, including online sampling coupled to analytical instruments as well as offline sampling on certain collection materials, the whole range of collection materials used for aerosol sampling, passive and active sampling and methods used for size resolution of the aerosol particles during sampling are some of the commonly known parameters that deserves a dedicated manuscript on the topic, which is beyond the scope of this book. However, a simplified overview of various ways of aerosol sampling is given in Figure 2.

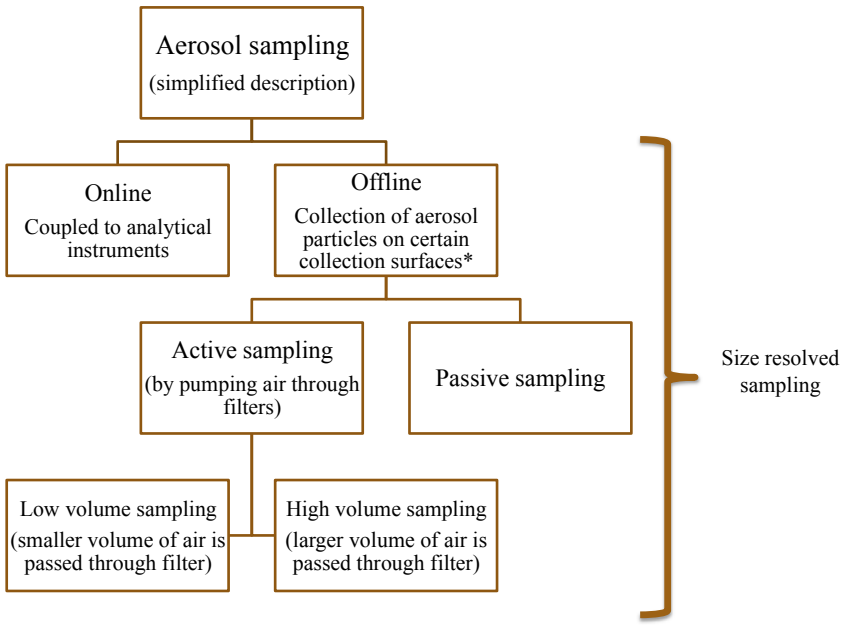



**Figure 2.** A simplified illustration of various types of aerosol sampling, the terms online and offline sampling refer to if the aerosol sample is analysed online or offline, respectively. Size resolution represents the aerodynamic size of aerosol particle, *e.g.* $PM_{10}$, $PM_{2.5}$.

## 1.4 Source apportionment

When quantifying the atmospheric aerosol loading of carbonaceous aerosols using thermo-optical methods, the total carbon (TC) can be divided into organic carbon (OC) and EC (Equation 1). EC originates from oxygen-starved combustion of all hydrocarbon-containing fuels. Most importantly here are EC from combusted biomass and fossil fuels, contributing to the measured concentrations of $EC_{BM}$ and $EC_{FF}$ respectively (Equation 2). Similarly, OC can be divided in $OC_{BM}$ resulting from biomass combustion and $OC_{FF}$ from fossil fuel combustion. In addition, biogenic primary emissions of OC, as well as oxidation products of volatile organic compounds of biogenic origin (BVOC) forming secondary OC, both contribute to the total OC aerosol loading ($OC_{Bio}$ in Equation 3).

$$TC = OC + EC \quad (1)$$

$$EC = EC_{BM} + EC_{FF} \quad (2)$$

$$OC = OC_{BM} + OC_{FF} + OC_{Bio} \quad (3)$$

A radioactive isotope of carbon, $^{14}C$, can be used to distinguish modern carbon emissions from fossil fuel emissions. This is due to the fact that today all biomass contains naturally produced $^{14}C$ as well as $^{14}C$ from nuclear blasts from 1945 and onwards until the atmospheric test ban in the 1960´ies. In contrast, fossil fuels are completely depleted of $^{14}C$, which has a half-life of 5730 years. Therefore, $^{14}C$ serves as emission marker for all modern carbon sources. Another important piece of the puzzle is estimation of $OC_{BM}$ that can be calculated by the help of levoglucosan (and methoxyphenols) that serves as emission marker for biomass burning. A simplified illustration of different fractions for TC and the works presented on various fractions is given in Figure 3.

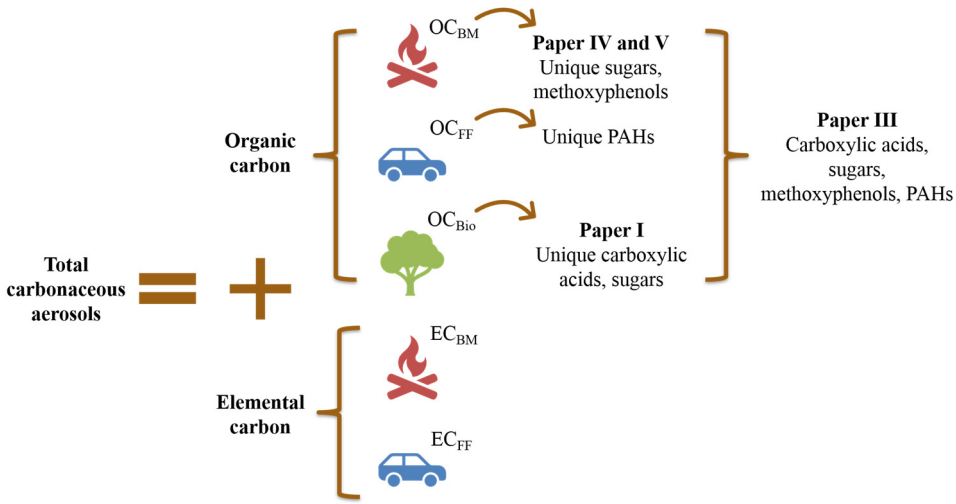
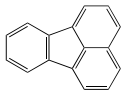
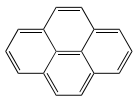
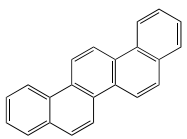
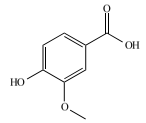
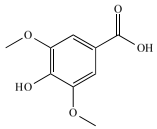
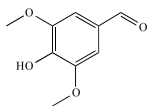
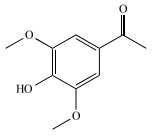
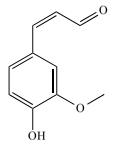



**Figure 3.** Description of various components that constitute carbonaceous fraction of atmospheric aerosols and emission markers for each component represented according to their chemical structures. An overview of the thesis with different works dedicated to emission marker(s) from certain sources is also shown.

A detailed description of different organic emission markers studied in this doctoral research are described (Table 1) with respective chemical structures, logP values, predominant sources of emission and common methods of sample preparation and analysis.

**Table 1.** A description of various organic emission markers from atmospheric aerosols studied in this work.

| Compound name | Chemical Structure | logP* | Predominant source | Compound class | Common methods of analysis for the compound class | |
|---|---|---|---|---|---|---|
| | | | | | Sample preparation | Chromatography and detection |
| Acenaphthene |  | 3.9 | Fossil fuels predominantly automobile emissions[23] | PAHs | Soxhlet extraction[23-25]<br>Ultrasonic extraction[26-28]<br>Hollow-Fibre Micro-Porous Membrane Liquid-Liquid Extraction (HF-MMLLE)[9] | GC–MS[9, 23, 25, 26, 28]<br>Liquid Chromatography coupled to Ultraviolet detector LC–UV[29]<br>Atmospheric pressure photoionization (APPI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR)[24, 27] |
| Fluoranthene |  | 5.2 | | | | |
| Pyrene |  | 4.9 | | | | |
| Picene |  | 7 | Coal burning[30] | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Vanillic acid |  | 1.4 | Biomass burning (lignin pyrolysis)[31, 32] | Phenolic acids | Ultrasonic extraction[33, 34]<br><br>HFLPME[35] | GC–MS[33, 35, 36]<br><br>GC coupled to Ion Trap Mass Spectrometry (GC–IT/MS)[34]<br><br>Supercritical Fluid Chromatography coupled to Mass Spectrometry (SFC–MS)[31]<br><br>Chemical Ionization Mass Spectrometry (CIMS)[32] |
| Syringic acid |  | 1 | | | | |
| Syringaldehyde |  | 0 | | Phenolic aldehydes & ketones | | |
| Acetosyringone |  | 0.2 | | | | |
| Coniferyl aldehyde |  | 1.5 | | | | |

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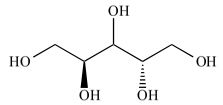
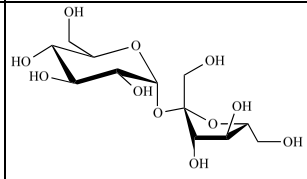
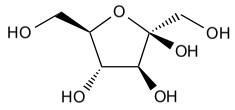
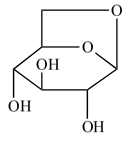
|---|---|---|---|---|---|---|
| Arabitol |  | -2.5 | Fungal spores[37] | Sugars | Ultrasonic extraction[9, 38, 39]<br><br>Solvent extraction by vortex agitation[40] | Gas Chromatography coupled to Flame Ionization (GC–FID)[37]<br><br>GC–MS[9, 39]<br><br>Liquid Chromatography coupled to Electrospray Ionization tandem Mass Spectrometry (LC–ESI/MSMS)[40] |
| Sucrose |  | -3.7 | Biogenic predominantly pollen origin[37] | | | |
| Fructose |  | -2.23 | | | | |
| Levoglucosan |  | -2.1 | Biomass burning[9, 30] | | | |

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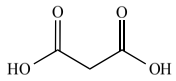
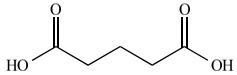
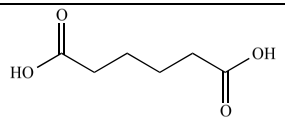
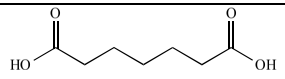
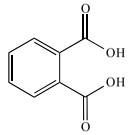
| Malonic acid |  | -0.8 | Anthropogenic[41] | Carboxylic acids | Ultrasonic extraction[34, 38, 42]<br><br>HFLPME[43] | GC–MS[42]<br><br>Gas Chromatography coupled to Ion Trap Mass Spectrometry (GC–IT/MS)[34]<br><br>Liquid Chromatography coupled to Electrospray Ionization Mass Spectrometry (LC–ESI/MS)[38] |
| Glutaric acid |  | -0.3 | Anthropogenic (photo-oxidation of unsaturated cyclic alkenes)[44] | | | |
| Adipic acid |  | 0.1 | Anthropogenic (photo-oxidation of unsaturated cyclic alkenes)[44, 45] | | | |
| Pimelic acid |  | 0.5 | Anthropogenic[45] | | | |
| Phthalic acid |  | 0.7 | Anthropogenic (Automobile and manufacturing of plastic)[41, 46] | | | |

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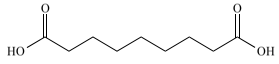
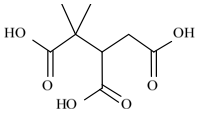
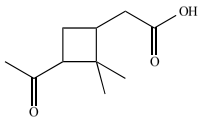
|---|---|---|---|---|---|---|
| Azelaic acid |  | 1.6 | Biogenic (sources like unsaturated fatty acids)[46, 47] | | | |
| MBTCA |  | -0.3 | Biogenic (secondary aerosols from monoterpene emissions)[20, 43] | | | |
| Pinonic acid |  | 1 | | | | |

*logP values collected from Pubchem database (**https://pubchem.ncbi.nlm.nih**)

## 1.5 Analytical challenges

In targeted analysis, identification and quantification of analytes have improved to a great deal with rapid development of chromatography and mass spectrometry in recent decades. An atmospheric aerosol sample is a complex matrix of organic and inorganic substances. Today aerosol sampling on membranes introduce some selectivity based on particle size and type of filters used for sampling. However, variables like seasonal and diurnal changes in emissions, daylight hours and relative humidity responsible for complex photo-oxidation reactions and other chemical transformations and collection of long-range transboundary aerosols can introduce immense complexity to chemical composition of the sample. For example, Hamilton *et al*.[48] isolated over 10,000 individual organic compounds in 10 μg of aerosol mass collected on quartz microfibre membrane using thermal desorption coupled to two dimensional gas chromatography and time-of-flight mass spectrometry (TD–GCxGC–ToF). Having the complexity of the matrix understood, the quantitative analysis of the analyte(s) of interest from a sample consisting of merely a few micrograms of semi-volatile organic compounds, various interfering inorganic salts and other substances is an unnerving job. Another challenge in targeted analysis of emission markers is quantitative analysis of compounds of interest at trace levels. High sample complexity and small sample size further add to these challenges. In a large number of scientific articles published on the analysis of atmospheric aerosols, usually the emphasis is on understanding the chemical composition and behaviour of aerosol particles and little efforts are made on the development of analytical methods with high sensitivity and selectivity. Therefore, it is highly desirable to improve analytical methods in terms of analyte enrichment, selectivity, sensitivity, accuracy and precision.

