# Determination of organic aliphatic and aromatic amines and acids in atmospheric aerosol particles

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O Presidente do Júri,

Porto, _____/_____/_____

# Preface

The investigational study described was carried out in the Laboratory of Analytical Chemistry of the Department of Chemistry, University of Helsinki, that belongs to the Finnish Centre of Excellence in Physics, Chemistry, Biology and Meteorology of Atmospheric Composition and Climate Change.

I want to express my deepest gratitude to Prof. Marja-Liisa Riekkola for the opportunity to work in a world-renowned center and to increase my knowledge in a field that fascinates me. It was definitely the best experience of my life.

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# Abbreviations

ACN – Acetonitrile

AP – Atmospheric pressure

APCI − Atmospheric Pressure Chemical Ionization

API − Atmospheric Pressure Ionization

APPI − Atmospheric Pressure Photoionization

CPC − Condensed Particle Counter

C8 – n-octyl

C18 – n-octadecyl

DAPPI − Desorption Atmospheric Pressure Photoionization

DMA – Differential Mobility Analyzer

ESI – Electrospray Ionization

GC – Gas chromatography

GLC – Gas-liquid chromatography

GSC – Gas-solid chromatography

HILIC – Hydrophilic Interaction chromatography

HPLC – High Performance Liquid Chromatography

IL – Ionic liquids

IT − Ion-trap

LI – Laser ionization

LLC – Liquid-liquid chromatography (LLC)

LOQ – Limit of Quantification

LSC – Liquid-solid chromatography (LSC)

MALDI − Matrix Assisted Laser Desorption/Ionization

MrM − Multiple Reaction Monitoring

MS – Mass spectrometry

PILS – particle-into-liquid sampler

PM – Atmospheric particulate matter

PTFE – Politetrafluoroethilene

ROG – Reactive organic gases

RP-HPLC – Reversed phase-High Performance Liquid Chromatography

Rsd – Relative standard deviation

RT − Retention time

SIM − Ion Monitoring Mode

SOA – Secondary organic aerosol

SrM − Selective Reaction Mode

TOF − Time-of-flight mass spectrometry

TST – Total Suspended Particles

# Symbols

*A* − Area

C − Concentration

Cc – Cunningham Correlation Factor

$C_M$ - mass transfer coefficient of the mobile phase

Dp – Physical size of particles

e – Elementary charges

EI – Electron ionization

K – Distribution coefficient

*k* – Retention factor

Mw – Molecular Weight

m/z – mass to charge

N − Number of points removed

*N* – Number of plates

V − Volume

Zp – Electrical mobility

σ – Standard deviation

η – Gas viscosity

λ – Mean free path of gas molecules

α – Selectivity factor

# Resumo

Os aerossóis são partículas ubíquas na atmosfera que afectam o clima e a saúde dos seres vivos por uma diversidade de processos extremamente complexos. O clima é afectado tanto directamente, por ocorrência de absorção e reflecção da radiação solar, como indirectamente, devido à alteração das propriedades das nuvens.

A compreensão dos processos de formação e do impacto das partículas de aerossóis nas diversas esferas que constituem o sistema terrestre é fundamental para o desenvolvimento de acções concertadas que permitam o conhecimento e controlo dos efeitos adversos associados aos aerossóis atmosféricos.

Neste trabalho de investigação foram desenvolvidos e aplicados novos métodos de colheita, tratamento e determinação analítica de amostras de partículas de aerossóis, com vista à análise química de aminas e ácidos orgânicos de interesse investigacional, por cromatografia líquida-espectrometria de massa (LC-MS), utilizando-se como fonte de ionização a ionização por spray de electrões (ESI).

As amostras de partículas de aerossóis ultrafinas, de diâmetro correspondente a 30nm, foram colhidas na floresta boreal de Hyytiälä, na Finlândia, por um Analisador de Mobilidade Diferencial (DMA).

A preparação da amostra consistiu na utilização de um procedimento de extracção assistida por ultra-sons, dinâmica, sendo também adicionado um padrão interno. Para a análise de aminas, o método de preparação da amostra consistiu na dansilação acelerada de pequenas aminas alifáticas e aromáticas, por ultra-sons.

Um novo método de determinação analítica de ácidos por cromatografia líquida-espectrometria de massa foi desenvolvido, utilizando-se uma coluna de cromatografia de interacção hidrofílica (HILIC) e um espectrómetro de massa do tipo *Ion Trap*, tendo permitido a separação de 11 ácidos presentes em aerossóis.

Para a determinação de aminas por LC-MS foi utilizada uma coluna C18 e um espectrómetro de massa do tipo Triplo Quadrupolo, sendo utilizado um método previamente desenvolvido, o que permitiu a identificação e quantificação de nove aminas em amostras de partículas de aerossóis.

Procedeu-se ainda à colheita de partículas de aerossóis em meio urbano por bomba de vácuo e por DMA, bem como à comparação da eficiência de amostragem dos filtros de Quartzo e de Teflon.

**Palavras-chave:** Aerossóis atmosféricos, Fontes de aerossóis, Processos de formação, Aminas alifáticas e aromáticas, Ácidos orgânicos, Amostragem em filtros, extracção, Cromatografia líquida-espectrometria de massa.

# Abstract

Aerosol particles are ubiquitous in the Earth´s atmosphere, affecting the climate and public health of living beings by a variety of extremely complex set of processes. Climate is affected both directly, by occurrence of absorption and reflection of solar radiation, and indirectly, through the changing of cloud properties.

The understanding of the formation processes and impact of aerosol particles in the various spheres that make up the Earth system is crucial for the development of concerted actions that enable the knowledge and control of the adverse effects associated with atmospheric aerosols.

In this research project, new sampling, processing, and determination methods of aerosol particles have been developed for the chemical analysis of organic amines and acids, of analytical interest, by liquid chromatography-mass spectrometry (LC-MS) , using electrospray ionization (ESI) as an ionization source.

Samples of ultrafine aerosol particles, corresponding to 30 nm in diameter, were collected in the boreal forest Hyytiälä, in Finland, by a Differential Mobility Analyzer (DMA).

Sample preparation consisted of applying an ultrasonic-assisted extraction, being added an internal standard to the samples. For amine analysis, the sample preparation method also involved an ultrasound based accelerated dansylation of small aliphatic and aromatic amines.

A new analytical method for determining acids by liquid chromatography-mass spectrometry was developed, using a hydrophilic interaction chromatography (HILIC) column and an ion trap mass spectrometer, having allowed the separation of 11 acids present in aerosols.

For the determination of amines by LC-MS was used a C18 reversed-phase HPLC column and a Triple Quadrupole mass spectrometer, being applied a previously developed method, which allowed for the identification and quantification of nine amines in aerosol samples.

In this project was also performed the sampling of aerosol particles in urban atmosphere, using two different techniques, by a vacuum pump and by DMA, as well as the comparison of sampling efficiency of Quartz and Teflon filters.

**Keywords:** Atmospheric aerosols, Aerosol sources, Formation processes, Aliphatic and aromatic amines, Organic acids, Filter Sampling, Extraction, Liquid chromatography-mass spectrometry.

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# 1. Introduction

## 1.1. The emergence of Environmental Chemistry - A global perspective

Environmental Chemistry is the branch of chemical science that deals with the sources, transportation, reactions, effects, and destinations of chemical species present in water, soil, air, and organisms, and the importance of technology in these events [1].

Before the 50's, concern with environmental degradation was practically scarce [2]. However, the growing preoccupation with environment and its limited resources has led to the emergence of environmental movements, which were intended to raise the awareness of society, government and companies for the harmful consequences of their actions to the environment [2]. These actions performed by people concerned about environment have conducted, in the 60/70 century, to the birth of modern environmentalism, and later, in the 80/90 of the same century, to the emergence of the concept of Green Chemistry [3].

Until about 1970, much of the work done in Environmental Chemistry was confined mainly to industrial level, by workers whose background formation was in other areas than chemistry [1]. For example, when pesticides were synthesized, some biologists have realized some of the undesirable effects of its use, and when detergents where formulated limnologists wondered why the previously normal lakes become shocked with stinking Cyanobacteria [1]. Despite of this long-term environmental effects, some of them fairly recent and severe like the problem of the hazardous wastes, a few chemists deal with substantial knowledge related to Environmental Chemistry during their academic education [1].

In recent years, specifically in the early 90's of the twentieth century, there was a significant progress in the importance attributed to Environmental Chemistry [1]. The industries became aware to the importance of including well trained environmental chemists in its staff to avoid penalties from regulatory agencies that was emerging, being fundamental for that purpose the development of instrumentation to control the industrial processes and products, in order to reduce and prevent pollution [1].

Chemists, thus, become extremely involved in issues related to environmental research, and academic chemistry has included the Environmental Chemistry as part of the academic education program [1].

In this sense, there has been a significant evolution in the manner of practicing chemistry, where it began to give attention not only to the ultimate goal, such as

obtaining a product by a process of synthesis, but also to the overall result of their production, including the need to ensure the mildness of the obtained product [4].

This change of paradigm in view of environmental protection resulted in a shift from a reactive posture to a preventive one, based in the conjugation of a diversified set of factors, not directly related to chemistry but that manifest themselves in the environmental, technological, economical and societal involucre with intrinsic factors of the chemistry itself [4].

Currently, the environment is definitely a major concern, where governments seek to find solutions and reach agreements on global environmental problems [5]. The reasons for this concern are numerous and supported by several indicators, such as the rapid growth in world population, the increase of fossil fuel energy consumption and the consequent release of $CO_2$ in large quantities into the atmosphere, depletion of resources, the use of pesticides and fertilizers to increase agricultural production, the increase of waste production, among many other factors, often interrelated and that jeopardize the quality of life of living beings or even its existence [5].

The study of environment is extremely hard due to their dynamic behavior, where physical forces and chemical reactions constantly change the planet through a wide variety of events, supported by natural phenomena like volcanoes, or by human action, for example, by emission of gases to the atmosphere that can react with ozone [5]. Indeed, to understand the environment, it is necessary to obtain a broad knowledge, which is not restricted only to scientific fields [5].

Thereby, in the future challenges of green chemistry, it will be extremely important to combine and understand the relationships between the specific objectives of chemical practices with the complex and diversified chemical involucres in which chemistry is part of. In this way, it can be possible to achieve a planned and sustained progress of anthropo-technosphere, simultaneously with the conservation of ecosphere, having as a driving force the evolution of scientific knowledge.

## 1.2. Analytical Chemistry and environmental research

Chemical analysis of environment is involved in all phases of environmental study, from problem recognition, monitoring, knowledge of its extent, implementation of control procedures, gathering information that is ascertained the compliance and efficiency of control procedures, among many other actions protagonized in environmental issues [5].

A major challenge in environmental chemistry research is the determination of the source and concentration of pollutants in environment [1]. However, due to the

physical-chemical characteristics of pollutants, chemical analysis can be extremely difficult and complex [1]. A clear example of this difficulty and complexity associated to the analysis of pollutants is easily understandable when we try to analyze atmospheric pollutants which, due to their referred physical-chemical characteristics, may consist of less than one microgram per cubic meter of air [1]. Significant levels of environmental pollutants may consist of only less than a few parts per trillion, which naturally implies the need of analytical instruments whose detection limit is extremely low [1].

Another problem in the analysis of trace amounts of analytes is related to the intrinsic complexity of Environmental Chemistry, because there is an enormous diversity of factors to take into account, many of them dynamical [1]. For example, in environmental analysis occur permanently changes of temperature, composition, intensity of solar radiation, input of materials, and many other factors that strongly influence the physical-chemical conditions of analysis [1].

Therefore, analytical chemistry is an extremely complex but fundamental tool to understand the nature, reactions and transportation of chemical species in environment [1].

## 1.3. Aerosols

Aerosols are disperse systems of small liquid and solid particles suspended in a gaseous phase, usually the air [6].

This definition applies to a wide range of clouds of particles found in nature, comprising clouds of particles of natural origin, for example sand storms or clouds formed from pollens, bacteria or viruses, and clouds of particles produced by anthropogenic activity, as the fumes containing waste resulting from coal combustion [7].

The aerosol denomination arises from military research during World War I [8], as a result of an increased public concern with the problems associated with pollution [7]. Nevertheless, the science of aerosols has always existed in the history of civilization, being strictly related to the history of air pollution [8]. For example, there are reports from ancient Rome that relate the dissatisfaction among Romans with the dirt present in the air, while in the year of 1273, in London, a severe case of air pollution by particles led to the ban of coal use [8]. Still, in a far longer period of time, were found traces of aerosols in mummified bodies with a black tonality lungs, dating from Paleolithic [9, 10].

The size of aerosol particles varies in the range from 0.001 to 100 micrometers, whereas particles greater than 100 micrometers are not outstanding in the air long enough so that they can be observed and measured as aerosols [11].

Given the small size of aerosol particles, their physical state becomes distinct from that observed for larger matter, being this phenomenon more pronounced for lower particle sizes until they reach a limit where the size is of the molecules themselves [7]. This occurs because when particle is in that distinct physical state, the number of molecules and atoms becomes so few that a large part of them are at the surface, whereby the own concept of surface becomes limited because structure of particle cannot be defined simply as solid or liquid but as a physical aggregate [7].

Thus, the spatial distribution and reactivity magnitude of particle surface depends on how individual molecules combine themselves to form a particle as a whole [7].

### 1.3.1. Sources and formation processes of Aerosols

Aerosols are ubiquitous in nature [11]. They comprise a complex mixture of low- and semi-volatile organic and inorganic compounds [12], and although they constitute a large portion of environmental aerosols rarely contribute significantly to the total mass fraction [13].

Atmospheric particulate matter (PM) may be formed from biogenic sources, as the case of particles formed by degradation of biomolecules and polymers, or from anthropogenic sources, as the compounds present in waste gases resulting from the combustion in motor vehicles or in waste treatment [14]. The composition, morphology, physical and thermodynamic properties of PM depends on factors such as time, location and season of the year [15, 16].

There are basically two processes of aerosol formation, the primary processes and the secondary processes, which form respectively the primary and secondary particles [17].

Primary processes emit aerosol particles directly from its source [17], consisting generally on mechanical and thermal process such as the combustion of fuels or heating of materials at very high temperatures [7]. These processes generally form particles larger than 2.5 micrometers [8].