# 2 Aims of the thesis

The aim of this doctoral research was to develop analytical methods of sample preparation and chromatography and to optimize mass spectrometry methods for targeted analysis of selected emission markers in atmospheric aerosols. The focus was given to improve the efficiency of analytical methods, *i.e.* analyte enrichment, method selectivity and sensitivity, precision, accuracy, low limits of detection and quantification.

## 2.1 Specific research questions

The research presented in this thesis was intended to address following research questions:

- Can the scope of dispersive liquid-liquid microextraction be extended for the extraction of MBTCA and how efficient – in terms of limit of detection of MBTCA – is the microextraction as compared to conventional extraction techniques?
- Can supercritical $CO_2$ be used to isolate EC and how efficient – in terms of percentage removal of OC and percentage recovery of EC from aerosol samples – is supercritical $CO_2$ method?
- How can supercritical fluid chromatography and mass spectrometry be exploited for the analysis of several emission markers – with a wide range of polarities – in a single run?
- How accurate and robust is aethalometer model – in terms of source apportionment of atmospheric aerosols – compared to GC–MS?
- Can smouldering fires be detected at an early stage with the help of emission markers from aerosol signature of the fires?

# 3 Sample preparation

*"All my life through, the new sights of Nature made me rejoice like a child" – Marie Curie*[49]

One of the most widely used methods of aerosol sampling is the collection of aerosol particles on various membranes and filters. For any pre-concentration and chromatography step, the analyte is first extracted into liquids that can range from water to organic solvents and solvent mixtures based on the chemical nature of the analyte(s). Usually such extractions are carried out by ultrasonication[9, 42, 50-52]. The extracts are re-concentrated before chromatography and mass spectrometry. This chapter is dedicated to extraction and microextraction methods applied to either solid aerosol samples collected on filters or to the liquid extracts obtained by ultrasonication. Microextractions can often be a good choice when the goals of sample preparation are enrichment of analyte and reduction of use of organic solvents. On the other hand, extractions (using larger volumes) also have advantages, including but not limited to, *e.g.* use of larger volumes of extraction fluids with repeated extraction cycles or continuous flow of solvent can enhance the extraction of analyte[53, 54].

## 3.1 Dispersive liquid-liquid microextraction (DLLME)

Rezaee *et al*[55] presented dispersive liquid-liquid microextraction (DLLME) for the first time in 2006. In DLLME, a small volume of a water-immiscible extraction solvent (≈ 50-500 μL) is mixed with an organic dispersion solvent and the mixture is injected into an aqueous sample (≈ 1-100 mL) to make a cloudy dispersion of numerous tiny droplets of extraction solvent. It provides larger surface area as compared to traditional liquid-liquid extraction (LLE). After the extraction is completed, the dispersion is disrupted usually by another injection of dispersion solvent or by centrifugation. The separated layer of extraction solvent is collected and used for further analysis (Figure 4). DLLME provides remarkably higher extraction yields in a very short time than conventional methods, *e.g.* Ding *et al*[56] reported 1 min DLLME method as compared to 120 min long method used for the extraction of preservatives in food. Since the introduction of DLLME, various modes of the method have been applied to environmental, biological and food

samples for trace analysis of PAHs, pesticides, fungicides, pharmaceuticals, phthalate esters, metals and other similar classes of compounds [56-61].

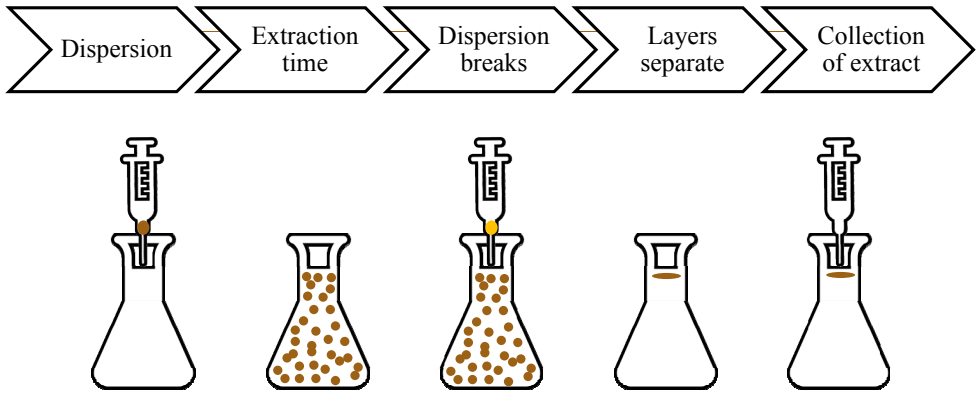



**Figure 4.** A simplified illustration representing different steps of dispersive liquid-liquid microextraction, brown liquid in syringe and later in the flask represents extraction solvent and dispersion made of extracting solvent, respectively. Yellow liquid in the syringe represents second injection of dispersion solvent that terminates the dispersion and extraction is completed, adapted and modified from Benqiong *et al*[62]

In early DLLME, the extraction methods were limited to the extraction of non-polar compounds due to the fact that an extraction solvent immiscible in aqueous solution supports the extraction of hydrophobic compounds only. Since then several modifications of DLLME were proposed including use of ion-pairing and chelating agents for the extraction of polar organic compounds and metals, respectively[63, 64]. It adds a new dimension to DLLME however adding more steps may cause loss of analyte in ion-pairing and chelating followed by their partition in extracting solvent, which may lead to decreased extraction efficiency and distribution kinetics that are beyond desirable in trace analysis. The study showed in Paper I presents an extension of DLLME for the extraction of MBTCA, a tri carboxylic acid. It was demonstrated that the chemistry of extraction solvent could be fine-tuned to extend the application window of DLLME to MBTCA (Paper I). The extraction solvent, 1-octanol, was modified by the addition of tri-n-octyl phosphineoxide (TOPO) for the extraction of MBTCA. Hydrophobic -octyl chains of TOPO enhance its solubility in 1-octanol whereas its phosphine group presents a tendency to attract polar moieties. Such additive-assisted extractions are suitable for the extraction and enrichment of MBTCA at trace levels. In some recent studies, Faraji *et al*[65] and On *et* al[59] reported extraction of halogenated compounds like halomethanes and haloacetonitriles using 1 decanol and dichloromethane as extraction solvetns, respectively. However, MBTCA being a highly polar compound (logP – 0.3) as compared to halogenated methanes and acetonitriles (logP ≈0.29-2.16) may require

a water-immiscible extraction solvent with highly polar characteristics. The DLLME method presented in Paper I followed by derivatization and GC–MS analysis provided a limit of detection of MBTCA of 0.12 pg/m$^3$ of air that was 41.6 and 10833 times lower than the limits of detection reported by Fu *et al*[66] and Zuth *et al*[67], respectively. Fu *et al*[66] performed aircraft based high-volume sampling followed by ultrasonic assisted extraction, derivatization and GCMS analysis. In this study the performed high-volume sampling using a flow rate of 78 L of air/min, which collects much larger mass of aerosol particles as compared to low-volume samples used in Paper I. Nevertheless, the lowest limit of detection of MBTCA – reported so far to best of our knowledge – was achieved.

DLLME is a green technique in term of reduced use of organic solvents that also cuts down the cost. However, it is important to investigate the compatibility of the extraction solvent and the additives with desired chromatographic and detection methods. Regarding the practical implications of DLLME, limited dilution of the sample can reduce matrix effects. It is recommended to use minimum 10 µL extraction solvent due to injection limitations in chromatography[68]. After the completion of the extraction, sometimes it can be tricky to collect the layer of extraction solvent especially while working with volumes less than 50 µL. Therefore, a custom-made glass container with narrow-neck or similar can be used to facilitate the collection of extraction solvent after the extractions.

## 3.2 Supercritical fluid extraction (SFE)

Supercritical fluids are known for over a hundred years. In analytical chemistry supercritical fluids got popularity in extractions and chromatography since the end of 20$^{th}$ century. A common way to describe a supercritical fluid is by phase diagram. As the phase equilibrium curve reaches to its end, the phase boundaries disappear at the critical point. Above the critical point, a substance presents physical properties *e.g.* liquid-like density, gas-like viscosity and diffusion coefficient intermediate to those of a liquid and a gas[69-71]. At this state the substance is called supercritical (Figure 5). In past, supercritical $CO_2$ has extensively been used for the extraction of a variety of analytes from biological, environmental and other samples[72-78]. The beauty of supercritical $CO_2$ lies in its gas-like viscosity, high diffusivity and tuneable liquid-like density above easily achievable and mild conditions *e.g.* critical temperature of 31.1 °C and critical pressure of 72.8 bars[79]. Tuneable density and a possibility to modify the polarity of supercritical $CO_2$ using co-solvents are the features that render it superior to many other extraction methods available today.

As discussed earlier, soot or so-called EC is an emission marker for oxygen-starved combustion. Isolation and estimation of EC has been a challenge for a long time.

Several organic and inorganic compounds are responsible for interfering in the estimation of EC by commonly used thermal optical methods. EC being huge and complex graphite-like structures, is insoluble in all known solvents[15, 19, 80]. Therefore, it is practically impossible to extract EC from a sample. An alternative can be chemical cleansing of small organic molecules from aerosol samples keeping EC intact for further analysis using thermal optical methods. Unfortunately, commonly used extraction methods are designed for the extraction of selected classes of analytes on the expense of destructive rupturing, heating, swelling and physical disintegration of a sample matrix. Contrary to the usual approach of extracting the analyte(s) of interest from a given matrix, supercritical $CO_2$ was used in Paper II for chemical cleansing of small organic molecules from aerosol samples.

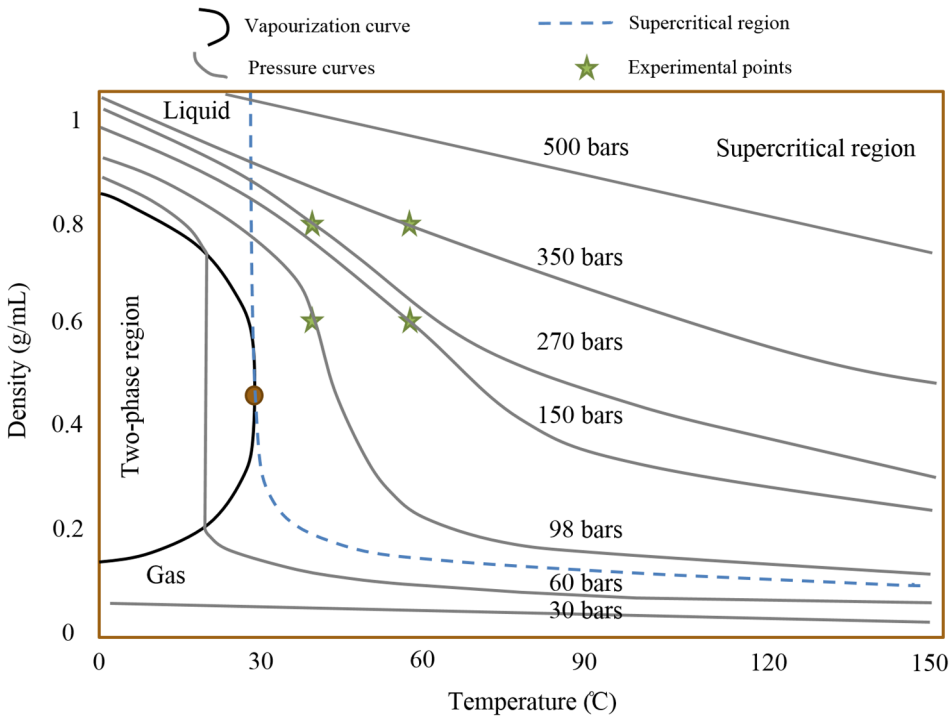



**Figure 5.** Temperature-density phase diagram of $CO_2$ representing critical point above which $CO_2$ presents supercritical properties, green stars represent screening experiments (neat $CO_2$) on two different densities of supercritical $CO_2$ (phase diagram adapted and modified from Bachu, 2003[81] with permission)

In Paper II, the extractions were designed to isolate EC on the quartz-fibre filters used for the collection of aerosol samples while having various classes of small organic molecules carried away with supercritical $CO_2$ modified with methanol. Figure 5 shows a phase diagram with experimental points (only neat $CO_2$) plotted against density and temperature of supercritical $CO_2$. Green stars represent

screening experiments performed on two different densities of supercritical $CO_2$, including 0.6 and 0.8 g/mL (the diagram was adapted and modified from Bachu 2003[81]). It was observed that the most significant variable was addition of methanol – as co-solvent to introduce polarity in the fluid – as compared to density and temperature of supercritical $CO_2$. Up to 60 % removal of OC using 10 % methanol in supercritical $CO_2$ indicates that at least 60% OC consisted of medium-polar organic compounds. Density of supercritical $CO_2$ affects its solvent properties, *e.g.* diffusivity. As no significant influence of density of supercritical $CO_2$ was observed, it may be inferred that due to very thin layer of particulate matter collected on filters, the density of 0.6 g/mL was sufficient for the extraction of OC.

It was also observed that the variables including direction of extraction cell (horizontal vs vertical), flow of supercritical $CO_2$ and presence/absence of glass bead, commonly used for homogenous mixing of sample and to increase surface area of sample, were the most influential factors in the isolation of EC. The optimised method was also compared with commonly used extractions carried out using water, ultrasonic assisted extractions using water in addition to extractions performed with dimethyl sulfoxide and dimethylformamide[82, 83]. The optimised method was more efficient as compared to existing methods, in terms of OC removal and EC recovery. As a future perspective of the work presented here, it can be interesting to perform $^{14}C$ analysis on the isolated EC from an aerosol sample to discriminate EC released from the combustion of modern and fossil origins as described in Chapter 1.

## 3.3 Derivatization using silylation reagents

In the studies presented in Paper IV and V, derivatization method from Genberg *et al*[9], using N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylsilyl chloride, was modified for levoglucosan by reducing the final volume to 25 µL. Reduction of volume is useful to avoid dilution and to achieve lower the limits of detection. Silylation reagents, *e.g.* N,O-bis(trimethylsilyl)trifluoroacetamide, react predominately with organic compounds containing –OH, –SH and –NH functional groups. Silylation of sugars, alcohols and carboxylic acids makes the compounds less polar, more volatile and thermally stable to be analysed by GC. A simplified reaction mechanism is given below where $R_3$ Si-X represents silylation reagent.

$$\text{Sample–OH} + R_3\,\text{Si–X} \rightarrow \text{Sample–O–Si–}R_3 + \text{HX}$$

Paper IV consists of a yearlong source apportionment study in southern Sweden. Levoglucosan was used as an emission marker for biomass burning and the data was used to evaluate aethalometer model for BC from wood burning. The aethalometer

is an instrument used for the estimation of carbonaceous aerosols by optical methods. A brief overview in terms of applications of emission markers, complexity of aerosol samples and challenges in quantitative analysis are described here. In this study a quartz membrane with 47 mm diameter was used to collect atmospheric aerosols using a flow of 2.3 $m^3$ of air/h for approximately 72 hours. The sample was divided into portions for various analyses. In a complex sample matrix, analysis of the compound of interest using very small sample sizes is challenging. The filter was divided for $^{14}C$ analysis, OCEC analysis and levoglucosan analysis. Figure 6 can be a good illustration of amount of sample available for the analysis of levoglucosan.


**Figure 6.** The picture shows an aerosol sample collected on 47 mm quartz-fibre filter. The darker circle on the filter represents the aerosol particle mass collected during sampling and the circular hole is a portion of filter punched out for analysis. The horizontal cylinder on top was the punh-cutter used to take sub-samples from the filter.

In a yearlong sampling (2014-2015), quantitative data of levoglucosan was found in a correlation of 0.71 with that of aethalometer derived wood burning data (Figure 7). The plot represents a comparison of source apportionment methods based on chemical composition of samples and amount of emission marker, levoglucosan, in the sample with that of BC from wood burning optically estimated by aethalometer. It is obvious that the two methods of source apportionment consider different properties of aerosol particles, *i.e.* chemical and optical, respectively. Hence, the correlation and similarity in trends obtained by the two methods validate the authenticity of source apportionment studies.

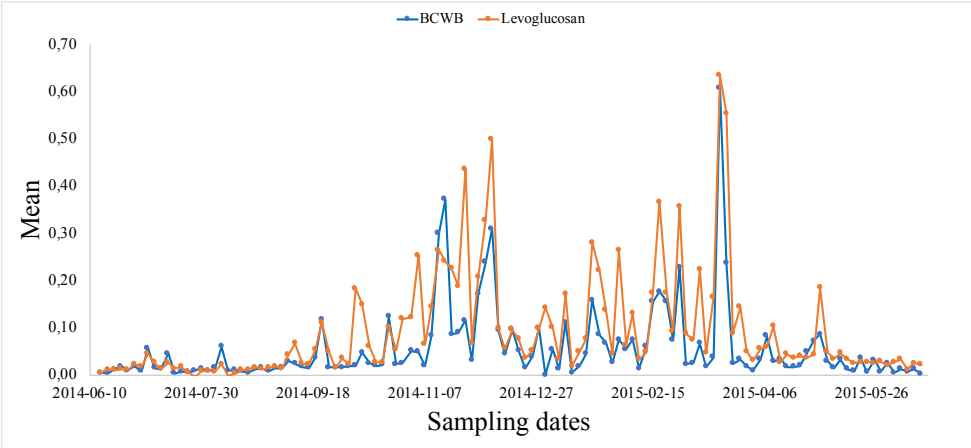



**Figure 7.** Red curve represents concentration of levoglucosan in the air analysed by GC-MS; blue curve represents aethalometer derived BC from wood burning shown as BCWB. The correlation of r^2 = 0.71 and the ratio between them (0.99) are in agreement with literature in terms of unit of levoglucosan per unit of EC (also known as BC) from wood stoves.

Paper V presents a study demonstrating early detection of smouldering fire in biomass with the help of emission markers in aerosol signature of the fire. Smouldering fire is flameless, low temperature fire that propagates in fibrous combustible materials[84], *e.g.* expanded polyurethane and biomass, for days and weeks unnoticed. Unfortunately, usual fire signatures including heat, radiations, smoke and gases are not suitable for the detection of smouldering fire and when the fire erupts in flames it can cause devastating consequences. In this study aerosol sampling of laboratory simulated smouldering fire in cotton stack was performed. Offline derivatization followed by GC–MS analysis proved that the early detection of smouldering fire could be possible by the analysis of levoglucosan in the aerosol emissions. Figure 8 represents a mass loss curve of the cotton stack used for laboratory controlled smouldering fire. It can be observed that levoglucosan, emission marker, can be detected in aerosol emissions before notable mass loss and even several hours before the cotton stack goes into flames.

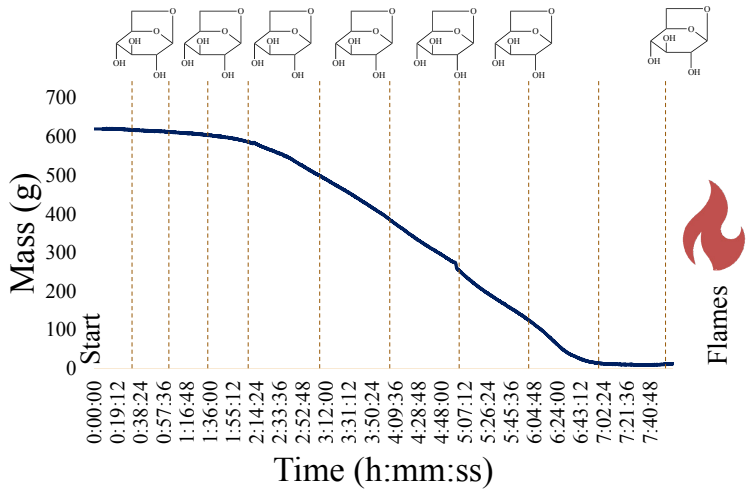



**Figure 8.** A mass loss curve during the whole experiment (8 hours), starting point to flaming point is shown. Vertical grids represent sampling points throughout the experimental timeline. Levoglucosan molecules represents that from 30 min onwards it was possible to detect smouldering fire till the cotton stack went into flames. Levoglucosan could not be detected in the sample collected after 7 hours, due to high baseline.

The experiment was monitored with 80 thermocouples placed all over the cotton stack. A simplified diagram of temperature curves and visual signal of smouldering fire represents > 300 °C temperature in various parts of the cotton stack that confirms thermal degradation of cellulose necessary for the evolution of levoglucosan (Figure 9). Interestingly, the visual signal of fire does not appear until after 3 hours.

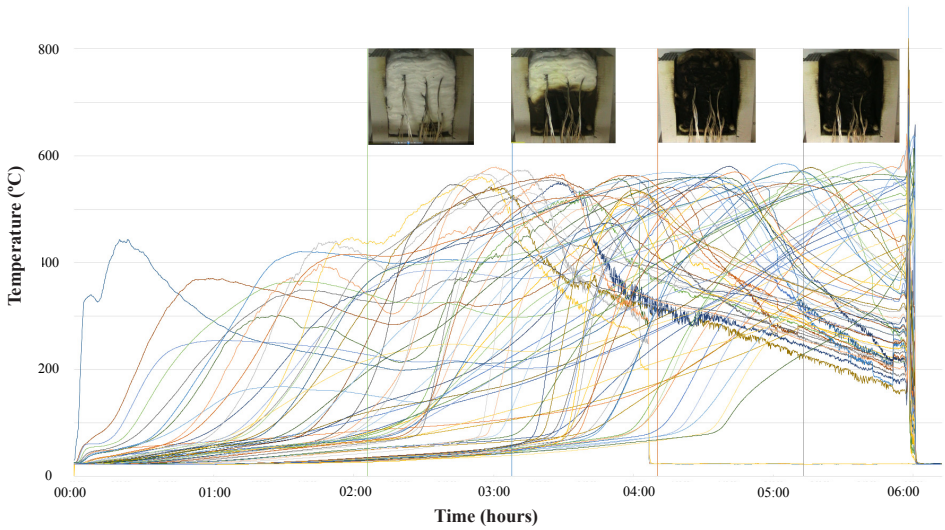



**Figure 9.** Readings of thermocouples as the smouldering wave develops during the experiment (8 hours). On top of the temperature curves different stages of smouldering in experimental timeline have been shown. The extreme left picture of cotton stack (around three hours) shows little visual identification of smouldering. Interestingly at that stage identification of levoglucosan confirmed smouldering in cotton. The last picture of cotton stack around six hours shows that the whole cotton stack has blackened as a result of smouldering. After eight hours the blackened cotton goes into flames.