In turn, secondary processes consist on gas-particle conversion events, such as nucleation, condensation and multiphase chemical reactions [18]. The formation of secondary atmospheric aerosol particles is more complex and starts with the production of nuclei with a nanometric size, from the gas phase, followed by a continuous growth of these nuclei until the formation of aerosol nanoparticles [19]. This growth process is simultaneously and dynamically accompanied by particle removing processes from atmosphere [20].

Particles of size greater than 10 nanometers grow more than particles of smaller size due to the strong Kelvin effect sensed by the particles of smaller size, in particular the increased surface curvature that lead to a less effective supersaturation [21].

Previous studies have demonstrated that between 12 to 50 percent of aerosol mass and about 50 percent of condensation nuclei are originated from forestry sources, which demonstrates the importance of carrying out studies on the composition of aerosol particles in this environment [22, 23].

### 1.3.2. Organic composition of Aerosol particles

Organic compounds may represent about 70% of total dry fine particle mass in lower troposphere, depending on the sampling site and its degree of pollution [24].

The number of organic compounds in aerosol particles can reach about a few hundred [25], having already been measured in the atmosphere between 10000-100000 different organic compounds, which clearly demonstrates the complexity of particles with respect to its composition [26]. These compounds are often present at trace levels, with concentrations generally less than a few nanograms per cubic meter [27].

Despite knowing the high organic matter content of aerosol particles, in reality very little is known about its exact chemical composition [18, 25], which emphasizes the need to obtain an inventory of organic compounds present in aerosol particles [27].

In a study made on a research station in the boreal forest of Hyytiälä, in Finland, it was suggested that formation and growth of aerosol particles over forests were led by condensable organic vapors emitted by vegetation [21].

A wide variety of organic compounds are emitted into the atmosphere from plants in forest environment [28]. Various studies have shown that species produced in oxidation reactions of biogenic organic compounds with OH radicals, ozone molecules or with $NO_3$ radicals are more polar, more water soluble, and have vapor pressures lower than their parent compounds, reason why they may play and important role in the formation of secondary aerosol particles [28].

Forests are a significant source of volatile organic compounds and aerosol particles, which is why there has been growing interest in study ultrafine particles formation in this natural environment [29]. This interest is exacerbated by the scarcity of information about the chemical composition of aerosol particles in forests when compared to the one available for urban areas [27].

In another study performed in a Finnish Forest, in 2003, it was verified that the concentration of organic compounds in aerosol particles was greatly influenced by the

direction of the air, since when it moves from a polluted area both the number of aerosol particles and the concentration of organic matter increase significantly but, as expected, even lower than the concentrations obtained in urban areas [27].

Coniferous trees that constitute most of the forest area emit significant amounts of reactive trace organic vapors, as a function of temperature and to a lesser extent of light [30]. Such gases are typically isoprenes, monoterpenes and sesquiterpenes [15, 31, 32], and its reactivity is a consequence of the structure of these molecules, particularly because of the fact of being unsaturated molecules with high reaction potential through its double bonds [33, 34, 35]. Subsequently, oxidation of these gases followed by conversion into particles contribute to a volume of about 1000-2000 particles per cubic centimeter in a size range of 40-100 nanometers [36, 37].

The products of low volatility formed from the oxidation of reactive organic gases (ROGs) condense or adsorb on the particles surface, and may also adsorb on particulate matter already formed, resulting in the addition of components to atmospheric particles [24].

These products modify the chemical and thermodynamic properties of aerosol particles, changing their behavior in the atmosphere, for example, increasing the water uptake in some other local areas or, conversely, decreasing it [24].

The mechanism of formation of organic products is as follows:

$$HC + OH \rightarrow \alpha_1 P_1 + \alpha_2 P_2 + \ldots$$

$$HC + O_3 \rightarrow \alpha_3 P_3 + \alpha_4 P_4 + \ldots$$

$$HC + NO_3 \rightarrow \alpha_5 P_5 + \alpha_6 P_6 + \ldots$$

$$P_i + OH \rightarrow Products$$

$$P_i + O_3 \rightarrow Products$$

$$P_i + NO_3 \rightarrow Products$$

being $\alpha_i$ the stoichiometric factor that relates the quantity of product i formed per amount of reacting hydrocarbons [28].

In the presence of a sufficient concentration of aerosols these products will partitionate between gas phase and particle phase, and particle phase is called secondary organic aerosol (SOA) [28].

The higher the concentration of organic compounds resulting from oxidation, the greater the concentration in particle phase [28].

In hydrocarbons containing about six or more carbons, oxidation products formed are semi-volatile [28].

It is estimated that the annual total biogenic organic emissions are in the order of 491-1150 GCT, exceeding the emissions from anthropogenic sources that are in the order of 98 GCT [38, 39].

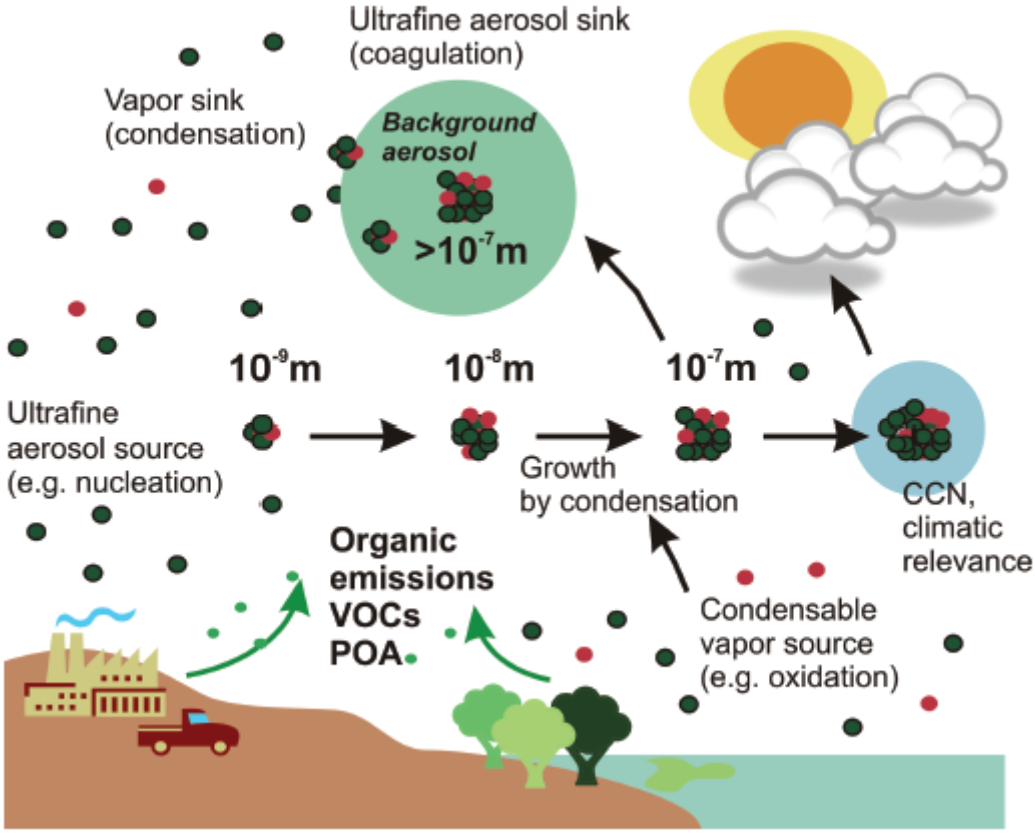


**Figure 1 – Organic emissions and dynamic processes involving atmospheric aerosol particles [40].**

## 1.3.3. The importance of atmospheric aerosols research

Nucleation and aerosol particle formation is an extremely important phenomenon that occurs in atmosphere because these particles affect the quality of life both locally and globally [41].

Local impact is related with the adverse effects for human health and other effects such as the reduced visibility [41].

In turn, global impacts are manifested mainly by the climate change [42] and in the hydrological cycle [41]. Additionally, aerosols participate in heterogeneous chemical reactions in atmosphere, thereby affecting the abundance and distribution of atmospheric trace gases [43, 44].

Climate is a highly dynamic and complex system, wherein aerosols play an important role [45]. With regard to the environment, aerosols may affect the climate by a multiplicity of processes, either directly or indirectly [17]. Direct effects are caused by

absorption or scattering of solar radiation that provoke a significant reduction in the total amount of sunlight that reaches earth's surface, with potential to influence both terrestrial climate and, naturally, all ecological system [17]. Indirect effects are related, for example, with changes of the properties of clouds [46].

A study has demonstrated that the formation of aerosol particles in the Finnish boreal forest caused a local disruption of radiation in a range between -5 and-14 $W.m^{-2}$, which can counteract the effect of forest albedo, defined as the relative amount of reflected light [36].

Concern about the adverse effects of aerosols on human health, particularly in urban areas, was accentuated in the last decade [47]. Due to their small size, aerosol nanoparticles can be deposited in respiratory tract, transported into the cells through the pores of the skin, and enter the bloodstream [48], being this phenomenon the reason why aerosols are associated with adverse cardiovascular effects [49].

Furthermore, aerosols are associated with some other adverse effects resulting from its deposition, like crop damage by acid deposition or the deposition of dirt on properties [8], and many others, for example, resulting from the fact that aerosols can contain potentially hazardous species ranging from microbiological, biological or chemical toxins [50].

All these effects are accentuated by the persistence and bioaccumulation characteristics of some aerosols in natural environments where they are found [17].

In sum, the science of aerosols has, nowadays, tremendous practical applications in the world [51].

The impact of atmospheric aerosols on global radiation that is emitted, whether direct or indirect, is crucial to understand the Earth's climate and anthropogenic contribution to its amendment [52].

Moreover, despite legislation at local and international level related with aerosols had already been introduced, to protect health and environment [11], urges the need to establish policies, standards and regulations based on practical knowledge that supports the decisions in that direction [51], being the analytical process of aerosols fundamental for that purpose.

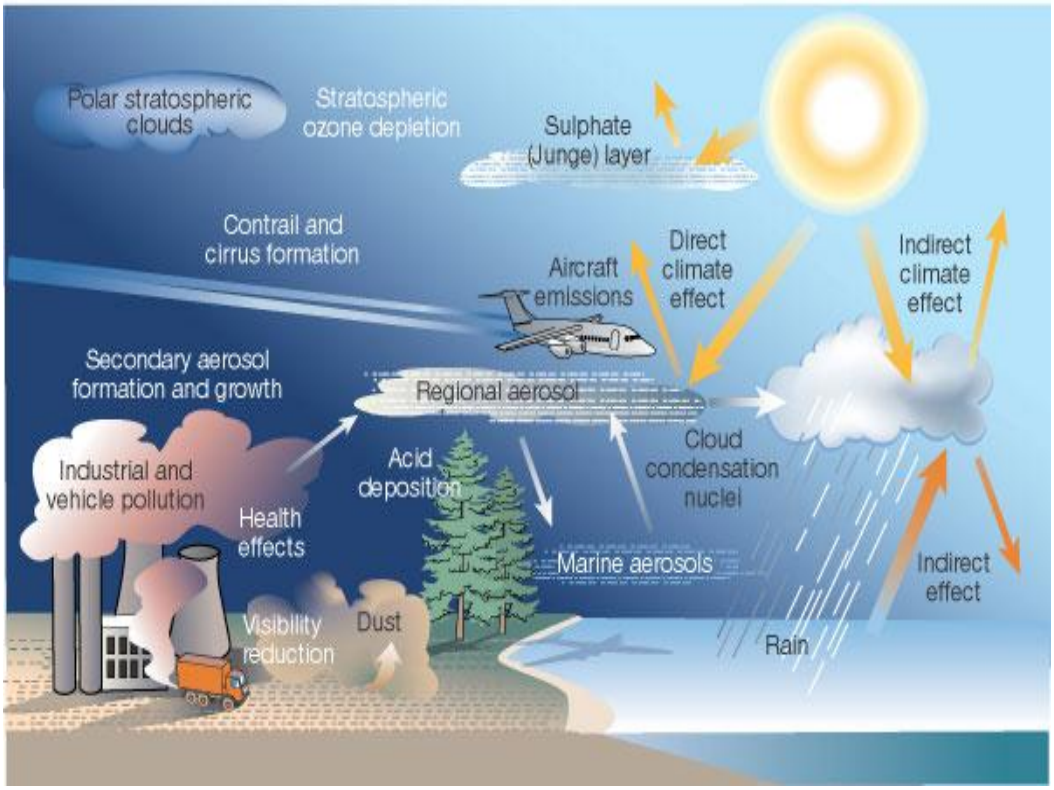


**Figure 2 – Sources and effects of aerosol atmospheric particles [53].**

### 1.3.4. Analysis of the impact of aerosols on climate and human health

Aerosol atmospheric particles and trace gases affect significantly the quality of life in several ways [54].

The analysis of the impact of aerosols on climate and human health is very problematic because they are particles whose concentrations depend greatly on a variety of factors such as the time and location [55], and whose effects depend on their size, chemical composition and concentration [50]. Understanding these variables is therefore crucial to determine factors as the levels of human exposure and corresponding effects on their health.

Particle size of aerosols is one of the most important parameters because it determines their physical nature, as well as the analytic procedure used for their identification and quantification [32].

The size is normally expressed by its diameter, although sometimes also its radius is used [1]. For a spherical particle the definition of diameter is relatively easy since it corresponds to the geometric diameter, however, for non-spherical particles it is more appropriate to define the particle size in terms of one or more equivalent diameters, like the equivalent volume diameter that is the diameter of the sphere having the same volume as the particle in question [7].

Adverse effects on human health are usually caused by particles whose diameter is less than 10µm ($PM_{10}$) or 2.5µm ($PM_{2.5}$), being also related with the chemical composition of the particles with referred sizes [56, 57].

The fraction of organic matter of aerosol particles tends to increase with decreasing particle size, whereby it is extremely important to relate the information about the size of the particles with its composition and their corresponding effects [18].

Sampling of ultrafine aerosol particles and the analysis of their composition is extremely challenging [12], making the analytical determination of organic compounds present in that particles extremely difficult as well [58]. The difficulty is related with the fact that many constituent compounds have very similar structures [59], extremely small masses and are found in trace concentrations [58], except during rapid growth events that follow nucleation [13].

Another issue is that, in order to understand the sources, transformations and the impact of aerosols, there is the need of methods which allow particle-by-particle chemical composition measurements [17].

Because of their inherent complexity, there is little knowledge about sources, mechanisms of formation, composition and properties of aerosols [60], and understanding their impact on climate and human health continues to be a science with an enormous associated uncertainty, which requires more scientific research all over the analytical process [61].