The study demonstrates how emission markers can help in early detection of smouldering fire. The study is presented as a proof-of-concept though and aerosol sampling followed by offline derivatization and GC–MS analysis is a time demanding procedure and practically impossible to use in real biomass storage with risks of fire. *In-situ* derivatization using TD–GC–MS can be considered as a speedy solution. Future perspective of such studies can be summarized as

1. quantitative analysis of levoglucosan sampled at smaller intervals that can be drawn as levoglucosan curve (concentration per cubic meters of air sampled) against mass loss as shown in Figure 8 to provide further information on emission of levoglucosan on various stages of smouldering fire
2. experiments using different sources of biomass *e.g.* cardboard and lignin products, agricultural waste used for energy production and other biomass based combustible materials
3. study of lignin-based emission markers *e.g.* methoxyphenols
4. development of online methods *e.g.* sensors and electronic noses, aerosol mass spectrometer and TD–GC–MS

Papers IV and V show offline derivatization using reduced volumes to avoid sample dilution. Furthermore, the application of emission markers to address practical

problems has been demonstrated. Some preliminary experiments were performed using a TD–GC–QqQ. Sheesley *et al*[85] and Grandesso *et al*[86] presented *in-situ* derivatization of organic compounds in TD using commercially available silylation reagents. Although no comparative study was performed between offline derivatization followed by GC–MS analysis and in-situ derivatization using TD–GC–QqQ, however, it is expected that *in-situ* derivatization using TD–GC–QqQ can provide lower limits of detection by avoiding multiple steps of extractions, evaporation like offline derivatization of the sample as reported by Grandesso *et al*[86] (here comparing only sample preparation methods).

## 3.4 Hollow-fibre liquid-phase microextraction (HFLPME)

HFLPME is a membrane extraction technique in which polypropylene hollow-fibre membranes of different thickness and internal diameters are used. The pores in the fibre walls are filled with an organic solvent/solution, a supported liquid membrane, and the lumen of the fibre is filled with an acceptor solution, designed by pH adjustment to trap certain analyte(s), selectively. The fibre is sealed from both ends and dipped in a pH adjusted aqueous sample, the donor solution. Continuous stirring of sample enables analyte molecules to come in contact with the fibre walls, pass through the organic phase present in the pores of the fibre and reach the acceptor solution. Often, the donor solution containing acidic or basic analytes is adjusted for pH to neutralize the analyte whereas the acceptor pH is designed to induce ionization of acidic or basic analyte molecules. The organic phase facilitates the transportation of analyte molecules from the donor solution to the acceptor solution. Hence a charged analyte is trapped into the lumen of hollow-fibre membrane (Figure 10). A constant gradient of analyte towards acceptor solution provides high enrichment and significant sample cleanup[9, 35, 43, 87]. The micoextractions designed in this way are known as three-phase hollow-fibre liquid-phase microextractions.

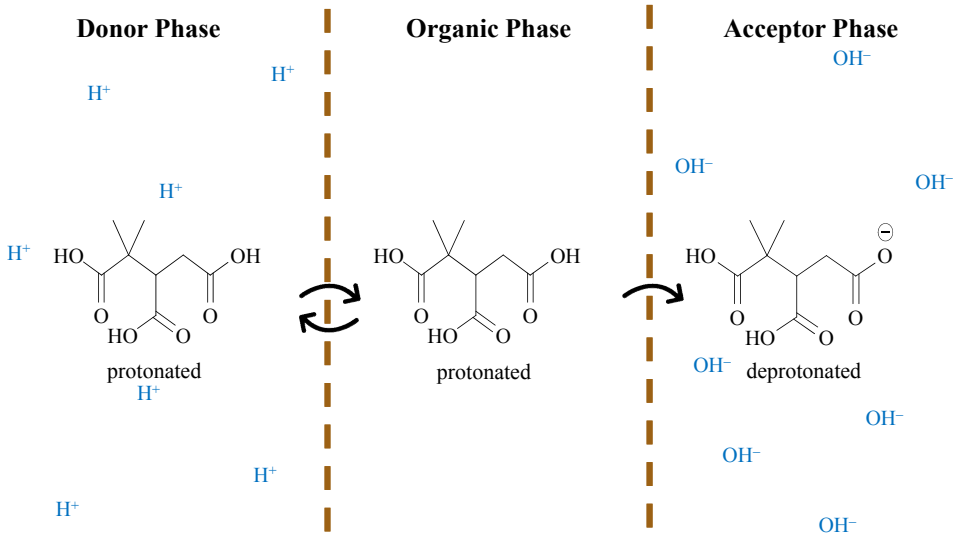



**Figure 10.** Transfer of MBTCA from donor solution (protonated form) to acceptor phase (deprotonated form) through a third organic phase impregnated in the pores of fibre membrane, adapted and modified from Ekman-Hyberg, P. [88]

HFLPME has been extensively used for the analysis of complex environmental samples[35, 43]. Over the last two decades several modifications were proposed to address analyte and/or sample related challenges. In this study, HFLPME was optimised for the extraction and enrichment of MBTCA with a modified organic phase in the pores of fibre membrane. Experiments were performed to find the most suitable type of hollow-fibre based on membrane thickness and internal diameter, length of fibre, type of organic phase in the pores of the fibre and suitable additive in the organic phase. Studies show that trioctylamine improves extraction efficiency when added to organic solvent for the preparation of supported liquid membrane for the extraction of citric acid on industrial scale. Citric acid-trioctylamine complex is formed in supported liquid membrane that requires presence of sodium ions in the acceptor phase to break the complex giving rise to high enrichment of analyte in acceptor phase[89]. Due to the structural similarity between citric acid and MBTCA, trioctylamine in addition to two more additives including TOPO and trioctylmethylammonium chloride (aliquat 336) was tested for hollow-fibre membranes. Acceptor phases of 0.1, 0.5, 1 and 2 molar $(NH_4)_2CO_3$ solutions adjusted to pH 9.5 by NaOH were tested. Donor solutions of milliQ water with pH adjusted to 1 and 3.5 by $H_2SO_4$ were tested. Two types of hollow-fibres were tested *i.e.* thick fibre (600 µm inner diameter, 200 µm wall thickness and 0.2 µm pore size) and thin fibre (280 µm inner diameter, 50 µm wall thickness and 0.1 µm pore size). Five different organic solvents were tested including, methyldecanoate,

dihexylether, octanol, 6-undecanone and n-undecane. Figure 11 shows screening of qualitative variables and suitable ranges of quantitative variables.

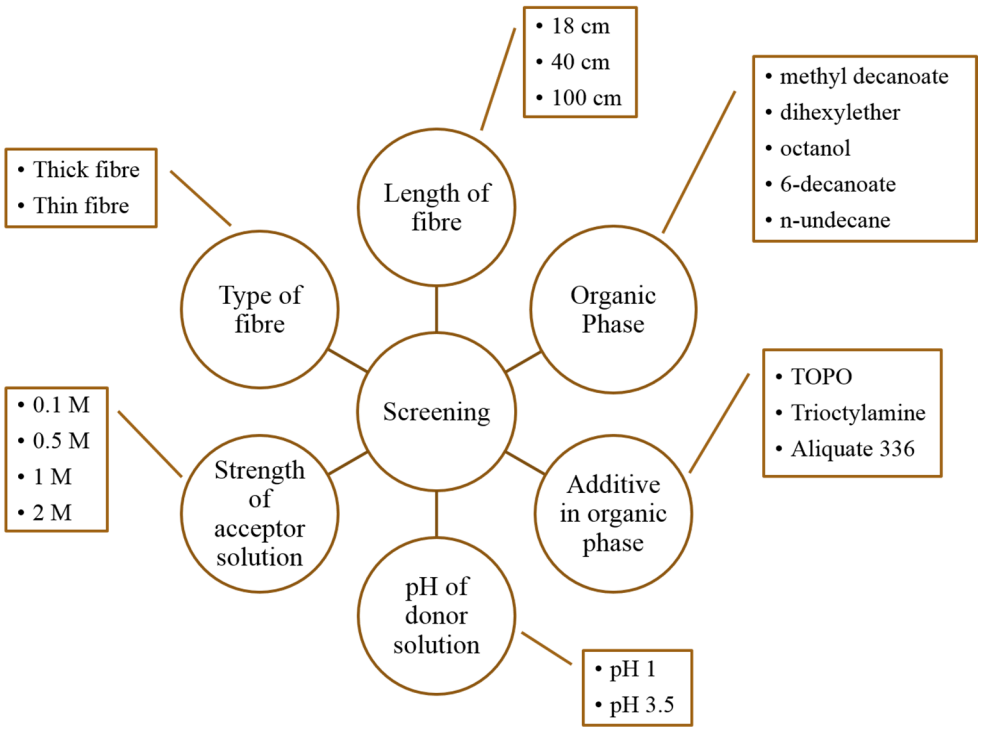



**Figure 11.** Screening experiments performed to select suitable qualitative variables *e.g.* type of fibre, type of organic phase and type of additive in organic phase. Quantitative variables *e.g.* length of the fibre, pH of donor solution and strength of acceptor solution were also screened for suitable working ranges.

The most suitable conditions of quantitative and qualitative variables screened were 18 cm long thin fibre impregnated with methyl decanoate containing TOPO as additive. Donor solution with pH 1 turned out more effective, which can be explained by pKa values of MBTCA, the lowest being 3.5[88]. MBTCA exists in deprotonated form at pH 1 that supports its interactions with TOPO and mass transfer. No significant variation was observed using different molar concentrations of donor solution. Therefore, it was inferred that 0.1 M $(NH_4)_2CO_3$ solution adjusted to pH 9.5 by NaOH was rich enough in sodium ions to break MBTCA-TOPO complex in the liquid membrane and retain deprotonated MBTCA. The most suitable fibre type, fibre length, organic phase, additive in organic phase and type of donor phase obtained from screening experiments were further optimized for stirring rate, extraction time and concentration of TOPO in organic phase using face-centred central composite design.

Multivariate optimization (CCF) of three quantitative variables i.e. stirring rate, extraction time and concentration of TOPO in organic phase showed that the most suitable conditions were stirring rate of 1000 rpm for 30 minutes using 5% TOPO as additive in methyl decanoate. Figure 12 shows contour plots describing interactions between the variables and optimum extraction conditions. Here, it is imperative to understand that the optimum conditions that end up on lower or upper limits of a chosen working range of a variable do not represent the best achievable conditions, statistically. It rather represents the flaws in experimental design with variable ranges selected inappropriately. However often it is limited to practical limitations. For example, in Figure 12 red curves represent highest extraction efficiency in terms of concentration of MBTCA. It can be seen that the stirring rate of 1000 rpm is the most suitable condition, which represents upper limit of the variable. It is possible to argue statistically on the validity of the model; however, the results would still be valid due to the fact that 1000 rpm was the maximum operating capacity of available magnetic stirrer in the laboratory. Similarly, optimum conditions for time and concentration of TOPO fall around lower limits of the variables. Both variables were tested for suitable working ranges before using multivariate design of experiment approach and it was found that the values below 30 min extraction time and 5% TOPO were not suitable. Hence, the most suitable experimental conditions included stirring rate of 1000 rpm with an extraction time of 30 min using 5% TOPO as additive in methyl decanoate, the organic phase.

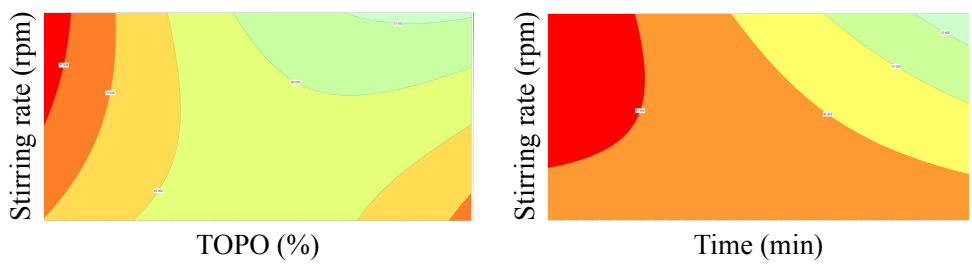



**Figure 12.** Contour plots representing interactions between stirring rate, amount of TOPO and time. Colour gradient from light green to red represents an increase in extraction efficiency (red being the highest) in terms of peak area of MBTCA obtained from extractions.

The extraction time obtained through multivariate design of experiment was shorter than a number of HFLPME methods optimized by one-factor-at-a-time approach[35, 43]. A detailed description on the ability of multivariate design of experiments to provide superior information as compared to one-factor-at-a-time approach of experiments is provided in chapter 5. Repeatability of HFLPME and ultra-high performance liquid chromatography coupled to electrospray ionization tandem quadrupole time-of-flight mass spectrometry (UHPLC–ESI–QToF) method was

found to be within 5.47 % RSD (n=6) using 10 mL donor solution spiked to 10 µg/mL with MBTCA.

It was observed that the optimised method showed poor or nil extraction efficiency for aerosol samples and donor solutions of lower concentrations. After repeated HFLPME, LLE were performed to investigate MBTCA mass transfer between donor-organic-acceptor phases. LLE were performed using spiked donor-organic phases and organic-spiked acceptor phases. The LLE experiments were performed using organic phases as methyldecanoate modified with 5% TOPO and methyldecanoate modified with 5% aliquate 336. The experiments were performed by shaking the two phases in a vortex shaker for 30 minutes. The samples were left resting for 15 minutes after extractions and subsamples from both top (organic phase) and bottom (donor/acceptor phase) were analysed by UHPLC–ESI–QToF. It was found that organic phased modified with TOPO had high affinity for MBTCA as more than 5 times higher amounts of MBTCA were detected in methyldecanoate modified with 5% TOPO as compared to donor solution. Based on the results of LLE, following deductions were made:

1. addition of TOPO in organic phase enhances the extraction of MBTCA in organic phase
2. high affinity of organic phase modified with 5% TOPO may pose resistance in the transfer of MBTCA from SLM to acceptor phase
3. on higher concentrations of MBTCA, the organic phase in the porous walls of hollow-fibre membranes gets saturated and a steady transfer of MBTCA to acceptor phase takes place

In conclusion, it can be stated that HFLPME is not suitable for the extraction of trace amounts of MBTCA. The technique is very well established and has been proven suitable for other organic acids in aerosols, for example, pinic acid and cis-pinonic acid[43].

# 4 Supercritical Fluid Chromatography and mass spectrometry

"*All of science is nothing more than the refinement of everyday thinking" – Albert Einstein*[90]

In the whole chain of analysis, starting from the sampling to the detection of the analyte, chromatographic separation is usually one of the most important parts of the job. Chromatography techniques including GC, liquid chromatography (LC) and/or supercritical fluid chromatography (SFC), can be seen in almost any analytical chemistry laboratory today. In the development of the work presented here, GC, LC and SFC were exploited based on the nature of the scientific problems. Although the journey went through interesting method development and educational experience of anatomical study of the instruments through routine maintenance as well as painstaking troubleshooting, the description of chromatographic analysis in this thesis is limited to SFC only. GC and LC are very well established and the most widely used techniques today for the analysis of organic compounds in atmospheric aerosols[50, 85, 91-96]. Use of LC and GC is presented in Paper I, IV and V. SFC being a relatively newer technique in terms of the availability of commercial instruments and method development has been focused in this text. Furthermore, an interesting discovery of metal complexes of MBTCA has also been discussed briefly under section 4.3. "Metal complexes MBTCA".

## 4.1 Supercritical fluid chromatography (SFC)

SFC has gotten popularity for rapid mass transfer due to high diffusivity leading to shorter run times, ability to separate non-volatile compounds without any prior derivatization like in GC and its greenness as compared to LC with extensive use of organic solvents as mobile phase[72, 97-99]. Invention of packed columns in SFC, use of co-solvents and additives in mobile phase, and back pressure regulator instead of capillary restrictor were breakthrough in the separation of organic compounds that changed the perception of capillary SFC being merely an extension of gas

chromatography[100]. Although the choice of chromatographic technique, *i.e.* GC, LC or SFC, can be exclusively determined by nature of the sample, nature of the analyte(s) and the goals of chromatography in question. However, a generalized comparison between GC and LC can be summarized as:

1. GC provides fast separations, higher plate number, narrower peaks and relatively high resolution
2. LC offers higher selectivity as both stationary and mobile phases interact with analyte molecules and it is compatible for non-volatile and thermally labile compounds

Packed column SFC can be consider a good alternative of GC and LC, for many applications, if not all, due to the fact that supercritical $CO_2$ possesses high diffusivity and tuneable density (as described earlier in Section 3.4). SFC provides faster separations and narrow peaks then most of LC methods. The mild critical temperature and pressure and possibility of modifying the elution strength of supercritical $CO_2$ with co-solvents makes SFC suitable for thermally labile compounds and compounds from a diverse range of function groups[101-104]. With modern SFC instruments, there are dramatically increasing number of studies on food, biological and other samples. Unfortunately, little efforts were made to explore the scope of SFC separations for organic compounds in atmospheric aerosols.

One of the biggest challenges in the study of emission markers is small sample size especially when dealing with low volume sampling of atmospheric aerosols. Often the sample size and the amounts of emission markers are too small to measure. Here, it is important to understand that chemical nature of emission markers vary to a large extent ranging from non-polar PAHs to polar organic acids and a large variety in between. Naturally, a comprehensive study of several sources of emissions would require more than one chromatographic analysis using both gas and liquid chromatography. Small sample size and trace amounts of emission markers have been a bottleneck in comprehensive chemical source apportionment of atmospheric aerosols. Paper III demonstrates the study on SFC method development for the analysis of various classes of emission markers in a single run. The classes of emission markers studied in this work include representative compounds of PAHs, methoxyphenols (phenolic acids, aldehydes and ketones), sugars and carboxylic acids with logP values ranging from –3.7 to 7 (Table 1, Chapter 1). Some recent studies reported the comparison of SFC separations for organic compounds with that of contemporary techniques[100, 105, 106]. SFC can cover a wider range of classes of organic compounds, based on polarity, as compared to contemporary techniques *e.g.* GC and LC (Figure 13). Packed column SFC allows addition of high ratios of co-solvents to pressurized $CO_2$ that can modify the elution strength of mobile phase and broadens the application to polar compounds[107]. This strength of SFC was

investigated in the present study for quantitative analysis of emission markers. The compounds of interest were selected based on the unique information they provide about their sources of emission; however, they are grouped according to their chemical structures and polarities, *e.g.* arabitol represents fungal spores, sucrose and fructose for mixed biogenic emissions (predominantly pollen) and levoglucosan for emissions from wood burning but they are grouped together as sugars.

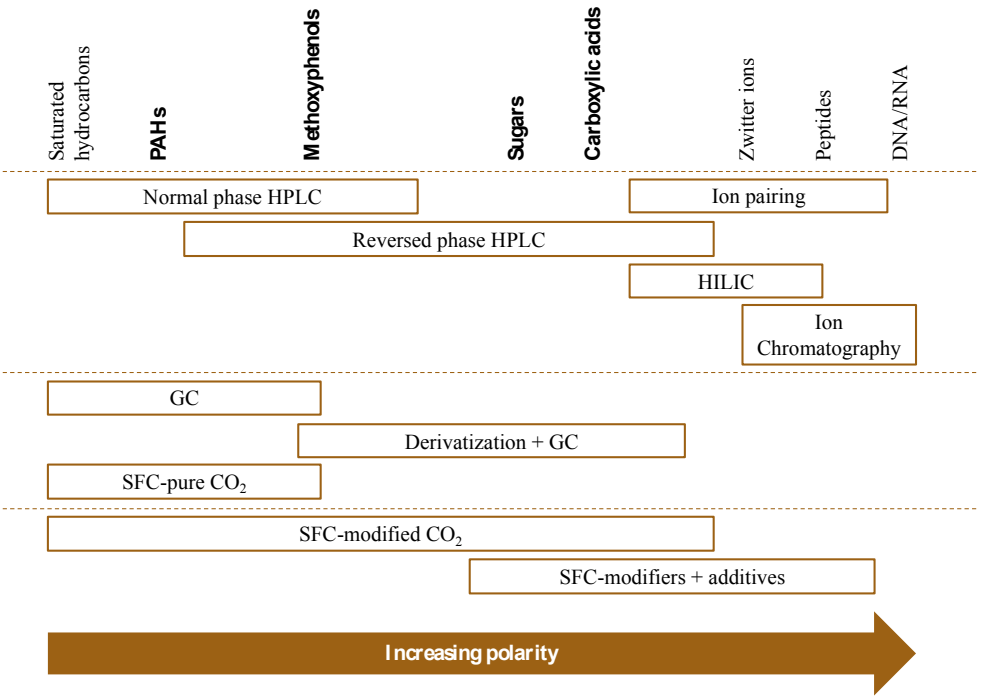



**Figure 13.** A comparison of various chromatography techniques and their areas of application in terms of polarity of analyte. The classes of organic compounds focused in the presented work are highlighted in bold. Adapted and modified from Vlckova, H. K.[105] and Berger, T.A.[106].

As the goal of the study was separation and estimation of several emission markers with a wide range of physical and chemical properties in a single run, emphasis was given to optimization of chromatography and detection methods. In such challenging separations a desirable chromatographic method should provide increased selectivity and reduced ion suppression/enhancement that can be achieved by:

1. chromatographic efficiency in terms of narrow peaks and rapid separation
2. method selectivity by optimizing stationary and mobile phases

As discussed earlier, high diffusivity of supercritical $CO_2$ provides faster separations and narrow peaks as compared to LC. However, a fully optimized method requires careful fine-tuning of temperature and pressure that control the density of the fluid and may have significant influence on retention of analytes as well as separation factors[99]. The wide range of available packed columns used for SFC offers a range of selectivity, *e.g.*, C18, 1-aminoanthracene (1-AA), 2-picolylamine (2-PIC), flourophenyl, diethylamine (DEA), diol and ethylene bridged hybride (BEH). Mobile phase consisting of $CO_2$ modified with methanol as co-solvent and 2-PIC column as stationary phase were used in the study. Challenges like poor retention of PAHs need further investigations and may be addressed by following strategies:

1. use of inert stationary phase with extended column length to separate PAHs using neat $CO_2$ followed by gradual increase in methanol contents for the elution of other compounds
2. use of tandem columns with orthogonal selectivity for separation of all compounds of interest

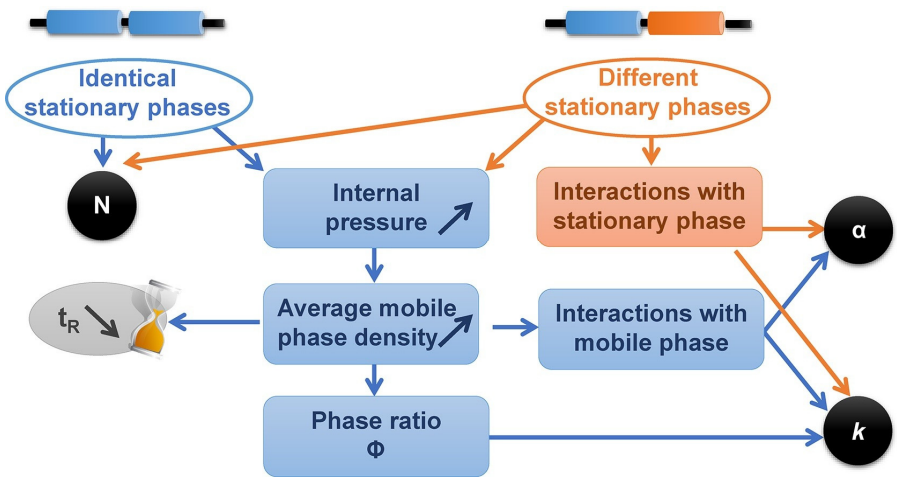



**Figure 14.** An illustration of theoratical consequences of tandem columns with identical as well as different stationary phases, reprinted from West *et al*[108] with permission.