### 1.3.5. Composition of atmospheric aerosol particles

Among the several families of compounds that are often found in atmospheric particles are included amines, acids, polyols, and aldehydes [52, 62, 63, 64].

Monosaccharides, including anhydrous monosacharides, also comprise a significant proportion of water soluble organic carbon in atmospheric aerosols [65]. During winter, some of the major components of aerosols are levoglucosan, manosan and galactosan, originated from the combustion of cellulose and hemicellulose when wood is used to heat townhouses [66]. These may also be founded in high levels during summer due to wildfires [66]. Also in the warmest period of the year, there are high concentrations of primary biological sugar alcohols, such as d-mannose, inositol, arabitol, glucose and fructose [67]. The reason for the high concentrations observed is related to the fact that these compounds are secreted by trees and shrubs during that season of the year [67].

In turn, monocarboxylic and dicarboxylic acids have also been identified as major constituents of aerosols, as a result of primary emissions [68] or photochemical

reactions [63]. Dicarboxylic acids are compounds of extremely interest to the characterization of atmospheric chemistry, as suggest having a major role in the nucleation process [69]. For its part, the importance of monocarboxylic acids is related to its possible oxidation, via intermediates such as hydroxy acids or ketocarboxylic acids to dicarboxylic acids, playing thereby, albeit indirectly, an important role in the nucleation process [70].

With respect to aldehydes, was recently discovered, in a study on the composition of atmospheric aerosols carried out in a Finnish boreal forest, the presence of pinoaldehyde as the most abundant compound [64]. Furthermore, β-caryophyllene, a compound formed from the oxidation of sesquiterpene precursor by atmospheric ozone, is also present in high quantities in aerosols and in gaseous phase [52]. This compound has an important role in aerosol particles growth [71]. Another compound of the family of aldehydes present in aerosol particles is benzaldehyde, which has a key role as an intermediate in photo-oxidation of some aromatic hydrocarbons, such as toluene, and is always present in detectable amounts in polluted urban areas [72].

Finally, alkylamines have also been identified both in biogenic and anthropogenic aerosol particles and in gaseous phase [62]. These compounds, after being in the particle phase, may starring subsequent reactions and form highly molecular weight compounds [62].

## 1.4. Scope of work

The aim of this study was the determination of organic aliphatic and aromatic amines and acids in atmospheric aerosol particles. To this purpose have been developed and applied sophisticated analytical methods in environmental chemical analysis.

The development of analytical methods which enable the determination of atmospheric particles composition is critical to understand their formation process and its environmental impact, in particular its implications on climate and public health.

This investigation project was carried out in the Laboratory of Analytical Chemistry of the Department of Chemistry, University of Helsinki, and was part of the project "Sophisticated Instrumental Techniques in the Elucidation of Organic Compounds in the Formation Process of Aerosol Particles." The laboratory belongs to the Finnish Centre of Excellence in Physics, Chemistry, Biology and Meteorology of Atmospheric Composition and Climate Change.

The instrumental technique used was the high performance liquid chromatography coupled with mass spectroscopy.

Samples used were collected in the boreal forest of Hyytiälä, located in Finland.

Were also performed other technical procedures such as the collection of aerosol and gas-phase samples for further analysis or the study of the filters used for sampling process.

This work allowed thus to acquire skills in all stages of the analytical process of aerosols, from sampling, sample preparation to its instrumental analysis and data treatment.

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# 2. Sample collection and preparation

## 2.1. Characterization of the compounds

Terpenes generally play an important role in the formation of biogenic secondary organic aerosols (SOA) due to its low saturation pressure and abundance in atmosphere, being therefore extremely important the study of the compounds formed from its oxidation in order to understand the processes that lead to the subsequent formation of aerosol particles [1, 2, 3, 4].

The organic fraction of ultrafine aerosol particles present in troposphere constitute more than a half of its total composition [5, 6].

Each volatile organic compound can form a wide variety of oxidation products which can subsequently contribute to the formation and growth of secondary organic aerosols [7].

The analytes studied in this research project consisted of amines and acids commonly present in atmospheric aerosol particles. Compounds of this type are extremely difficult to analyze for various reasons, including low sensitivities of detection or variable ionization during the analytical procedure [8].

Their derivatization is however easy, which can provide increased efficiency of their separation and subsequent analysis [8].

### 2.1.1. Acids

From acid constituents present in atmospheric aerosol particles the compounds in high oxidation states, such as monocarboxylic acids, dicarboxylic acids and keto acids are compounds of great analytical interest because of its low saturation pressure, which gives them the potential to form aerosols [9].

Its potential is related to the fact that these acid products commonly present in gas phase can form secondary organic aerosols, given its reduced vapor pressure [9], either by condensing/partitioning in pre-existing particles or by nucleation [10].

### 2.1.2. Amines

Recent studies on the composition of atmospheric aerosol particles have demonstrated the importance of nitrogen compounds to aerosol chemistry and its participation in particle growth process [11].

The nitrogen-containing organic compounds most frequently and abundantly present in atmosphere are aliphatic amines of low molecular weight (containing

between one and six carbons) aniline, nitroaromatic compounds, amino acids and amides [11, 12, 13, 14].

The most common biogenic sources of these compounds include the degradation of biomolecules and polymers, and cellular metabolism [12].

In turn, some of the most common anthropogenic sources are volcanic fumes, vehicle combustion, combustion of waste, livestock industries, among others [12].

The contribution of amines in aerosol samples is quite small except for particles of 30nm which may be associated with the occurrence of acid-base reactions during particle growth or with oxidation processes that may modify chemically these compounds [15].

## 2.2. Sampling

Aerosol sampling consists in the separation of particles from a given volume of air, in order to allow the analysis of their relevant physicochemical properties [16].

Sampling is an extremely problematic step of the analytical process of aerosol particles [15]. One of the aspects that make it extremely difficult is that, during this process, aerosol particles continue to grow [15]. Furthermore, aerosol sampling is dependent on factors such as the particle size, the season of the year and the place of sampling [11]. Sampling process should be as short as possible to avoid the occurrence of volatilization and the formation of positive or negative artifacts from oxidation reactions, absorption or adsorption of gas phase compounds [17].

Particle size is the most important property when carrying out sampling [16]. Before the analytical procedure, aerosol particles are usually separated by size, so that it is possible to obtain the chemical composition as a function of particle size [17].

The small mass of ultrafine aerosol particles is a limitation for analytical measurement instruments, mainly because of the time required to perform a collection of a representative sample in quantities exceeding the detection limit of the analytical instrument, this problem being more pronounced in real time analysis [18]. This difficulty is exacerbated by the fact that ultrafine atmospheric aerosol particles have extremely low concentrations [19], except during rapid growth events that follow nucleation, making their sampling extremely problematic [20].

Some particles also have lifetimes of only minutes or hours [21], and not always analytical instruments are capable of effecting the analysis of particle composition at a particular moment [2, 3, 22, 23].

Time resolution of off-line sampling techniques tends to be unsatisfactory, even when takes place the measurement of the daily variations of chemical species in study [24].

Clearly, there is the need to develop new sampling techniques which are less sensitive to artifacts, allows for better time resolution and which can be coupled on-line with the techniques used for detecting organic compounds in aerosols [24].

This need for new techniques is urgent to study nanometer-size aerosols, where the mass of organic carbon in particle phase can be much lower than the mass of carbon present in gas phase [25]. Actually, it is observed that, even after a collection time of several days, the total aerosol mass may be in the order of ten to one hundred nanograms, well below the quantities of organic carbon mass observed in gas phase [25].

### 2.2.1. Filters

Filters generally used to collect aerosols are made of quartz or Teflon, since they permit the circulation of gas between the filter, being the particles accumulated on its surface [11].

When the collection of aerosol particles is performed off-line, collection time can vary from a few hours to several days, depending on factors such as the concentration of compounds in aerosol particles, the number of particles in the air, the detection limit of the method used in analytical determination, or the sampling flow rate [11].

Quartz filters are most commonly used because adsorption is much higher than in Teflon filters for the reason that there is a wide range of possible interactions with quartz filter, including dipole-dipole interactions, electrostatic, and others, regardless of particle size [25]. Furthermore, these filters have a larger surface area, increasing the amount of adsorbed aerosols, with the drawback that also adsorb more artifacts from gaseous phase [26].

For some compounds was also observed the dependence of adsorption with the vapor pressure of the compound [27]. However, Parshintsev J. et al. (2011) has demonstrated, in a comparative study of quartz and teflon filters for collecting aerosol particles, that this evidence was not applicable to all compounds, and even was predominant the non-dependence between adsorption and vapor pressure for the majority of the compounds studied [25].

In the same study it was found that none of the filters can be considered the most suitable since both not totally prevent the occurrence of chemical changes in the sample and undergo modifications in physical-chemical characteristics of the material

[25]. It has been found that Teflon, for example, after adsorption of aerosol particles, undergoes a variation of polarity, as this filter, non-polar, begins the adsorption of polar compounds after collection of aerosol particles, which function as an additional filtering chromatographic stationary layer comparable in terms of adsorption behavior to the one verified for quartz filters, of polar nature [25].

Thus, this study has demonstrated that both filters are suitable for collecting aerosols previously separated according to a defined specific size by using a differential mobility analyzer, if conducted simultaneously the collection of gas phase samples and the subtraction of the value obtained for gas phase filters to the one obtained for aerosol filters [25].

It was however established that quartz filters has a higher adsorption capacity than Teflon filters, as was expected [25].

A substantial amount of gaseous phase compounds can be adsorbed on the filter, causing an overestimation on the concentration of the compounds present in the particles studied, being the overestimation more pronounced in the case of nanometer-size aerosols [28]. Moreover, the variation in the concentration of other chemical species present in gas phase during sampling, as occurs for example with atmospheric ozone, may lead to the occurrence of chemical reactions with the substances adsorbed, leading to a change in the composition of atmospheric aerosol particles which is intended to quantify [26]. Quartz filters, due to its higher adsorption capacity, will therefore adsorb more compounds from gas phase during sampling, producing positive artifacts, which will result in an overestimation of organic carbon mass present in atmospheric aerosol particles [28].

A possible solution to overcome these problems might be the use of two quartz filters, one for aerosols and another as a secondary filter, allowing to know and subtract the resulting excess of inherent overestimation [26]. Another possibility is to use Teflon filters for collecting aerosols and quartz as a secondary filter [27].

However, the two options have drawbacks, including the high adsorption of compounds from gas phase in the first filter, especially quartz, so that the secondary filter does not provide reliable information about the contribution of gas phase to the total amount of organic compounds present in the filter for aerosols [25].

However, many researchers consider negligible the amount of organic compounds that are not part of the constitution of aerosol particles but which are adsorbed in the aerosol filter from gas-phase [26].

Other researchers consider also that losses by volatilization are much more significant than the gain associated with adsorption, suggesting that secondary filter

should be placed in front of the primary filter to obtain a higher accuracy of the measurement process [26].

The combination of a primary filter for collecting aerosols with a secondary filter for collecting gaseous phase or for retention of analytes suffering evaporation from primary filter can, therefore, improve the sampling efficiency [26]. Another possibility is to use a Denuder-filter-adsorber system, where the Denuder will remove organic compounds from gas phase before sampling in the primary filter, the primary filter will retain only the organic compounds of aerosols, and the adsorbent will capture all the organic compounds of particle phase that suffer volatilization from the primary filter [26].

In this case, the efficiency of the adsorbent and Denuder should be 100% or at least known with a high degree of certainty from the measurement of total organic carbon concentration before and after the Denuder and the adsorbent, in an environment free of particle flow [26].

### 2.2.2. Sampling techniques

The sampling process of gases can be developed for a specific range of compounds or for the analysis of all compounds present in a given location [29].

There is a wide variety of techniques used for aerosol sampling [30].

Traditionally, aerosol particles were collected on filters and subjected to extraction processes using organic solvents in order to release the compounds from the filter for further analysis [17].

Conceptually, the adsorption on filters is similar to the gas-particle partitioning, where the filter acts as adsorbent and the organic compounds as adsorbates [31]. When aerosols are pumped through the filter, condensable compounds are exposed to a large surface without any initially adsorbed compound [26]. Then, condensable organic compounds associated with atmospheric particles enter in a dynamic equilibrium with gas phase [26]. The equilibrium between adsorbent phase and adsorbed phase occurs after the adsorption of condensable organic compounds, being partitioning between gas phase and sorbent phase dependent on factors such as the atmospheric temperature, the surface area of the adsorbent, the partition coefficient and the presence of adsorption competitors [26].

However, this technique has significant limitations, such as the long sampling time which may cause changes in the concentrations of analytes [18]. Furthermore, this technique is inadequate for studying processes of short duration, since time resolution of the data obtained is unsatisfactory, requiring a high sampling time to obtain sufficient

quantities of analytes that exceeds the detection limit of the available instrumentation [17].

Currently, sampling can be accomplished by instruments such as impactors, electrostatic precipitators, filtration pumps, systems for particle growth in supersaturated water vapor, or a particle-into-liquid sampler (PILS) which combines the particle growth in supersaturated water and the impaction onto a quartz plate [15].

The analytical results obtained from a particular sample are extremely dependent on the method chosen for the sampling process [29]. Sampling instruments used to collect particles of reduced dimensions are evaluated in terms of performance by its ability to select, specifically, particles having a given size after the step of collecting gas containing the analytes under study [16]. The geometry, size and transmission section between the gas inlet and the filter are specifically defined to allow penetration characteristics that meet the range of particle fraction pretended [16].

Sampling techniques of aerosols can be divided into four groups, defined according to the type of sampling as on-line or off-line, and according to the size of the sample particles as total suspended particles (TSP) or size separated fraction ($PM_{10}$, $PM_{2,5}$) [32].

The most used techniques are based on off-line sampling with size separation because they are easier to apply and have acceptance by environmental protection authorities [Directive 2008/50/EC] [32].

Sampling of aerosols with sizes between $PM_{10}$ and $PM_{2,5}$ is generally performed in filters or impactor plates, resorting to the Cyclone and Impactor techniques [32].

From the size and average density, the number of particles can be measured using a condensed particle counter (CPC), and the obtained value can be subsequently converted into an absolute value expressed as quantities of aerosol per nanogram, which can increase the amount of information obtained from the samples [25].

In this research project were used the filtration and differential mobility analyzer sampling methods.