In SFC, low mobile phase viscosity as compared to LC supports the use of columns with extended lengths. However, with an increase in column length, internal pressure and mobile phase density increase that affect solvent strength and phase ratio[109]. Therefore, it is highly uncertain to predict the outcome of such experiments. Similarly, use of extended column lengths, as proposed earlier, needs to be investigated. Another option is use of tandem column with different selectivity as reported by West *et al*[108]. They presented use of tandem columns for the study of

impurity profile of drugs. In this context, further experiments are required to achieve a good resolution of the candidate emission markers.

## 4.2 Mass spectrometry

Selection of ionization source and m/z analyzer is based on type of analytes in question, goal of detection and available resources. Zhou *et al*[110] and Cox *et al*[111] presented a comparison of three most commonly used ionization sources, i.e. electrospray ionization, (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI). ESI is one of the most commonly used ionization sources that has ability to ionize polar and semi-polar compounds[111]. On the other hand, APCI is used to ionize compounds with relatively lower molecular masses as compared to ESI and semi-polar moieties. Non-polar to medium polar compounds can be best ionized by APPI[110]. Based on these studies the best choice of ionization source would be APPI however due to limited resources APCI and ESI were tested for the selected emission markers in both positive and negative modes. Comparison of APCI and ESI provided following information:

1. APCI was found to be suitable for the ionization of non-polar compounds *e.g.* pyrene, picene, flouranthene and anthracene in negative mode, however, the ionization of polar compounds, *e.g.* levoglucosan, arabitol, MBTCA and all carboxylic acids (Table 1) could not be performed in both positive and negative modes
2. ESI turned out to be suitable for the ionization of all carboxylic acids, sugars, methoxyphenols, aldehydes and ketones in negative mode, however, ESI did not work for PAHs as expected

Based on the results, it was decided to use Diode Array Detection (DAD) for the detection of PAHs and ESI for rest of the compounds

Often the choice of m/z analyser is motivated by the goal of analysis and/or available resources. The available mass spectrometers included triple quadrupole mass spectrometer (QqQ) and QToF. Often QqQ is the technique of choice when the goal is to obtain low limits of detection in targeted analysis. Pozo *et al*[112] reported a study on the comparison of QqQ and QToF hyphenated with LC for the detection of anabolic steroids in doping control analysis. Their findings showed that QqQ was more sensitive than QToF in terms of limits of detection for targeted compounds. Multiple reaction monitoring (MRM) in QqQ also reduces interferences hence providing higher selectivity[113]. QToF on the other hand is suitable for non-targeted analysis for its accurate mass capabilities and high sensitivity in scan mode as described by Pozo e*t al*[114].

SFC–DAD–ESI–QqQ method was optimized for the emission markers of interest, as presented in Paper III. The step-by-step scheme of method optimization is shown in Figure 15. Use of DAD for the identification of PAHs is of course a compromise and limits of detection obtained from DAD may not be low enough as compared to ESI–QqQ. It can be interesting to study ionization of different classes of emission markers using APPI.

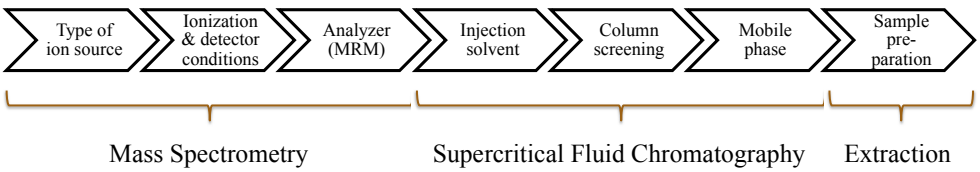



**Figure 15.** Schematic description of optimization of SFC–DAD–ESI–QqQ method. The second step represented as ionization and detector conditions includes optimization of ESI and electron multiplier as well as DAD settings

## 4.3 Metal complexes of MBTCA

MBTCA is a tricarboxylic acid and secondary organic aerosol originating from monoterpenes. Since the discovery of MBTCA as emission marker by Szmigielski at el[20] in 2007, a number of studies have been dedicated to identification, quantitative analysis and to uncover physical and chemical properties of the compound[66, 115]. As discussed in chapter 3, extensive studies were carried out for trace analysis of MBTCA. During these studies interesting complex formation behaviour of the compound was discovered in ESI–QToF. Kostenidou *et al*[116] reported physical and chemical properties of MBTCA. Elm *et al*[117] described cluster formation behaviour of MBTCA, however, the discovery of its complex formation behaviour was reported for the first time. A comprehensive description of MBTCA-iron(III) complexes is given in Paper I. During the course of study MBTCA and its complexes were observed in both positive and negative ESI modes. Experiments were performed with various combinations of capillary and cone voltages in both positive and negative ESI–QToF. The goal was to test if formation of MBTCA-iron(III) complexes can be minimized by a suitable ESI method. If this assumption would be true, changing capillary and cone voltages should have suppressed/enhanced the formation of complexes. As shown in Figure 16, none of the combination of capillary and cone voltage suppressed formation of the complexes, nor the peak representing MBTCA was completely consumed in complex formation. As MBTCA exists in trace amounts in the atmosphere it is

recommended that the quantitative analysis should be performed by taking MBTCA and its iron(III) complexes in account.

It is not fully understood yet if such metal complexes also exist in the atmosphere. The hypothesis needs to be investigated and if such interactions are observed in the atmosphere, it can open new research questions towards atmospheric organo-metallic chemistry.

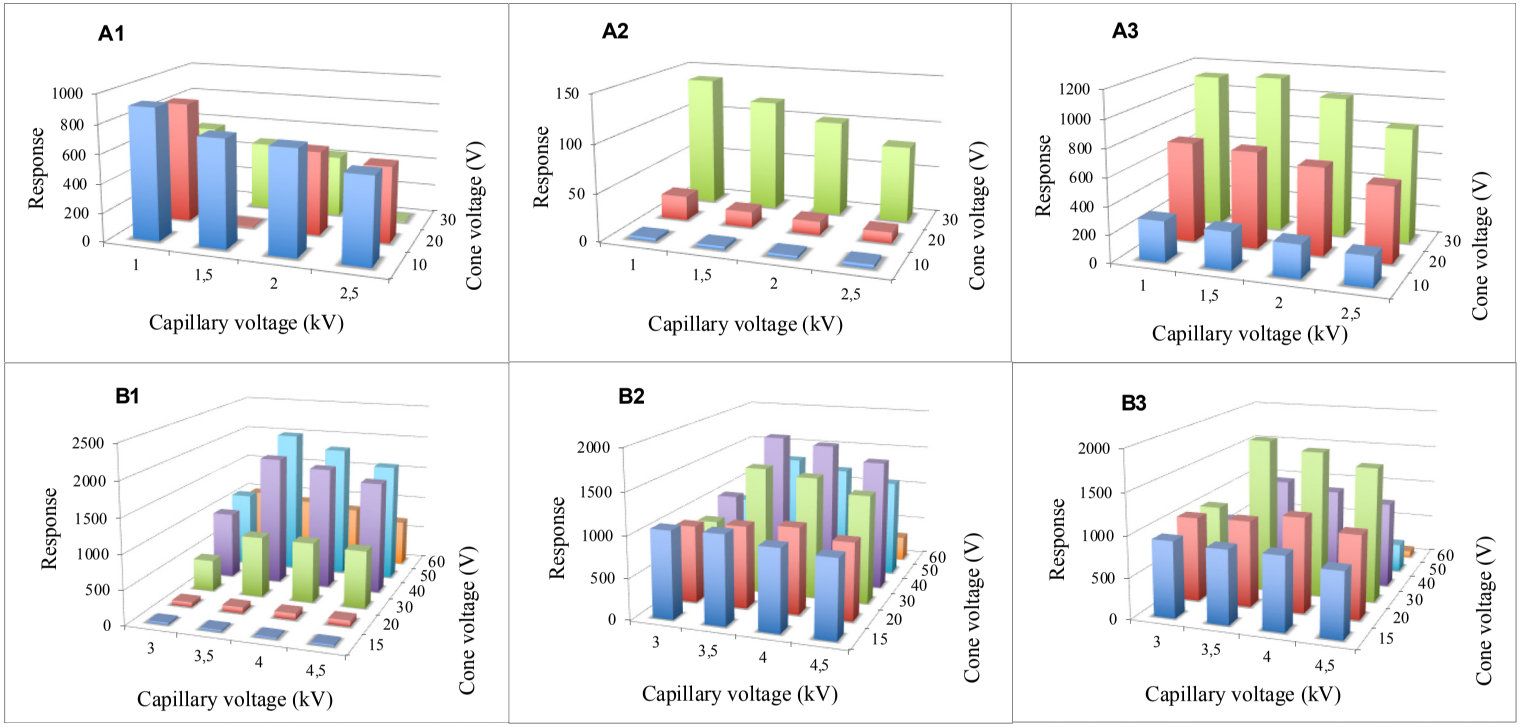



**Figure 16.** A description of response obtained from MBTCA and its complexes in ESI–MS (20 ppm MBTCA dissolved in milliQ water was analysed by direct infusion ESI–MS), top row represents experiments performed in negative mode whereas bottom row shows experiments performed in positive mode. Response of MBTCA, $[2MBTCA–4H–H_2O+Fe]^-$ and $[2MBTCA–4H+Fe]^-$ on different combinations of capillary and cone voltages is shown in figure A1, A2 and A3, respectively. Response of MBTCA, $[2MBTCA–2H+Fe]^+$ and $[3MBTCA–2H+Fe]^+$ against various combinations of capillary and cone voltages in given as B1, B2 and B3, respectively.

# 5 Design of experiments

*"To consult the statistician, after an experiment is finished, is often merely to ask him to conduct a post mortem examination. He can perhaps say what the experiment died of" – Ronald A. Fisher*[118, 119]

Human beings have a natural tendency to think and assimilate several variables affecting a desired outcome. For example, in a routine shopping episode, it is rational to think about price, style, durability and usefulness of a product before buying it. Similarly, a driver is expected to take safety measures by judging the speed of an object, visibility, road conditions and several other factors, all at the same time. Such ability to understand the effects of several variables influencing an outcome constitutes human judgment and prediction, which together with overall intellect of human beings, are the root of today's technological development. Unfortunately, this instinctive thinking ability was ignored in science for a long time. The first book on design of experiments was written by Fisher in 1935[120] and the subject started to get attention of scientists in certain fields over the past eighty years. Despite of this, one-factor-at-a-time approach has been the common choice of many chemists for the optimization of experimental conditions. It is therefore necessary to explain how the designed experiments provide superior scientific information as compared to one-factor-at-a-time approach.