### i. Filtration

Filtration is the most used technique for aerosol sampling because of its simplicity of application, flexibility and above all because of the lower costs associated with their acquisition and use when compared to other sampling techniques [33].

The purpose of filtration is to remove from the atmosphere a representative sample of aerosols by retaining them on a filter or on a porous medium [33].

The transfer of aerosols from a medium where are dispersed into the filter allows to obtain a more compact sample in terms of composition, which will facilitate their storage, transport, preparation, and detection during chemical analysis [33].

Adsorption on filters is particularly advantageous when it is desirable to collect samples for subsequent analysis involving the application of gas chromatography [29].

In its simplest version, the filter mechanism comprises a pump and a support for the filter, being also possible to couple a flow regulator, a gauge and a flow meter [33].

The air containing aerosols is then forced, by the pump, to circulate isokinetically through the filter, and the filtered content is selected by setting parameters such as the pore size of the filter, the air flow rate through the filter, among many other factors [33].

Sampling using only one filter does not perform the separation of particles by size, although virtual impactors could be applied to remove larger particles [34].

### ii. Differential mobility analyser

A good way to prevent the interception of artifacts during the sampling process is to use a differential mobility analyzer (DMA), wherein filtration of particles with a specific size takes place at atmospheric pressure, being the pressure drop below to that observed when using impactors, which make evaporation negligible [15].

The DMA is constituted by two concentric metal electrodes, the inner electrode and the outer electrode [35]. DMA principle consists in applying a controlled negative charge to the inner electrode, ranging from 1 V to 10 kV, being the outer electrode electrically grounded, producing an electric field between the two electrodes. [35]. Aerosols are introduced at the top of DMA device, flowing down through the annular space between the electrodes [35]. Then, aerosols surround the inner core of sheath air, without mixing, and the electric field causes positively charged particles to be attracted through the sheath air to the negative charged collector rod, where particles are collected [35].

Because the charge state of aerosols is not generally known, aerosol particles are forced to pass through a neutralizer before the DMA [36]. In the neutralizer, aerosols will be exposed to an ion cloud, that is overall electrically neutral but that is composed by positive and negative ions [36]. This will permit to quantify the fraction of particles that exist in a given charge state, by altering the charge state of particles to obtain a known charge distribution [36].

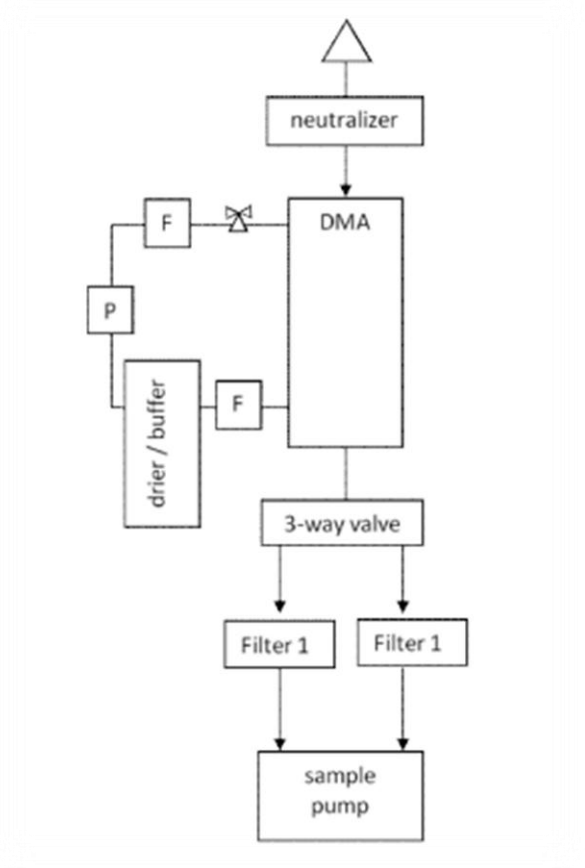


**Figure 3 – Differential Mobility Analyzer [25].**

DMA is introduced before the filter for size separation of atmospheric aerosol particles retained in the respective filter [20, 37].

Separation is based upon the electrical mobility of charged particles when subject to an electric field [25]. The relationship between electrical mobility (Zp) and physical size of the particles (Dp) is given by:

$$Zp = \frac{neCc}{3\pi\eta Dp},$$

where n is the number of elementary charges (e) of the particle, η is the gas viscosity, and Cc is a correlation factor called the Cunningham Correlation Factor that can be determined through the formula:

$$Cc = 1 + \frac{\lambda}{Dp}[2.514 + 0.800\exp\ (-\frac{0{,}55Dp}{\lambda})],$$

where λ is the mean free path of gas molecules [38].

Then, is applied a voltage corresponding to a specified particle size, thus effecting a pre-selection of the particles to be analyzed before they are retained in the filter [25].

With the application of DMA is possible, with reliability, to collect particles of a size less than 100 nanometers [25], thus enabling to obtain information about the

chemical composition of these particles and to understand how nucleation and particle growth events occur in earth's atmosphere [39].

Sampling process must necessarily be as short as possible to avoid formation of artifacts and significant changes in the concentration of certain compounds [18].

## 2.3. Sample preparation

After sampling of aerosol particles in filters, it is necessary to make its extraction from the filter in order to perform the determination process.

Furthermore, many analytes in trace amounts, such as analytes constituents of aerosol particles, given their low concentrations, require a pre-concentration step or a step to remove interferents prior to the analytical determination [40].

Several techniques may be used for the treatment of the samples to be analytically determined.

Off-line techniques for sample preparation are the most commonly used but, given the high associated uncertainty, it has been studied the use of on-line techniques for that purpose [9]. For example, some techniques operate at high pressures and temperatures (e.g. Supercritical Fluid Extraction or Accelerated Solvent Extraction) which can lead to the oxidation of the sample, thus compromising the reliability of analytical determination [32].

After extraction, it is common to proceed to the aerosol compounds concentration using a rotary evaporator or by exposing the sample to a nitrogen flow, the latter having the advantage that will protect the sample against oxidation and volatilization of organic compounds [32].

In this study the techniques used for sample preparation included ultrasonic-assisted extraction and derivatization, being also added an internal standard.

### 2.3.1. Ultrasonic-assisted extraction

Ultrasonic-assisted extraction is an extraction technique which involves the application of ultrasonic vibrations in order to increase contact between sample and solvent used in the extraction process [41]. For this purpose, an extraction chamber containing the sample filter is placed in an ultrasonic bath [41], proceeding to an ultrasonic-assisted extraction, intermittently or continuously, for a period from a few minutes to a few hours [42]. This technique is relatively fast but efficiency is lower when compared to other extraction techniques [41].

Generally, ultrasounds have a frequency around 20kHz, and involve cycles of compression and expansion through a medium [43]. Expansion sends the molecules

away from each other while compression causes their approach [43]. In a liquid sample, the expansion cycles lead to the formation of bubbles or cavities, being the process of formation, growth and collapse of the bubbles named cavitation [43]. These cycles produce extremely high temperatures and pressures, in the order of 5000°C and 1000 atm, however, given the small size of the bubbles compared to the total volume of fluid, the heat produced is quickly dissipated [43].

For samples with low analyte concentrations, it is recommended to perform the extraction more than once with the same amount of solvents, being sometimes necessary, after the completion of the extraction, to execute the filtration or the centrifugation of the extract as well as a washing step prior to the final determination step [41].

As with other techniques ultrasonic-assisted extraction can be performed statically or dynamically [41].

The advantages of ultrasonic-assisted extraction are the reduced execution time, good efficiency and accuracy, low solvent consumption and the possibility of extraction from a low amount of sample [41]. Additional advantages include the use of relatively simple laboratory equipment and the ability to simultaneously perform multiple extractions [42]. Furthermore, it is a technique that has a low cost [32].

In turn, main limitations have to do with the fact that it may occur decomposition of some compounds after ultrasonic-assisted extraction [41] and the requirement that sample matrix has to be permeable to the solvent used, although exist methods that increase the permeability of matrix to the solvent such as heating or dispersion of matrix [44].

### 2.3.2. Derivatization

Sometimes, before or after chromatographic separation, it is necessary to modify chemically the analytes in the sample to enhance the separation process of its components, in a process called derivatization [45].

The purpose of derivatization step is to reduce the polarity of the species, increase the response on the detector and hence sensitivity, and selectively increase the detector response to some components of the sample [45].

Thus, derivatization may improve the analytical determination by making liquid chromatographic separation easier due to the referred reduction of polarity, increasing or modifying the detector response to different compounds and allowing the detection and quantification of analytes whose spectral bands are initially overlapped [8].

Furthermore, derivatization can also protect labile compounds which might be destroyed during the analytical procedure [8].

Derivatization allows also to improve the initial extraction of analytes, generally carried out by adjusting the pH to which the analyte is ionized by reacting reversibly the analytes with an auxiliary compound that modifies this property [8].

Derivatization processes can be divided into two types, pre-column derivatization and post-column derivatization [46]. The post column derivatization consists in mixing the effluent from the High Performance Liquid Chromatography column with the derivatization reagent before it enters the detector [46].

The process most commonly used is the pre-column derivatization, as it allows the reaction and washing to be performed manually or off-line, simplifying the required liquid chromatography system [8]. Besides, it makes possible a greater combination of solvents, reaction times can be longer and allows the use of excess reagent [8].

Pre-column derivatization, despite being the most frequently used because of its good applicability, it also has some disadvantages compared to post-column derivatization, such as the fact that some samples yield more than one reaction product, increasing the complexity of the chromatogram, the derivatization reaction has to be completed, or pre-column derivatization may be slower than when performed automatically post-column [8].

### 2.3.3. Internal standard

The accuracy of results is increased by incorporating a certain amount of internal standard in the sample and by using calibration standards [45].

Subsequent graphic representation of the ratio analyte area/area of internal standard versus concentration of analyte will allow to obtain a more accurate quantitative determination, reducing uncertainties related with the preparation and introduction of sample in the analytical instrument [45].

Generally, the internal standard is an analogue of the analyte, stable and isotopically labeled, containing, for example, deuterium atoms [45].

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# 3. Sample analysis

## 3.1. Introduction

The complexity of atmospheric aerosol particles implies that the analytical techniques used during its determination need to be powerful enough to obtain information about its composition and consequently about its formation processes [1]. Furthermore, the compounds of interest present in aerosol particles are part of a complex mixture, necessitating therefore a method for their separation to enable their subsequent detection, having been developed for this purpose the chromatographic methods [2].

One of the greatest advantages of chromatography is its flexibility, allowing the use of several types of mobile phases and stationary phases, which increases the range of analytes that can be separated and analyzed [3].

In turn, the main limitation of chromatography is the inability to provide an unambiguous identification of the constituents of the mixture by itself, even when separation is successful, because identification is based on comparison of the retention times of analytes in the sample with the retention times of reference compounds tested under similar experimental conditions, requiring therefore a combination of the chromatographic method with a more reliable method for detection and quantitation [2]. In this regard, the combination with Mass Spectrometry (MS) has emerged as an important alternative, because its mass spectrum is specific enough to permit the identification of compounds with a high degree of confidence [2]. Moreover, it has also received the acceptance as a routine technique over the past decades due having enabled to make separations faster and more efficient when compared with other conventional chromatographic techniques [4].

The principle of coupling the chromatographic method with mass spectrometry consists in separating the analytes in the mixture, and then, after removal of volatile solvent, transferring the analytes to the mass spectrometer [2] where the signal of the analytes is measured as a function of time, obtaining a chromatogram which will allow the identification of analytes and its quantification [5].

Several on-line instrumentation systems have been developed to permit the analytical determination of sample analytes [6]. Although these systems eliminate some of the drawbacks found in manual methods also have some limitations and are not universally applicable to all analytes, as well as the cost is generally higher than other instrumental systems [6].

Recently has been growing interest in the miniaturization of analytical devices owing to its greater sensitivity and speed, reduction of sample and solvent consumption

and low production costs [7]. In mass spectrometry, it has been sought the miniaturization of ionization sources [7], especially regarding the Electrospray Ionization (ESI) technique since is generally the most used for atmospheric pressure ionization [8]. However, the miniaturization of electrospray ionization technique has demonstrated poor stability of the signal and the inability to ionize somewhat polar and nonpolar compounds, and for that reason has been sought the miniaturization of other ionization techniques such as Atmospheric Pressure Chemical Ionization (APCI) and Atmospheric Pressure Photoionization (APPI) [7].

In this research project for analysis of amines and acids by Liquid Chromatography-Mass Spectrometry (LC-MS) was used the ESI technique for ionization of analytes at atmospheric temperature and pressure. With respect to the chromatographic separation method consisted in using a Hydrophilic Interaction chromatography (HILIC) column, whereas for analysis of amines resorted to a C18 column. The detection was carried out using an Ion Trap type mass spectrometer for the analysis of acids and a Triple Quadrupole type mass spectrometer for the analytical determination of amines.

## 3.2. Principles of chromatography

Chromatography is a physical method of separation in which components are distributed between two immiscible phases, a mobile phase, which may be a liquid, a gas or a supercritical fluid, and a stationary phase, which may be a solid, a gel or a liquid [2].

A chromatographic system consisted generally of four components, the injector, the mobile phase, the stationary phase and the detector [2]. Furthermore, it is installed a furnace where the column is placed with a temperature programmer and controller, allowing analysis to occur under suitable temperature conditions [9].

The distribution system, the part of chromatographic system where solutes are distributed between the two phases, can take the form of a column, of small diameter, comprising a tube in which stationary phase is coated on the inner surface or where a given particulate material is packaged [9]. Mobile phase flows under pressure through the column, allowing the sample mixture to compete for the adsorption sites, which are primarily saturated by the more strongly adsorbed solutes, followed by the solutes with less interaction with the adsorbent [9].

The mass transfer coefficient of mobile phase, $C_M$, is proportional to the square diameter of the packaged material particles, whereby the column efficiency increases dramatically by decreasing the diameter of particles [5].

Then, the sample solutes are eluted in a reverse order of magnitude of the interaction forces between solute and stationary phase [9]. Eluent should be present in low concentration in the mobile phase, and the interaction with analytes must be strong enough to permit the breaking of adsorbent-analyte interactions for subsequent analytical determination by the detection system in the form of spectral bands [9].

The distribution of analytes in the chromatographic system is thermodynamically driven to the equilibrium between stationary phase and mobile phase, and this occurs when the amount of molecules that bind to stationary phase is equal to the number of molecules acquiring higher kinetic energy than the potential energy of its interaction with the stationary phase, allowing its release into the mobile phase [9].

Distribution coefficient (K) is determined by the relative affinity of solute for the two chromatographic phases, being higher for solutes that interact strongly with the adsorbent phase and, therefore, will be retained for a longer period of time by the stationary phase [9]. In turn, the affinity of solute for the adsorbent phase is determined by the intermolecular forces between solute and adsorbent such as dispersive, polar and ionic forces [9].

Retention time of analytes depends on the distribution coefficient between the two phases of chromatographic system, whereby, to separate the various components, the distribution coefficient value for analyte constituents of the sample should be sufficiently different to allow separation, but cannot be too low, which would lead to an incomplete retention, neither too high, which would result in prohibitive retention times [5].

As a consequence of differences in migration rate for the compounds along the chromatographic system, these are separated into discrete bands, allowing their identification and quantification [5].

Band dispersion corresponding to the elution of a solute should be constrained so that each solute is eluted slightly, which can be obtained by appropriate selection of the physical properties of the column, such as dimensions, diameter of particulate material, mobile phase flow rate, among others [9].

Broadening of spectral bands can occur because of problems outside the column, for example, related with the transportation of mobile phase on the open tubular systems such as on the injection system tubes, on the detection system and on the tubes that connect components of the chromatographic system [5]. The reason for broadening, in this case, is related to the occurrence of differences in flow rate between liquid layers adjacent to the tube wall and the layers at the center of the tube, because liquid moves faster in the center than in the peripheral zones of the tube [5]. In Gas

Chromatography (GC) this effect is overcome by diffusion, but diffusion in liquids is relatively slow, being checked more often the broadening of the bands [5].

The classification of chromatographic methods is based on the physical nature of mobile phase [2]. Thus, chromatographic methods can be divided into two types, liquid chromatography, wherein mobile phase is a liquid, and gas chromatography, wherein mobile phase is a gas [2]. In turn, both can be divided into two subgroups according to the physical nature of stationary phase, in particular gas-solid chromatography (GSC) and liquid-solid chromatography (LSC), if stationary phase is a solid, or gas-liquid (GLC), and liquid-liquid chromatography (LLC), in case of stationary phase is a liquid [9].

GC and LC techniques generally require a derivatization step to enhance the separation efficiency and the detection sensitivity of less volatile analytes, and to permit its separation in a reasonable period of time [5].

## 3.3. Liquid chromatography

Liquid chromatography coupled to mass spectrometry is a widely used technique for environmental analysis, given its capacity to identify and quantify a wide range of analytes such as primary pollutants and their transformation products [10].

Chromatography enables, sometimes in a one step process, the separation of a mixture in its individual components, yet providing a quantitative estimate of each constituent [9].

Two features of liquid chromatographic separation are the differential migration of compounds present in the sample, which is related to the different rates of the compounds when moving along the column, and the spreading of solute molecules along the respective column [6].

Main advantages of liquid chromatography include the reduction of analysis time, the convenience when it is desired to analyze complex mixtures of compounds, reduction of sample losses, and the ability to perform an accurate quantitation by the use of internal standards, among many others [2].

This technique also has several advantages towards GC, namely the fact that there are two phases in liquid chromatography capable of selectively interact with analytes, while in GC there is only one, as well as the existence of a wide variety of available stationary phases and the occurrence of separation at lower temperatures [6].

## 3.3.1. Chromatographic system

Generally, high performance liquid chromatography consists of five components, a reservoir of mobile phase, a pump, an injector, a column and a detector [2].

A pre-column of approximately 5 cm in length or a filter between the injector and the column may also be used to protect column from clogging caused by particles from solvent, sample, or the wear of the pump [3]. Pre-column can also provide some separation, and is generally composed of the same material used in the column [3]. If clogging occurs, pre-column can easily be removed, cleaned, and packed again with the adsorbent material [3].

### i. Pump

Pump must provide stable and constant flow rates [2], in a range between 0.1 and 10 ml/min, with the optimal flow rate being dependent on the type of interface used and the diameter of HPLC column [2]. Furthermore, it should allow the achievement of pressures in the order of 6000 psi and be corrosion resistant [5].

Provision of mobile phase to the column should be reproducible and because in LC columns are used small particles, which offer substantial flow resistance through the column, is necessary to use high pressure pumps [6], which is why the equipment for HPLC tends to be more elaborate and with higher costs than for other types of chromatography [5].

### ii. Injector

Injector used in HPLC, unlike in gas chromatography, is almost exclusively from one type, the loop injector of nominal volume [2]. Generally, the sample volume injected ranges from 5 to 50 µl [3].

The functionality of the injector is to introduce the liquid sample in a liquid stream [2]. While the loop is filled, mobile phase is pumped onto the column at a predetermined flow rate, enabling the equilibrium between column and mobile phase to occur [2]. Most systems, however, have nowadays automatic injection systems from sample vials [5].

Quantitative accuracy depends on the extent to which the loop is filled repeatedly, being important for their proper filling to ensure that no air bubbles are introduced, which may also affect the response stability of mass spectrometer [2].

### iii. Mobile phase

The relative interaction of analytes with both mobile phase and stationary phase will determine their retention characteristics [2].

HPLC requires that analytes are soluble in the mobile phase [2]. Sometimes to effect adequate separation of compounds, it is necessary to use a mixture of solvents, of variable composition, as a mobile phase, depending on the characteristics of the analytes in the mixture [2].

Viscous solvents are avoided since they require higher pressures and longer times to pass through the column, resulting in a broadening of the peaks and in a low resolution [3].

The elution of analytes can be isocratic, wherein composition of mobile phase is constant, or from a gradient, wherein composition varies over the time of separation [2].

Elution from a gradient is preferably used for the separation of mixtures of analytes having a wide range of characteristics [3]. In this type of elution, the elution bands that occur early in the chromatogram can be improved, and compounds retained more strongly to the column are eluted in a shorter time, being the corresponding bands shortened which results in an increased sensitivity [6].

Additionally, minor components that cannot be detected in isocratic separation may be detected when applying gradient [6]. The change of mobile phase composition during elution can be continuous or stepped [6]. Thus, gradient elution may permit the separation of analytes that are not separable by isocratic elution and to reduce the time required for separation [3].

Either in isocratic elution as in gradient elution, peak area increases significantly with the decrease of flow rate, whereas the height of the peak is, in turn, not greatly affected by the variation of this analytical parameter [11].

Modifications in mobile phase can lead to a reduction of peak height in the case of causing a greater retention of analytes in the column, with a consequent increase in the retention time, but without affecting however the peak area [6].

Buffers are often used to control the degree of ionization of analytes, providing a greater reproducibility of the retention and of the analytical response [2]. Generally, buffer solutions used in LC-MS are volatile, such as ammonium acetate, to prevent deposition of molecules at the interface and/or at the source of mass spectrometer, which would reduce the performance of the detector [2].

### iv. Stationary phase

With regard to stationary phase, is generally comprised of a chemically modified silica column, and the chemical modification determines the polarity of the column [2].

Columns used have generally a length of 10 to 30 cm and a diameter of 3 to 10 mm, being normally made of stainless steel [3].

The pH of buffer solution is extremely important for conservation of the column and is common to verify the occurrence of some degradation with the extensive use of columns, with consequent increase in the background noise and with a reduction of chromatography performance [2].

Column efficiency increases dramatically with the reduction of the bundled particles diameter [5]. However, the pressure difference required to maintain an acceptable flow rate of mobile phase is inversely proportional to the square of the particle diameter, which limits the particle size of the columns used [5].

The thickness of stationary phase affects the retention character and the capacity of column, being normally used thicker columns for more volatile analytes, allowing them to be retained for a longer period of time [5].

### v. Detection system

With regard to the detection, it is generally advantageous the coupling of a mass spectrometer, due to permit differentiation of compounds with similar retention characteristics and to identify and/or quantify components whose chromatographic resolution was weak or absent [2].

Globally the advantages of LC-MS are the possibility to detect and quantify a wide range of analytes, from molecular weights lower than 1000 Da to above 100000 Da, and the fact that mass spectrometer provide a more definitive identification of all HPLC detectors due to allow the determination of molecular masses of analytes [9]. Furthermore, the selectivity of mass spectrometer is high, enabling the quantification of analytes whose chromatographic resolution was poor and the use of isotopically labeled analytes as internal standards, which, with high sensitivity, enables quite accurate and precise quantitative determinations [9].

The efficiency of analytes separation by LC during analytical process depends on a variety of operating conditions, such as the column type, mobile phase used, length and diameter of the column, flow rate of the mobile phase, temperature, and sample volume, among others [6].

Furthermore, due to the possibility to occur evaporation, oxidation, among other changes in mobile phase, the solvent used should always be fresh in order to increase the reproducibility of separation [6].

Even small changes in mobile phase composition can cause significant changes in the retention time of analytes, resolution and height of the peaks [6].

The performance requirements of modern LC instruments include versatility on the type of samples that can be analyzed, low scan times, which implies the use of high efficiency columns and high mobile phase flow rates, reproducibility, the ability to control the operating parameters, sensitivity, well defined peaks and high response intensity in the detector [6].

LC-MS interface must also allow the possibility to choose the LC method and the operating mode of mass spectrometer, to increase analytical efficiency and to maintain the integrity of chromatographic separation [6].

The final goal is to achieve a highly efficient separation and quantification in order to obtain accurate quantitative data [6].

### 3.3.2. Classification of liquid chromatography

Liquid chromatography is often classified, based on the separating mechanism or type of stationary phase, in partition, adsorption, ion exchange, size exclusion, affinity, and chiral chromatography [5].

The latter three are not of great interest in analysis of aerosols, as size exclusion chromatography is generally used to solutes with molecular weights above 10000 Da, affinity chromatography is widely used for isolation of biomolecules, and chiral chromatography is applied for separation of enantiomers [5].

In this investigation project columns used were based on partition and eventually on adsorption chromatography, and for that reason only these types of chromatography techniques will be described in some detail.

#### i. Partition chromatography

Partition chromatography is a technique in which both stationary phase and mobile phase are two immiscible liquids [5].

Liquid sorbents are generally chemically bound to the column, resulting in extremely stable packaged phases, being these columns denominated liquid-bonded-phase columns [5]. Supports for the majority of bonded-phase packings are prepared from rigid silica or silica-based compositions, in the form of uniform, porous,

mechanically sturdy particles, commonly having diameters of 1.5-10 mm [5]. Silica surface is hydrolyzed and generally consists of chemically reactive silanol groups [5].

Partition chromatography can be classified into normal or reverse phase based on the relative polarities of mobile and adsorbent phase [5].

### *a) Normal-phase partition chromatography*

Normal-phase partition chromatography consists of using polar sorbent phases, while mobile phase is composed of relatively nonpolar solvents such as hexane [5]. The less polar components are eluted with lower retention times, while an increase of polarity of mobile phase results in a decrease of elution time [5].

In normal phase columns, the R group of siloxane is generally a polar functional group such as a cyano, amino or diol group [5]. In this study, however, this type of partition chromatography was not used.

### *b) Reversed-phase partition chromatography*

In reversed-phase chromatography the stationary phase is non-polar, usually consisting of hydrocarbons, being the mobile phase based on relatively polar solvents such as methanol or acetonitrile [5]. Thus, first eluted components are the most polar and increasing the polarity of mobile phase increases the elution time [5].

The R groups of siloxane used in reverse phase columns are usually n-octyl (C8) and n-octadecyl (C18) [5]. The advantages of using longer chains are that allows obtaining more retentive columns and using larger sample volumes [5]. The pH is an important variable for the stability of the column, because a pH greater than 7.5 can lead to the hydrolysis of siloxane groups or to silica solubilization in mobile phase as silicates, while a pH less than 2 can lead to acid hydrolysis of siloxane groups, with consequent deterioration or destruction of the column [5]

Ionic liquids (IL) can also be used in LC as a mobile phase and adsorbents [12]. The use of IL as stationary phase is particularly interesting, since it allows for greater selectivity [13].

Ionic liquid chromatography consists in a subset of reversed phase chromatography, in which easily ionizable species are separated on reverse-phase columns [5].

In this type of chromatography, is added to the mobile phase an organic salt containing an organic counter-ion of considerable size, such as a quaternary ammonium ion, which will act as an ionic pair [5]. Separation occurs when counter-ion forms a non-charged ionic pair with the ionic solute of opposite charge, which is then

partitioned by an apolar stationary phase, occurring the differential retention of analytes, depending on the affinity of ionic pair for the stationary phase [5]. Another alternative is the reversible binding of counter-ion to the neutral stationary phase giving it a specific charge and occurring, below, the separation of oppositely charged analytes by the formation of complexes between analytes and the counter-ion bounded to the stationary phase [5].

Most stationary phases used in Reversed phase-High Performance Liquid Chromatography (RP-HPLC) consist of imidazole or pyridine functionalized silica [13]. In turn, counter-ions generally consist of chloride, bromide, tetrafluoroborate and hexafluorophosphate, which have an important role in the separation process [13].

Silica based stationary phases are widely used due to its favorable physical characteristics, namely high mechanical strength, proper surface area and narrow size mesopore distributions [13].

The major advantages of reverse phase separation is the fact that water, an inexpensive and nontoxic solvent, can be used as the mobile phase and mass transfer is faster [5].

However, this type of columns also has an adverse effect related to the possible formation of residual silanols [13].

Solutes are eluted according to their increased distribution coefficients with concern to the mobile phase [9].

### *c) Hydrophilic interaction liquid chromatography*

Hydrophilic interaction liquid chromatography (HILIC) is a high efficiency liquid chromatography separation method that allows an effective separation of small polar compounds [14].

The mechanism of HILIC involves partitioning of analyte between mobile phase and stationary phase, although recent studies admit the contribution of both partition and adsorption processes [15].

Interaction forces responsible for the retention and selectivity of HILIC separations include hydrogen bonds, which depend on the acidity or basicity of the analytes, electrostatic interactions and dipole-dipole interactions [16].

HILIC stationary phases usually consist of silica in the silanol or siloxane form, or silica modified with polar functional groups, although polymer-based stationary phases can also be used [14]. Functional groups commonly used are diverse, and include diols, cyano, amino, amides or alkylamides groups, or even polymeric structures, among others [14].

With concern to mobile phase generally consists of polar water miscible organic solvents, such as acetonitrile, and a small percentage of water [17], although any aprotic solvent miscible with water can be used [14].