## 5.1 Multivariate analysis

Multivariate analysis is one of the statistically established tools that can be used in chemistry to analyse the effects of more than one variables on one or more experimental outcomes such as extraction efficiency, synthetic yield and product purity etc[121]. A chemometrician cannot use multivariate statistical tools efficiently without a good understanding of the chemical process, factors affecting it and the experimental outcome. Therefore, a multivariate method, *e.g.* design of experiments, should not be seen only as a shortcut to a desired outcome, it should rather be considered as a tool to better understand all the variables and their interactions in the selected ranges of the operation. Let's take the example of coffee

brewing. Coffee brewing can be simplified to three variables, *i.e.* coffee to water ratio, temperature of extraction and brewing cycle time. In this case the desired outcomes would be enhanced flavour and aroma of coffee. Following the available literature on coffee brewing, ranges of temperature, brewing cycle time (for French press coffee) and coffee to water ratio have been selected as 70-100 °C, 1-5 min and 5/160-15/200 g coffee/mL water. The flavour and aroma are rated on a sensory scale of 1-5 (Figure 17).

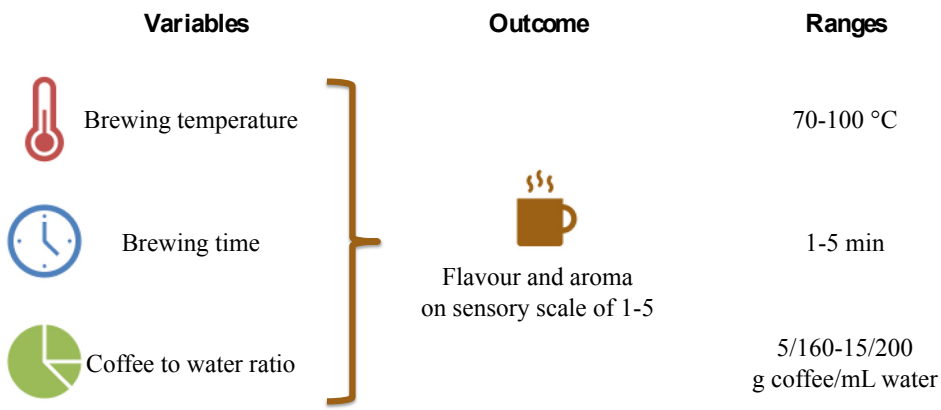



**Figure 17.** Variables affecting coffee flavour *i.e.* brewing temperature, brewing cycle time for French press coffee and coffee to water ratio with experimental ranges to be tested for best flavour. The desired outcome is to score the flavour and aroma on a sensory scale of 5 (1-5 ascending with improved quality).

We know from the available knowledge that brewing temperatures above 98 °C can burn the coffee and most commonly used temperatures are between 90-96 °C. Similarly, for a French press coffee the most suitable time is between 2-4 min and suggested coffee to water ratio is around 10 g coffee/180 mL of water. If the brewing experiments are performed according to one-factor-at-a-time approach, it is assumed that all the variables affecting coffee flavour are independent of each other and give a linear response, which is not true as brewing temperature and time are dependent variables. Similarly, coffee to water ratio may have interactions with other variables, *e.g.* brewing time and temperature, and for a cup of coffee rated 5 on sensory scales interactions of all variables may be important factors to consider. Real life chemistry may include many interactions and hence be much more complex then brewing a cup of coffee. A hypothetical plot between two variables, A and B, shows a non-linear response in the form of red curves (Figure 18). If the variables are optimized by one-factor-at-a-time approach and the best value for variable A appears to be “x” then the response to variable B is constant over all levels, therefore, it can be deduced that variable B has no influence on the experimental outcome, which would be false. On the other hand, if variable B is optimized first and the best value turns out to be “y” then the response of variable

A will be constant over all levels. This may mislead and mask important scientific information.

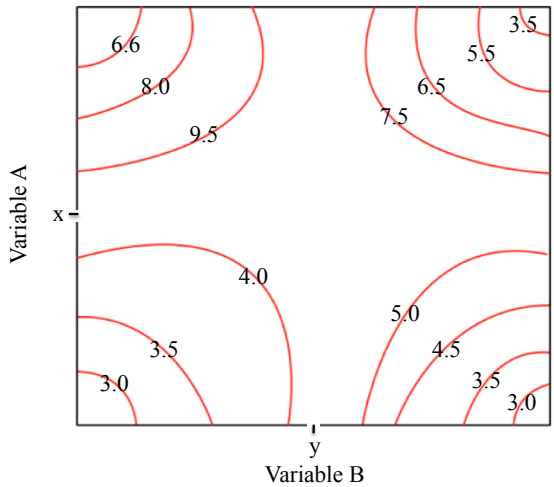



**Figure 18.** A hypothetical interaction plot between variables A and B, red curves with numbers show changes in response with different values of A and B.

This chapter will throw light on some of the multivariate designs used in the scientific work presented in this thesis.

### 5.1.1 Full factorial design

Full factorial designs are the simplest and can be considered as the base of many complex experimental designs. Generally, they are constructed to investigate variations in experiments at two levels of the variables under study *i.e.* low and high. Consider the example of coffee brewing, all variables are framed together in a multidimemsional space and the experimental points on the corners describe experimental response on low and high values of brewing temperature, brewing time and coffee to water ratio as shown in Figure 19. For an interaction model, at least one experiment is performed at the centre point of the cube, whereas triplicate experiments at the centre point provide information on experimental variations as well. The experimental response is recorded as a singular observation, *e.g.* flavour of the coffee. As the number of variables increases, the complexity of the design increases. Since the design is built for the variables studied at two levels, total number of experiments can be calculated as $2^k$, where k represents number of variables under study.

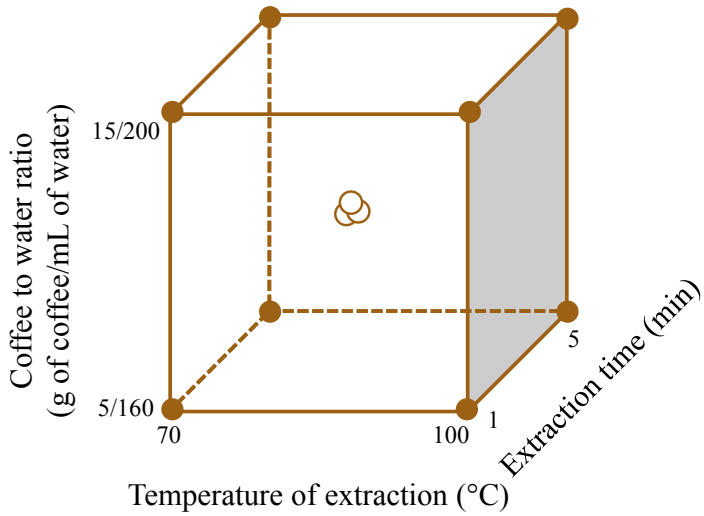



**Figure 19.** Full factorial design constructed for coffee brewing experiments with three variables and two levels each. Triplicate experiments are designed at the centre of the cube to investigate interactions between variables as well as experimental variabilty.

Now consider the coffee brewing example using one-factor-at-a-time approach, there are few hundred experiments needed to optimize brewing conditions (depending upon the step size) and still there is a possibility that potential interactions will be ignored. On the other hand, design of experiments requires 11 experiments to optimize three variables (full factorial design with 8 experiments on corners and 3 replicates in the centre). The strength of these designs lies in their simplicity and reduced number of experiments. Full factorial designs are easy to construct and interpret even without the help of fancy computation packages. These designs are commonly used for two to four variables; however, the number of experiments increase dramatically with every new factor included in the design.

In Paper III, optimization of ESI–QqQ was achieved by design of experiments in two steps. Initially screening of the revealed that two variables, *i.e.* electron multiplier voltage and makeup flow were the influential ones for a high analyte response (in terms of peak area). Based on the information from screening design, suitable experimental ranges of Electron Multiplier Voltage (EMV) and makeup flow were selected and the two variables were optimized by a full factorial design. The optimal conditions achieved were EMV of 1200 V and makeup flow of 0.1 mL/min. A total explained variance of 93% [$R^2(Y) = 0.93$] and a cross-validated predictability of 80% [$Q^2(Y) = 0.80$] was obtained. The description of screening design is given in following section.

### 5.1.2 Face centred design

The face-centred central composite design (CCF) is one of the most widely used designs in the family of central composite designs. CCF design can also be considered as an extension of the full factorial design. A CCF constructed for three variables is shown in Figure 20. It can be seen that eight experimental points on corners are designed similar to that of full factorial designs. There are six face-centred points and at least three replicates at the centre of the design space. The six face centred points render a design space with three levels for each investigated factor *i.e.* low, middle and high.

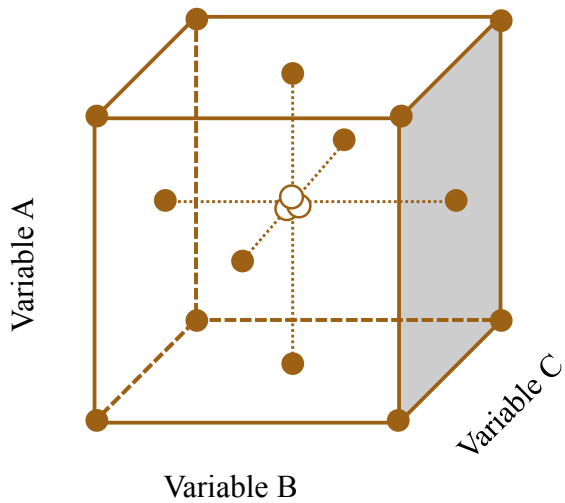



**Figure 20.** CCF design constructed for three variables A, B and C. Experiments are performed on corners of the cube representing low and high experimental values, experiments are performed in the centre of all six faces as well as in the centre of the cube.

In this thesis CCF was used in the following studies:

1. CCF model was presented in Paper I, where a dispersive liquid-liquid microextraction method was optimized for four variables *i.e.* extraction time, amount of salt, volume of extracting solvent and volume of dispersing solvent against peak area of MBTCA as a response using UHPLC–ESI–QToF. The amount of MBTCA extracted was taken as a response and validity of the model was evaluated in terms of total explained variance of 94% [$R^2(Y) = 0.94$] and a cross-validated predictability of 67% [$Q^2(Y) = 0.67$]. The variables were programmed as codded variables. The advantage of using coded variables in any design is that the results will reflect the effects, independent of factor units. A glimpse of codded units used is shown in Table 2, however, the description of different matrices based on

codded units for various designs is beyond the scope of this chapter, a pedagogic description can be found elsewhere[122].

**Table 2.** A description of codded units corresponding lower, middle and higher values of independent variables.

| Independent variables | Levels(coded units) | Levels (uncoded units) |
|---|---|---|
| Time (min) | -1, 0, 1 | 1, 8, 15 |
| Amount of salt (% w/w) | -1, 0, 1 | 0, 12.5, 25 |
| Volume of extracting solvent (μL) | -1, 0, 1 | 150, 225, 300 |
| Volume of dispersing solvent (μL) | -1, 0, 1 | 400, 550, 700 |

2. A CCF design (n=19) was used for screening seven variables in mass spectrometry i.e. EMV, gas temperature, gas flow, capillary voltage, nozzle voltage, nebulization and makeup flow. As described earlier, CCF designs are usually a common choice when the purpose of the study includes investigation of interactions between the variables. Although, as a screening design, the variable interactions are not the goal of the study, CCF can still be a good choice to reduce number of experiments and cost. A full factorial design for seven variables will require 128 experiments ($2^7 = 128$). On the other hand, screening of seven variables can be performed by CCF by 19 experiments (16 experiments and 3 replicates at centre point). Total peak are of the compounds was used as a response. A total explained variance of 92% [$R^2(Y) = 0.92$] and a cross-validated predictability of 72% [$Q^2(Y) = 0.72$] was obtained.