Relative strengths of solvents in the elution process of polar analytes can be summarized as follows:

Acetone<isopropanol-propanol<acetonitrile<ethanol<dioxane<DMF-methanol <water [14].

Generally additives are also used to increase separation efficiency [14]. Additives used to improve separation of ionizable analytes include buffers, such as ammonium acetate or ammonium formate, that will allow separation to occur at a defined ionic strength and pH [14]. The pH should be adjusted so that ionizable analytes are in their ionic form, making their retention in the separation column more effective [14]. Adverse effects of not use a buffer solution upon separation of analytes in ionic form include the obtainment of asymmetrically peaks, the occurrence of peak broadening or the poor recovery of analytes from stationary phase [14].

An increase of the concentration of buffer solution results in a decrease of the retention time of polar analytes, while a decrease of concentration results in an increased retention of said analytes [14].

For neutral analytes is unnecessary to use additives such as buffer solutions [14].

With regard to elution mode, HILIC separations can be performed in either isocratic or gradient elution, being that gradient mode consists in start elution with a high proportion of organic solvent, increasing then the proportion of an aqueous solvent for a defined period of time [17].

Some advantages of HILIC include greater sensitivity in ESI-MS, better retention on column for highly hydrophilic, ionic and polar compounds, shorter separation times by the possibility to use higher flow rates due to lower back pressures when using mobile phases with high organic and low water content [18], the ability to analyze compounds in complex systems, the good solubility of analytes in polar mobile phases used in HILIC, the fact that there is no need to use reactive ionic pair reagents that have a high cost, and the easy coupling of column to a mass spectrometer [14].

### ii. Adsorption chromatography

Adsorption chromatography can be classified in the same way as partition chromatography, being in this way governed by the same principles, but in this type of chromatography the adsorbent used is a solid phase [5].

### 3.3.3. Developing chromatographic method

The development of a chromatographic method tends to be more complex in LC than in GC, because in LC the components of the sample interact both with stationary phase as well as with mobile phase [5].

Selection of the column is based on the proper equilibrium of intermolecular forces between solute and stationary phase [5].

In turn, the selection of mobile phase is performed based on three parameters, namely, number of plates (*N*), retention factor (*k*), and selectivity factor (α) [5].

Before analytical determination of analytes, the method must be optimized by adjusting the operating conditions [19].

Most of analytical instruments have software that permits an automatic adjustment of some parameters, which gives maximum sensitivity and resolution as possible within a range of interesting values [19].

It is extremely important to subject the column to a purge step before performing the isolation and characterization of trace compounds [6].

Several factors determine the selection of the analytical method, such as the nature of the sample, the selectivity required for separation, the experimental convenience, the experience with the method, among many other considerations [6].

The knowledge of physicochemical properties of analytes in sample is also extremely important to select the method [6].

Even extremely efficient systems may not provide a desirable separation of mixtures containing a large numbers of compounds, and an optimal separation can be unsuccessful because selective separation can often be only optimized for simultaneous analysis of a limited number of compounds [6].

It is unlikely that the combination of stationary phase with mobile phase is adequate for all analytes present in the sample, and one should seek a compromise between them to allow reliable determination of the largest number of compounds as possible [6]. To increase efficiency, different mobile phase compositions, or columns of greater length and containing smaller particles should be tested to increase resolution [6].

In addition, for complex mixtures of analytes, it may be advantageous the combination of various chromatographic methods in order to take advantage of the selectivity inherent to each method [6].

## 3.4. Mass spectrometry

Mass spectrometry is a widely used technique in the analysis of trace organic compounds [19] and is probably the most powerful technique available for rapid identification and structural characterization of compounds [6].

The ionization techniques most commonly used are those based on electron ionization (EI) and laser ionization (LI) [20, 21].

EI has the advantage to produce an extensive ionization, but may provide a significant amount of fragment ions, which can be problematic in complex mixtures [22].

In turn, LI is a softer technique which produces less fragmentation and has a higher sensitivity and selectivity, having however some limitations as low repeatability of the laser energy or the fact that some aerosol sample molecules cannot absorb laser radiation at an applied specific wavelength causing the sample to be rapidly heated, which reduces its fragmentation [22].

After chromatographic separation and ionization, analytes are directed to one optical section, where solvent is evaporated and analytes are focused and accelerated toward the mass spectrometer [3].

MS is considered a universal technique of detection with potential for a highly selective mode of operation, being suitable for direct analysis of gaseous and particulate phases in the air due to their rapid response, excellent sensitivity and possibility to obtain structural information of the analytes under investigation [23].

Spectrum, depending on the ionization source used, may be constituted by one ion, often the molecular ion, or more ions including fragments of the molecular ion [19]. From spectrum, is then possible to obtain qualitative and quantitative information of sample analytes [23].

The use of chemometric tools for data analysis and use of Tandem techniques are extremely important to solve complex spectral information [23]. Furthermore, a high mass resolution, allowing to obtain the molecular formulas of analyte ions, associated with a soft ionization, to ensure a less fragmentation and less complex spectra, may make easier the analytical process [23].

The application of mass spectrometry to the analysis of atmospheric aerosols is a very efficient technique, which allows the analytical determination to occur in a reduced analysis time and, for some analytes, without the need for pretreatment of the sample [24]. Particles collected off-line on solid surfaces, such as filters or impactor plates, can be analyzed by MS using traditional desorption techniques or new desorption/ionization techniques, such as desorption atmospheric pressure photoionization (DAPPI) [23].

Desorption and ionization steps are extremely important for analysis of aerosols by mass spectrometry [22].

Recently, has been introduced new analytical methodologies based on online systems involving the integration of different analytical steps, such as extraction, washing, separation and detection in a closed system, usually automated [25]. These systems permit to correct several problems associated with analytical process performed off-line, adding benefits such as faster analysis, and more sensitivity and reliability [25].

Qualitative information about aerosols can thus be obtained on-line by various techniques of mass spectrometry [26]. However, off-line techniques continue to be generally used for that purpose [27].

For extremely polar compounds and non-volatile compounds, analysis cannot be performed by GC without prior chemical derivatization and are generally applied other techniques such as HPLC, ion chromatography, capillary electrophoresis, among others [24].

The intensity of mass spectrum peaks is dependent on factors such as the energy of ionization beam, the location of sample relative to the ionization beam, the pressure and temperature of the sample during analysis, the overall geometry of mass spectrometer, among others [5].

Frequently, chemical analysis of aerosols by MS is based on particle-to-particle analysis [11, 28], the main advantage of this technique being its real-time methodology [22]. Thus, a continuous flow of aerosols is directed to the mass spectrometer, while gas molecules are pumped out from the spectrometer inlet by a differential pump [22].

However, this technique has some disadvantages such as the need for high vacuum capacity, to allow sufficiently reduced pressures for MS analysis, and the loss of ultrafine particles of very small size which behave as a gas, being pumped out from spectrometer inlet by the differential pump [22].

### 3.4.1. Methods used for the detection and quantification

Methods used in this investigational project for analysis of aerosol particles were based in the coupling of a high performance liquid chromatograph with a mass spectrometer.

#### i. LC-MS

The most used method to study aerosols composition is GC-MS, especially given the ease of coupling GC with MS and the high availability of reference spectra for

this method [4]. However, GC-MS has an important drawback, namely the fact that extremely polar compounds generally have to be subjected to a chemical derivatization process, which, besides being time consuming, may affect the reproducibility of analysis, and residues of derivatization reagents may interfere with the analytical procedure [4].

Recently, LC-MS has taken an interest in analysis of polar compounds [4]. In this analytical method, using an ESI ionization source enables an efficient ionization of analytical compounds, while an ion-trap mass spectrometer (ITMS) allows obtaining a relatively high sensitivity in full-scan acquisition mode, as well as the ability to perform $MS^2$ analytical tests [4].

However, identification of compounds by ITMS can be difficult, especially in complex samples containing co-eluting compounds with the same molecular mass as the analytes, being used to counteract this limitation the Time-of-flight mass spectrometry (TOFMS), which allows to obtain greater sensitivity over the entire spectrum, high resolution power and a high accuracy in determination of molecular weight for obtaining the elemental composition of unknown compounds [4].

Thus, combination of LC-ITMS/MS with LC-TOFMS is a powerful tool for identification of unknown compounds in complex samples, where ITMS allows obtaining structural information about analytes while TOFMS provides accurate information about the molecular weight and the molecular formula of unknown compounds [4].

### 3.4.2. Ionization sources

The first step prior to obtaining a mass spectrum consists in the formation of analyte ions in gaseous form, being the appearance of spectrum obtained dependent on the type of ionization source employed [5]. The choice of ionization source in accordance to its sensitivity, linearity, repeatability and reproducibility is the key factor during the development of a method [29].

Traditionally, ionization technique involves the collision of electrons with organic compounds or other molecules, and their subsequent ionization, being the ionization products conducted subsequently to the detector and the spectrum of resulting fragments obtained [19].

There are two types of ionization methods, the gas-phase sources and the desorption sources [5].

In gas-phase sources sample is first vaporized and then ionized [5]. The ionization consists on the bombardment of compounds with a beam of electrons,

leading to the formation of the corresponding molecular ion, which given their excess energy will fragment, and consequently will permit to obtain a characteristic spectrum [19].

Gas-phase sources are especially suitable when analytes are able to lose electrons easily [19]. However, this technique has limitations, such as the fact that molecular ions, or some of the resulting fragments, are sometimes too unstable to be detected, or too stable for the occurrence of fragmentation [19]. Furthermore, leads to obtaining a spectrum with a higher degree of ionization, which can be a disadvantage since sensitivity decreases, and in case of absence of molecular ion in the spectrum another technique may have to be used [19].

In turn, in desorption sources, sample is directly converted into gas phase ions by supplying energy, in various forms, to the solid or liquid sample [5].

The greater advantage of desorption sources is their possibility of application to nonvolatile and thermally labile compounds, while gas-phase sources are almost restricted, when without application of derivatization, to the ionization of thermally stable compounds having a boiling point less than 500°C, which in practice implies that analysis is restricted to compounds with molecular weights below $10^3$ Da [5]. Furthermore, spectrum is very simple, generally constituted only by the molecular ion, which may be found also in its protonated form [5].

Ionization sources are also classified into soft or strong [5]. Soft ionization sources cause little fragmentation of analytes, while strong ionization sources impose strong enough energy to leave analytes in a state of high excitation, which leads to the breakage of bonds [5]. A high ionization essentially provides structural information of the compounds to be analyzed, whereas a soft ionization allows identifying the analyte, by obtaining accurate information about its molecular weight, and enabling their quantification [5].

Ionization can occur at high pressures or at atmospheric pressure (API), depending on the ionization source used [30].

A problem in analysis of aerosols is concerned with the ionization techniques based on pulses that, when applied, lead to a slowly mass scan rate [22].

### i. Atmospheric pressure ionization

Sources of atmospheric pressure ionization include more common techniques such as Atmospheric Pressure-Matrix Assisted Laser Desorption/Ionization (AP-MALDI), electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization [31].

Accordingly, ionization of sample takes place at atmospheric pressure, the ions being then transferred into the vacuum of a mass spectrometer by an atmospheric pressure interface [31].

Generally, since pressure difference is relatively high, are used two intermediate vacuum compartments between ionization source and mass spectrometer, connected by lenses with very small orifices, the skimmers [31].

The width of skimmers is an extremely important parameter that should be high enough to allow the entrance of as more analytes as possible to increase sensitivity, but small enough to maintain the proper vacuum [31].

The pressure of compartments is gradually reduced by high capacity pumps, and ions circulate in the intermediate compartments, with increasingly reduced pressure, through the skimmers until they reach the mass spectrometer [31].

Desorption should be maximized in order to gain sensitivity, and formation of clusters must be controlled since analytes must be in a stable and well defined form [31].

Another extremely important feature is the configuration of API source [31]. Firstly, API sources possessed an axial configuration, but, recently, orthogonal configuration has been widely adopted because, in this configuration, orifices are not saturated with solvent, so that only ions are directed to the inlet, allowing to increase the orifice size [31]. This increase in orifice size, combined with a reduction of noise, offsets transmission losses due to orthogonal configuration, allowing for greater sensitivity [31]. In addition, flow rates can be higher and orifice is better protected against possible contamination or clogging [31].

The great advantage of API is the easy on-line coupling of separation techniques with mass spectrometer [31]. Furthermore it allows the sample to be more easily introduced into the mass spectrometer, as the procedure of introducing the sample in a high vacuum is eliminated [31].

### *a) Electrospray ionization*

Electrospray ionization is one of the most used ambient temperature and pressure techniques, whose principle consists in pumping the sample solution at a rate of a few microliters per minute from a stainless steel capillary needle exposed to a considerable voltage of some kilovolts [5].

The applied voltage will lead to the formation of a charged spray comprising fine droplets of solution, which is directed to a desolvation capillary where evaporation of solvent occurs and where analytes acquire a charge [5].

As a consequence of evaporation drops became increasingly smaller and with a greater charge density, until they reach a point called Rayleigh limit, where surface tension no longer supports the charge and a Coulombic explosion occurs leading to a division of the drops into small droplets [5]. This process is repeated until all solvent is removed and analytes became multiple charged [5].

Mass spectrum is characterized by its low fragmentation, even for molecules of considerable size and thermally labile, as there is little energy retained by analytes after ionization [5].

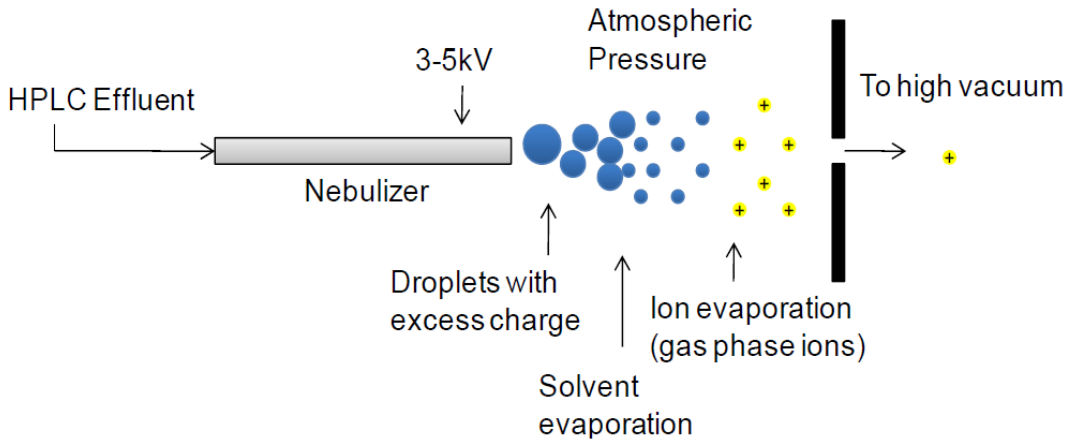


**Figure 4 – Electrospray Ionization [32].**

The greater advantage of this technique, in addition to the wide range of analytes that can be ionized, is the fact that ions formed are multiple-charged, so that its m/z value is small enough to allow detection of sample analytes with a wide range of molecular weights [5]. Another important advantage is the possibility to coupling ESI with HPLC or capillary electrophoresis columns [5].

Due to the low fragmentation associated, the structure of analytes is difficult to identify, and tandem MS can be applied for that purpose [5].

### 3.4.3. Mass spectrometers

Chromatographic techniques are usually based on retention time for identification of sample compounds, which is problematic in terms of accuracy [19]. The use of a mass spectrometer as a detector after application of chromatographic techniques enables to obtain a spectrum which, depending on the compound and the ionization mode, will allow achieving a more precise analytical result [19].

In mass spectrometry, ions are distributed according to their mass/charge ratio [5]. Ideally, this analytical instrument should be able to distinguish small differences in molecular mass and to permit the passage of a sufficient amount of ions to generate an ion current that can be measured faster [5].

Applications of MS are innumerous [5]. The identification of pure compounds is possible by determination of their molecular weight, their molecular formula, for evaluating fragmentation patterns in order to obtain information about its functional groups, or by comparing the analyte mass spectrum with those obtained in previous studies [5]. The analysis of mixtures is another application of MS by coupling with a chromatographic technique, implementing a tandem technique or by coupling a tandem with a chromatographic technique [5].

The main advantages of MS are the ability to identify various fragments of a molecule, to analyze small sample volumes [6], and the qualitative specificity and quantitative sensitivity of information obtained [19].

Some of disadvantages are related to the limited databases containing information about organic compounds of analytical interest, to the fact that for some compounds the relative abundances of ions in the spectra may vary slightly depending on the instrumental conditions, and the harder differentiation between isomers when compared to other conventional techniques [19].

Quantification can be made from a single ion, a group of ions, or using the total detected signal [19].

Mass spectrometer is the suited detector for LC since it allows to obtain both quantitative and structural information about sample analytes [30]. However, there are some problems in their coupling such as the fact that there is a difference of pressures conventionally used in HPLC and MS, generally being about two to three orders of magnitude higher in HPLC, or the difficulty to vaporize thermally labile analytes, requiring the installation of an interface that involves a compromise between its operating conditions [30].

Mass spectrometers designed to couple with HPLC require also a vacuum system designed to produce extremely low pressures in all instrument components to avoid collisions of analytes with atmospheric constituents [5].

### i. Ion-Trap Spectrometers

Ion-trap analyzers consist of a ring-shaped central electrode and a pair of electrodes around it [5]. A voltage is then applied to the central electrode, which leads to the circulation of ions of a given m/z in a stable orbit at the cavity around the ring [5]. As the voltage is increased, the orbits of ions with higher molecular weight became stable, whereas the lighter ions collide with the walls of the ring-shaped electrode [5].

Ions can be stored for a large period of time and in a wide mass range, being then ejected to the transducer by a technique called mass-selective ion ejection,

consisting of a sequential ejection of ions according to their mass by increasing the high frequency voltage applied to the ring electrode [5].

The advantages of Ion-Trap analyzers are their robustness and low cost compared to other mass analyzers and the low detection limits [5]. Moreover, sensitivity difference between full-scan mode and monitoring mode for a selected ion is small [19]. However, ions can only be scanned for a limited range of mass per charge unit [19].

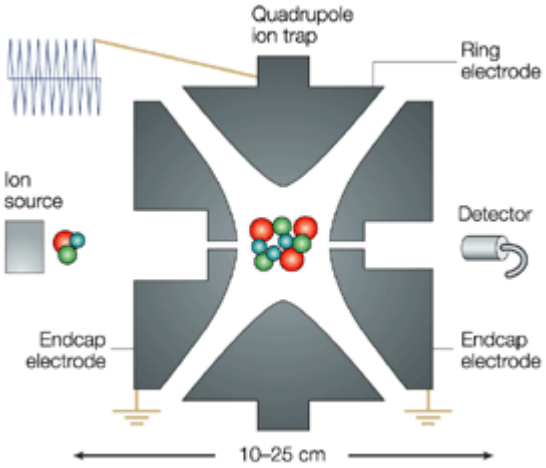


**Figure 5 – Ion trap [33].**

## ii. Quadrupole spectrometers

This analytical tool comprises four parallel cylindrical rods, which function as electrodes [5]. Opposed cylindrical rods are electrically connected, being one pair connected to the positive terminal of a voltaic source and the other to the negative terminal [5]. Ions are accelerated between the cylindrical rods by a potential difference of +5 to +10 V, being dc and ac voltages on the rods increased simultaneously to maintain their ratio constant [5]. Only ions of a certain m/z ratio hit the transducer, and the remaining ions are converted to neutral molecules by impact with the cylindrical rods [5].

This technique allows to obtain several analytical information, such as the elemental composition of the sample, the molecular structure of the compounds, the qualitative and quantitative composition of complex samples, and the ratio of isotopic atoms in the sample, among others [5].

Quadrupole technique is widely used in the analysis of aerosols [22].

The advantages of its use include low scanning time, particularly useful for coupling chromatographic methods [5], allowing to reduce the analysis time [19], and their robustness [5].

### 3.4.4. Tandem mass spectrometry

Tandem mass spectrometry is an analytical method that consists in multiple steps of mass spectrometry selection [19], allowing to obtain a mass spectrum of pre-selected fragment ions [5].

It is a widely used technique in the treatment of complex samples, particularly in samples containing many interferents [19].

Thus, MS acts as a pre-washing stage, and may replace or be performed before GC [19].

Sample is injected and eluted, and analytes to be determined are primarily ionized as gently as possible, being the non-fragmented molecules sent to the mass spectrometer [19]. Then, the non-fragmented molecules, denominated precursor ions, are forwarded to an interaction cell [5]. In this interaction cell, analytes are spontaneously decomposed or fragmented by collision with a reagent gas or with a beam of laser radiation, and resulting fragments, the product ions, are sent to a second mass spectrometer where they are detected [19].

There are two types of tandem spectrometry, namely Tandem mass spectrometry in space and in time [5].

In the first type, two independent mass analyzers are used in two distinct spatial regions [5]. An example of these instruments is the triple-quadrupole [5].

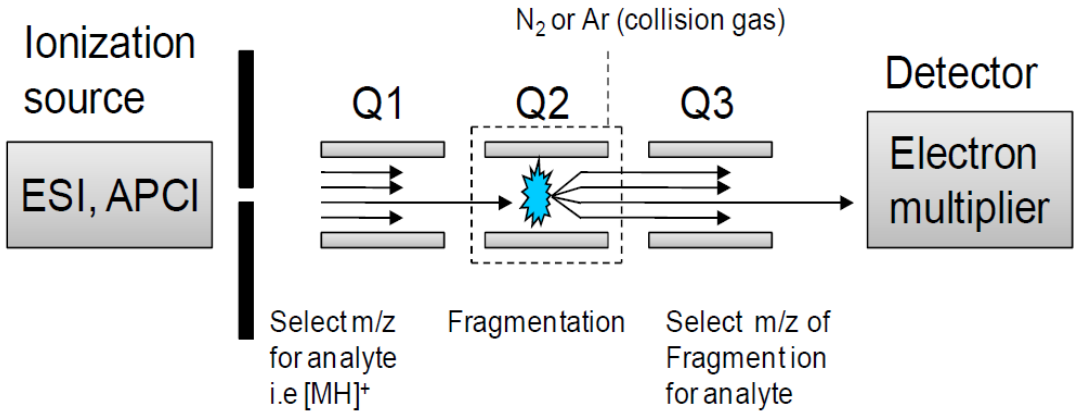


**Figure 6 – Triple-Quadrupole mass spectrometer [32].**

In this instrument, sample is introduced into a soft ionization source, and resulting ions are accelerated towards the first quadrupole which acts as a filter [5]. Then, the selected ions are forwarded to the second quadrupole, consisting of a collision chamber where dc voltage is not applied, occurring the dissociation of pre-selected ions in the first quadrupole [5]. The function of this quadrupole is to capture the precursor ions and their products at a relatively high concentration of collision gas [5]. Finally, in the third quadrupole, precursor ions and product ions formed in the collision

cell and selected are analyzed [5].In turn, Tandem mass spectrometry in time consists in the formation of ions in a given spatial region, being removed after a certain period of time the ions without analytic interest, while selected ions are dissociated and analyzed in the same spatial region [5].

This technique allows obtaining easily a spectrum of the product ions, however, the determination of precursor ions is harder than in tandem in space [5].

These processes may be repeated to allow for $MS^n$ analytical determinations [5].

Tandem techniques are extremely useful for the differentiation of analytes with the same molecular weight due to each analyte has a unique and characteristic fragmentation pattern [23].

### 3.4.5. Acquisition modes

#### i. Full scan mode

In full scan mode the entire mass range is analyzed, and full spectrum obtained allows unequivocal identification of compounds [19].

However, this mode has lower sensitivity since the number of ions to be analyzed is greater, so that the time spent by the detector to each individually ions is lower [19].

#### ii. Selected ion monitoring mode

In the selected ion monitoring mode (SIM), instrument is programmed to analyze only certain ions of analytical interest [19]. When sample composition is unknown, this method is not so advantageous, but is a good technique to confirm the presence of certain ions in the sample, having high signal intensity [19].

By performing SIM technique, the detection limit can be improved about 10 times on quadrupole instruments, being these instruments particularly suited for acquisition of specific ions in different areas of the chromatogram, allowing the analytical determination of samples containing a large number of analytes [19].

#### iii. SrM and MrM modes

Data acquisition mode for analytical quantitation of fragment ions of analytes by tandem instruments is known as selective reaction mode (SrM) [34].

When fragment ions of more than one analyte are simultaneously analyzed by LC-MS/MS, acquisition mode is, therefore, denominated multiple reaction monitoring (MrM) [34].

During the analysis of aerosol particles, given the fact that they consist of several compounds of analytical interest, MrM mode is normally used for the instrumental analysis process.

## 3.5. Identification and quantification of analytes

The chromatogram obtained using a chromatographic technique provides only some qualitative information about sample species, such as their retention times under certain experimental conditions, that can permit the determination of the presence of certain compounds whose identities are known or determine its absence or presence in amounts below the detection limit if sample does not produce a peak at the same retention time corresponding to the standard [5].

However, the use of an analytical detection method such as mass spectrometry allows the obtainment of a greater amount of information about sample constituents [5].

Quantification by MS consists in optimize the analytical conditions of mass spectrometer in order to measure one or more defined m/z values, being the ionic current measured as a function of time, originating a graphic that consists of a series of peaks characteristic of the ions to be analyzed [5].

Peak area obtained is directly proportional to the concentration of sample components, allowing a quantitative determination of analytes from the sample [5]. Another method of quantification is the determination of concentrations from peak height of analytes [5].

As for certain mixtures it is possible to identify the peaks corresponding to specific m/z values of sample components, it is also possible to prepare a calibration curve for determination of unknown analytes, being necessary to use an internal standard to improve the accuracy of obtained results [5].

Quantification is based on the comparison of the values obtained for analytes peak area with peak area values obtained for external standards solutions with a concentration close to the analytes, to obtain a calibration curve consisting of the signal intensity as a function of concentration [5].

The height of the peak can be used as an alternative to the area value with the advantage that its value is more easily measured and more accurate for peaks without broadening [5]. However, obtained value is inversely proportional to the width, whereby to obtain accurate results it is necessary that conditions used for the column do not cause variation of the width of the peak during standards and sample analysis period [5]. Also, peak height is conditioned by changes in retention time and efficiency of the

column, but the area value is considered more suitable since it is independent of spectral band broadening effects [5].

The addition of an internal standard and determination of analyte or external standard area divided by internal standard area will make analysis more precise, since uncertainties associated to the introduced volume are avoided [5]. However, for comparison to be effective, the peak corresponding to the internal standard must be well separated from peaks obtained for other sample constituents [5].

Accuracy and precision of results depend on the proper control and definition of analytical operations, from sampling to data processing [6]. In particular, accuracy of results obtained is highly conditioned by the ability to perform a calibration of the system with reliable standards and by minimizing the overlapping of spectral bands [6]. The definition of appropriate operating conditions, in turn, is essential for obtaining a good precision [6].

It is also important to perform the analysis of zero samples to ensure a sufficient reduction of interfering effects during analysis [6].

Reproducibility is a major problem in trace analysis [6]. Relative reproducibilities of approximately 10% are considered acceptable for analysis of compounds present in trace amounts [6].

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# 4. Experimental procedure

## 4.1. Chemical products

The chemical products used in this investigational study included 15 amines and 16 acid compounds used for preparation of standards, to the analysis of amines and acids in atmospheric aerosol samples.

Solvents used for preparation of samples included methanol (Sigma-Aldrich) and acetonitrile (VWR). For the extraction process were used methanol (Sigma-Aldrich), ammonium acetate (Sigma-Aldrich) and deionized water. In the derivatization process, chemical products used were dansyl chloride (Sigma-Aldrich), borate buffer prepared from di-sodiotetraborato-10-hydrate (Merck), acetone (Merck) and deionized water.

As mobile phase, apart from acetonitrile (VWR) were also used acetic acid (Merck), ammonium acetate (Sigma-Aldrich), formic acid (Sigma-Aldrich) and ammonium formate (Sigma-Aldrich) in deionized water.

The deionized water used both in sample preparation, mobile phase and in the derivatization process was previously ultrapurified in a Milli-Q system (DirectQ-UV, Millipore Corp., USA). Additionally, all reagents and solvents used were of analytical grade or HPLC suitable.

### 4.1.1. Acids

The acids studied are shown in Figure 7. These have a diversified structure, from aromatic and non-aromatic short chain acids to long-chain acids.

The study of these acids is related to the fact that they are often found in atmospheric aerosol particles because of its low saturation pressure, giving them the ability to condensing/partitioning in pre-existing particles or to form new nuclei, which leads to the formation of secondary organic aerosols.