3. As described earlier (Chapter 3), CCF was also used to optimize HFLPME conditions for three quantitative variables i.e. stirring rate, extraction time and concentration of TOPO in organic phase showed that the most suitable conditions were stirring rate of 1000 rpm for 30 minutes using 5% TOPO as additive in methyl decanoate (contour plots with variable interactions are shown in section 3.4).

### 5.1.3 D-optimal design

D-optimal designs belong to the family of optimal designs. They are non-standard designs constructed with the help of computational algorithms. Unlike full factorial and CCF, D-optimal designs are used to deal with problems that do not fit so-called standard designs, *e.g.* when the variables are distributed in irregular domains or there are constrains in variable settings. Imagine the example of coffee brewing. To introduce complexity to the problem another variable *i.e.* type of coffee, is added. Three different types of coffee beans are used, *e.g.* Arabian coffee, South American coffee and Italian coffee. Type of coffee beans is a qualitative variable whereas the variables like brewing temperature, brewing time and coffee to water ratio are quantitative in nature. Evidently, qualitative variables cannot be treated in a similar fashion to quantitative variables with a programmable range of low-to-high values. Hence, so-called standard experimental designs, *e.g.* full factorial and CCF, cannot be used. D-optimal designs offer flexibility to construct a design space for complex problems. A similar example of optimization of a method for qualitative and quantitative variables is presented by Ekman-Hyberg, P.[88]. In this study, preliminary experiments using HFLPME were performed using D-optimal design. Qualitative variables like type of organic phase in the pores of the fibre membranes and quantitative variables like stirring rate, extraction time and amount of TOPO added to organic phase were optimised.

Often D-optimal designs are more interactive in three-dimensional demonstration. A simplified D-optimal design is shown in Figure 21. Let's consider a hypothetical example, using a supercritical fluid extraction system. In a hypothetical scenario, the extractions can be programmed for a temperature ranging from 40-100 °C and a pressure between 70-400 bars, however, it is not feasible to use a temperature of 100 °C together with 400 bars pressure settings due to the risk of pressure overbuilt. Burst disk, the safety kill switch, can rupture stopping the extractions. A simple bi-variable D-optimal design for temperature and pressure is presented. It is obvious that the given experimental conditions cannot be constructed using so-called standard designs.

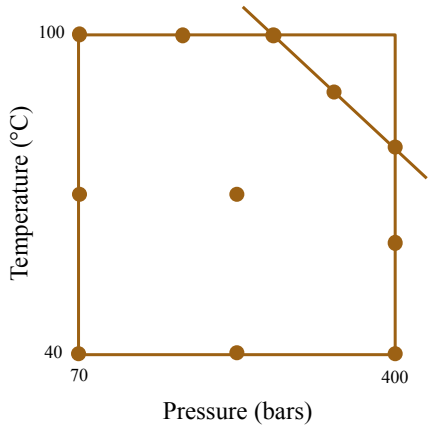



**Figure 21.** D-optimal design constructed for a hypothetical experimental setup with two variables *i.e.* temperature and pressure used in supercritical fluid extractions.

Based on the aforementioned examples we can deduct that design of experiments:

1. adds to scientific knowledge by providing information about possible interactions between the variables (one-factor-at-a-time approach will also find the optimum if enough experiments are conducted, which can be several fold higher than the number of experiments performed by design of experiments)
2. describes the whole experimental space, thereby facilitating identification of optimum conditions, hence, improves the experimental yield
3. reduces number of experiments, time and cost

Like any other tool, design of experiment may also go wrong at times. It is important to understand the scientific process, experimental variablity and method of quantification of the experimental outcome. Design of experiement can give no or false information when:

1. experimental variablity is very high, *e.g.* if a sample is taken from a chemical process to test the effect of process conditions and variability of the analytical method used for testing is higher then the variability of the chemical process under observation
2. measurement of experimental outcome/response can be easily misquantified, *e.g.* in the coffee brewing experiement if the sensory analysis of five different coffee machines is done by one judge

3. experiments are not randomised and systematic errors come in effect, *e.g.* absorption of moisture in weighing experiments

Model validation parameters can be used to identify problems like 1 and 2, however, problem 3 cannot be overcome if the experiments are not randomized.

## 5.2 The design selection

There is almost always a design that fits the purposes. Working with experimental designs can be exciting and the added information provided by the experimental designs can be appealing. Who does not want to explore the interactions between different variables in a process under study, or their significance in controlling experimental outcome besides the obvious attraction of reduced number of experiments? However, none of the experimental design presented here can be used as one-fit-for-all problems. As a chemometrician, it is very important to have good understanding of an experimental setup and all the variables involved. The choice of the experimental design should be motivated by the nature and needs of the experimental setup. As a rule of thumb chemometrics is only good when seen as "chemo$_{(1st)}$ and metrics$_{(2nd)}$".

# 6 Conclusions

*"If I have a thousand ideas and only one turns out to be good, I am satisfied" – Alfred Nobel*[123]

The main objective of this thesis was to develop and optimize analytical methods for the analysis of selected emission markers with focus on higher selectivity, sensitivity, precision, accuracy, low limits of detection and low limits of quantification. In this quest efforts were devoted to separation methods, *i.e.* sample preparation and chromatography. The study presented in **Paper I** showed that the scope of DLLME could be extended for the extraction of MBTCA by fine-tuning the chemistry of extraction solvent. The limit of detection of MBTCA obtained by DLLME followed by derivatization and GC–MS analysis was 0.12 pg/m$^3$ of air that was 41.6 and 10833 times lower than the limits of detection reported by Fu *et al*[66] and Zuth *et al*[67], respectively.

In the study described in **Paper II**, the use of supercritical $CO_2$ for the isolation of EC from OC was investigated. The results showed that supercritical $CO_2$ method significantly enhanced the removal of OC and recovery of EC – in comparison to conventional method used for the removal of water-soluble organic compounds – up to nearly 60% OC removal and 80% EC recovery. The dynamic mode of supercritical $CO_2$ extraction may increase percentage OC removal.

The results presented in **Paper III** guide towards a method for the separation and quantitative analysis of several emission markers with a wide range of polarities ranging from logP – 3.7 to 7.in a single run. The method was studied using SFC–DAD–ESI–QqQ. Identification of PAHs was performed using DAD whereas phenolic acids, phenolic aldehydes and ketones, sugars and carboxylic acid were studies using ESI–QqQ. It was found that separation of PAHs and other polar compounds in a single run was challenging and the method needed further improvements in terms of column and mobile phase selectivity for the separation of PAHs. Furthermore, QqQ may provide lower limits of detection as compared to DAD. As PAHs could not be ionized by ESI, it would therefore be worthwhile to investigate ionization and quantitative analysis of the selected emission markers with SFC–APPI–QqQ.

The aim of the study presented in **Paper IV** was to evaluate aethalometer model for source apportionment of carbonaceous aerosols against the results obtained from

chemical analysis of samples. Aethalometer works on the principle of optical properties of aerosols. It was observed that aethalometer model was suitable for a yearlong source apportionment for quantifying wood burning and variable biogenic carbonaceous aerosols at Vavihill site located in southern Sweden. The aethalometer model overestimated fossil carbonaceous aerosols due to possible interference of biogenic carbonaceous aerosols. Therefore, it is highly desirable to continue the study for the evaluation of aethalometer model by chemical analysis of aerosol samples for biogenic carbonaceous aerosols.

The findings reported in **Paper V** demonstrate that levoglucosan can be used as a tracer for early detection of smouldering fire. Levoglucosan signal was observed in first 30 minutes of initiation of smouldering fire, before any visual signs of the fire. The study was presented as a proof-of-concept that also highlighted the need for faster methods of analysis for early detection of these fires in biomass storages.

# 7 Outlook

*"With faith, discipline and selfless devotion to duty, there is nothing worthwhile that you cannot achieve" – Muhammad Ali Jinnah*[124]

Doctoral study is time limited and the tenure is not enough to quench one's thirst of science, unfortunately. Therefore, I leave several unanswered research questions to future scientific works, *e.g.* 1) under what conditions MBTCA forms complexes with iron(III) and if it undergoes complexation with other metals too? Are there other organic compounds with similar behaviour and are today's analytical methods underestimating them in aerosol samples due to the fact that their metal complexes are not known and not estimated for quantitative analysis? 2) in the study of EC, EC was isolated from the compounds interfering in thermal optical analysis. It is well known that the presence of interfering compounds may be a source of bias in the estimation of EC. In future it can be interesting to perform $^{14}C$ analysis of isolated EC to distinguish fossil and modern sources of combustion. Currently $^{14}C$ analysis of EC provides such information, however, such analysis is usually conducted on aerosol samples containing both EC and OC, leaving a need for methods that can isolate EC from organic compounds. 3) How will the future of early detection of smouldering fire look like? There can be fast and robust methods for early detection of these fires in the field as a continuation of just proof-of-concept study presented here. A calorimetric sensor array detection or aerosol sampling on thermal desorption tubes followed by TD–GC–MS analysis should be tested for their potential, until selective sensors for smouldering fire are developed.

# 8 Thank you

There are several people who have contributed to my scientific learning, personal development and a happy life. The most important person in five years journey of of my PhD was my principal supervisor, **Maggan** (Margareta Sandahl). In the world of doctoral research, people share supervisor related jokes and scary examples, but I haven't experienced any of them. Maggan has always been very supportive and friendly. It's remarkable that I never managed to make her angry. Her feedback was always precisely crafted to help me progress in this journey. She trained me to think systematically in my projects and gave me the opportunity to find my own research path. Thank you Maggan for all your support and encouragement especially in the times when I was down. You always trusted in me and gave me confidence to fight against the odds.

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I grew up in a huge family of several uncles, aunts and cousins. For most of my uncles, aunts and the society around me having higher education was not a popular fashion. I might have ended up getting marry at an early age and working for rest of my life in an effort to bring bread on the table. It was my family – my mother, father and sisters – who stepped forward to break the cycle of ignorance and supported me for my education at the top institutions of the country, even when the resources at home were limited. I am thankful to my family who supported me in my education and gave me the opportunity to get enlightenment and change my life. My late mother, who was never given a chance to go to school, was no doubt a great leader and teacher who dedicated her whole life for my education and the education of my elder sisters and taught us to stay positive and hopeful no matter what. I wish I could show this book to her, "Mama! I got it !!! I became a PhD" and could see her sitting in the first row in my PhD defence, smiling with proud. Nevertheless, I am happy that besides all my weaknesses, I did not give up and never will. I will not forget the lesson of positive attitude and hope.

I have always liked dogs, in fact I never thought of hugging a cat in my life. It was spring 2018 when I half-heatedly agreed to adopt Marjenka (it's a long story). I did not realize when we became good friends. She was loveable and playful. As I moved closer to my PhD defence, our friendship grew stronger. In last few months, I tried to hide my anxiety from colleagues and friends but couldn't hide it from her. Everyday when I reached home from Kemicentrum, I found her close to the entrance of my apartment ready to welcome in her own way. In long nights when I was working on my laptop until dawn, she was a soft ball of fur often sitting in my lap giving me company and comfort. It may seem funny and probably bizarre to some, reading an acknowledgement about a cat. She too was the least concerned when I read this part to her, strange!!! Instead of being happy that I honoured her companionship in my doctoral thesis, she preferred to lick her paw. Guess her love is so pure that she doesn't need it.

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*"With faith, discipline and selfless devotion to duty, there is nothing worthwhile that you cannot achieve".*

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