In Table 1 are expressed the molecular weight, purity, CAS number, safety and risks and producer for all acids.

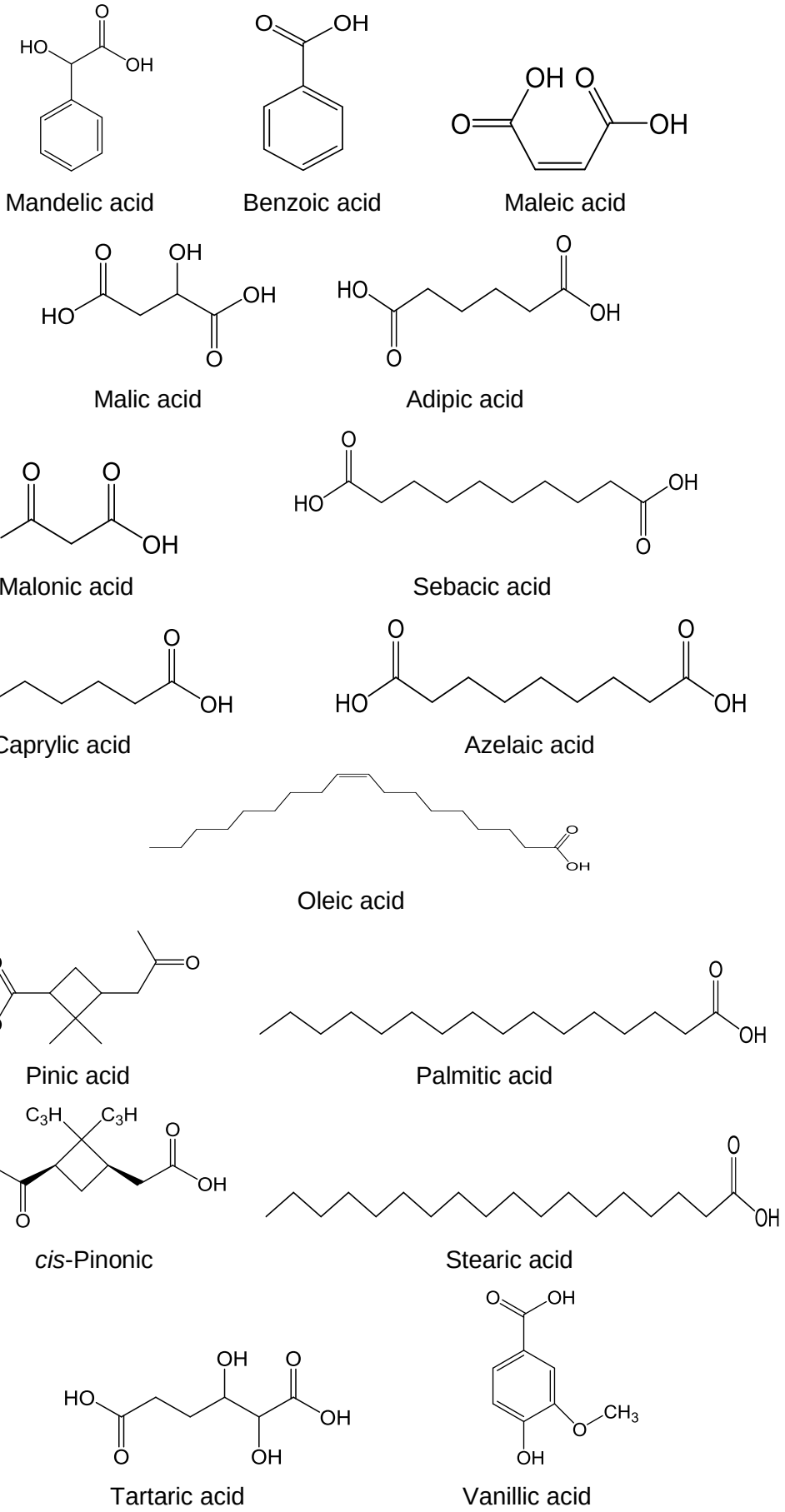


**Figure 7 – Acids studied in the project.**

Table 1 – Acids studied and major features.

| Chemical product | MW (DA) | Purity | CAS number | Safety & risks | Producer |
|---|---|---|---|---|---|
| **Adipic** | 146.14 | ≥ 99% | 124-04-9 | R: 36<br>S: 26 | VWR |
| **Azelaic** | 188.22 | 98% | 123-99-9 | R: 36/37/38<br>S: 24/25-36-26 | Sigma-Aldrich |
| **Benzoic** | 122.12 | ≥ 99.5% | 65-85-0 | R: 22-36-42/43-36/37/38-40-63-43-23/24/25-45-41-37/38-20/21/22<br>S: 26-45-37/39-24-22-36/37-24/25-23-53-36 | Sigma-Aldrich |
| **Caprylic** | 144.21 | ≥ 99.0% | 124-07-2 | R: 34<br>S: 26-36/39-45-36/37/39-25-27 | Sigma-Aldrich |
| **Maleic** | 116.07 | ≥ 99% | 110-16-7 | R: 22-36/37/38<br>S: 26-28-37-28[a] | Sigma-Aldrich |
| **Malic** | 134.09 | ≥ 99% | 6915-15-7 | R: 22-37/38-41-36/37/38<br>S: 26-39-37/39-36 | Sigma-Aldrich |
| **Malonic** | 104.06 | ≥ 98% | 141-82-2 | R: 20/22-41-36/37/38-22<br>S: 26-36/39-37/39-36 | Sigma-Aldrich |
| **Mandelic** | 152.15 | ≥ 99.5% | 90-64-2 | R: 36/37/38-22<br>S:22-24/25-37/39-26-36 | VWR |
| **Oleic** | 122.12 | ≥ 99% | 112-80-1 | R: 23/24/25-34-40-43-36/37/38-38<br>S: 36/37-37/39-26-36 | Sigma-Aldrich |
| **Palmitic** | 256.42 | 99% | 57-10-3 | R: 36-36/38<br>S: 26-37/39 | Sigma-Aldrich |
| **Pinic** | 186.00 | | unknown | | |
| ***cis*-Pinonic** | 184.23 | 98% | 61826-55-9 | R: 36/37/38<br>S: 26-36 | Sigma-Aldrich |
| **Sebacic** | 202.25 | 99% | 111-20-6 | R: 36/37/38<br>S: 26-36 | Sigma-Aldrich |
| **Stearic** | 284.49 | ≥ 99.5% | 57-11-4 | R: 38-36/37/38-11<br>S: 37/39-26-16 | Sigma-Aldrich |
| **Tartaric** | 150.09 | ≥ 99.7% | 526-83-0 | R: 36 | Sigma-Aldrich |
| **Vanillic** | 168.14 | ≥ 97.0% | 121-34-6 | R: 36/37/38<br>S: 37/39-26-36 | Fluka |

### 4.1.2. Amines

The amines studied are represented in Figure 8. As for acids, the choice of these amines is due to be often found in aerosol particles, possibly participating in particle growth process.

However, further studies are being performed to confirm that functionality.

In Table 2 are expressed the molecular weight, purity, CAS number, safety and risks and producer for all amines.

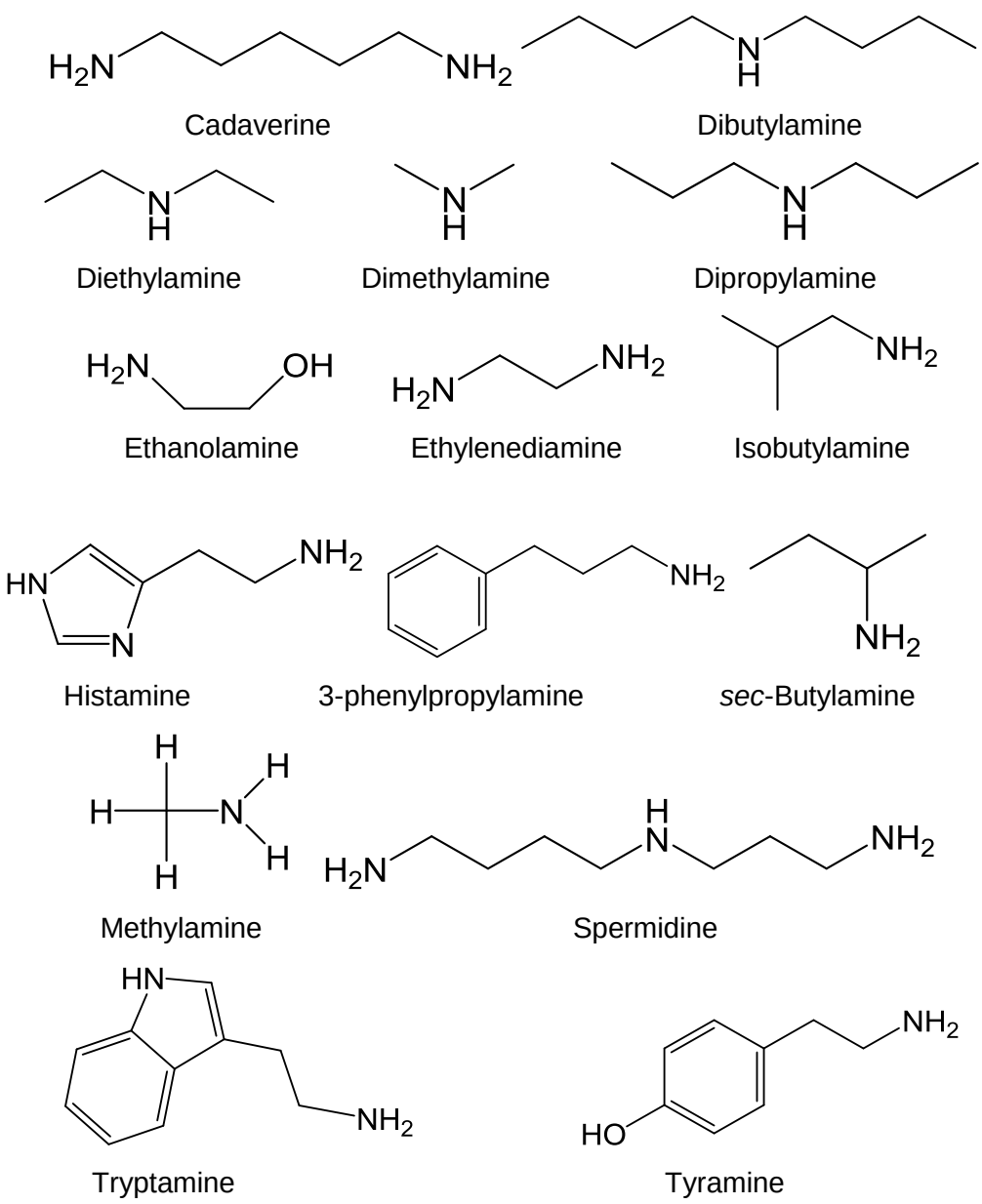


**Figure 8 – Amines studied in the project.**

**Table 2 – Amines studied and major features.**

| Chemical product | MW (DA) | Purity | CAS number | Safety & risks | Producer |
|---|---|---|---|---|---|
| **Cadaverine** | 102.18 | ≥ 99.0% (AT) | 462-94-2 | R: 34-36/37<br>S: 26-36/37/39-45-25-27 | Fluka |
| **Dibutylamine** | 129.24 | ≥ 98.0% | 111-92-2 | R: 10-20/21/22<br>S: 45-36/37/39-28A-26-23 | Fluka AG Buchs AG |
| **Diethylamine** | 73.14 | ≥ 99.7% (GC) | 109-89-7 | R: 11-20/21/22-35<br>S: 16-26-29-36/37/39-45-3 | Fluka |
| **Dimethylamine** | 45.08 | 33% (in absolute methanol) | 124-40-3 | R: 12-20-37/38-41-34-20/22-11-39/23/24/25-23/24/25<br>S: 3-16-26-29-36/37/39-45-39 | Sigma-Aldrich |
| **Dipropylamine** | 101.19 | ~99.0% (GC) | 142-84-7 | R: 11-20/21/22-35<br>S: 16-26-36/37/39-45 | Fluka AG Buchs AG |
| **Ethanolamine** | 61.08 | ≥ 99.5% | 141-43-5 | R: 20/21/22-34-39/23/24/25-23/24/25-10<br>S: 26-36/37/39-45 | Sigma-Aldrich |
| **Ethylenediamine** | 60.10 | ≥ 99.5% (GC) | 107-15-3 | R: 10-21/22-34-42/43<br>S: 23-26-36/37/39-45 | Fluka |
| **Histamine dihydrochloride** | 111.15 | ≥ 99.0% (AT) | 51-45-6 | R: 22-36/37/38-42/43<br>S: 22-26-36/37 | Fluka |
| **Isobutylamine** | 73.14 | ≥ 99.5% (GC) | 78-81-9 | R: 11-22-35<br>S: 26-36/37/39-45 | Fluka |
| **Methylamine** | 31.06 | 40% (in $H_2O$) | 74-89-5 | R: 12-20-37/38-41-34-20/22-11-39/23/24/25-36/37/38-23/24/25<br>S: 7-16-26-36/37-45-29-36/37/39-3/7-3-39-33 | Sigma-Aldrich |
| **3-phenyl Propylamine** | 135.21 | ≥ 98.0% (GC) | 2038-57-5 | R: 34-37<br>S: 26-36/37/39-45 | Fluka AG |
| ***sec*-Butylamine** | 73.14 | ≥ 98.0% (GC) | 13952-84-6 | R: 11-20/22-35-50<br>S: 9-16-26-28-36/37/39-45-61-28A | Fluka |
| **Spermidine** | 145.25 | ≥ 99.5% (GC) | 124-20-9 | R: 34<br>S: 26-36/37/39-45-27 | Fluka |
| **Tryptamine** | 160.22 | > 98.0% | 61-54-1 | R: 20/21/22-36/37/38-41-37/38-22<br>S: 24/25-36/37/39-36-26 | Fluka |
| **Tyramine** | 137.18 | ≥ 99.0% | 51-67-2 | R: 36/37/38<br>S: 26-36-37/39 | Fluka |

### 4.1.3. Other Chemicals

The chemicals used in the process of extraction and preparation of samples are shown in Figure 9. All chemicals have an adequate purity for HPLC analysis.

In Table 3 are expressed the molecular weight, purity, CAS number, safety and risks and producer for all chemicals.

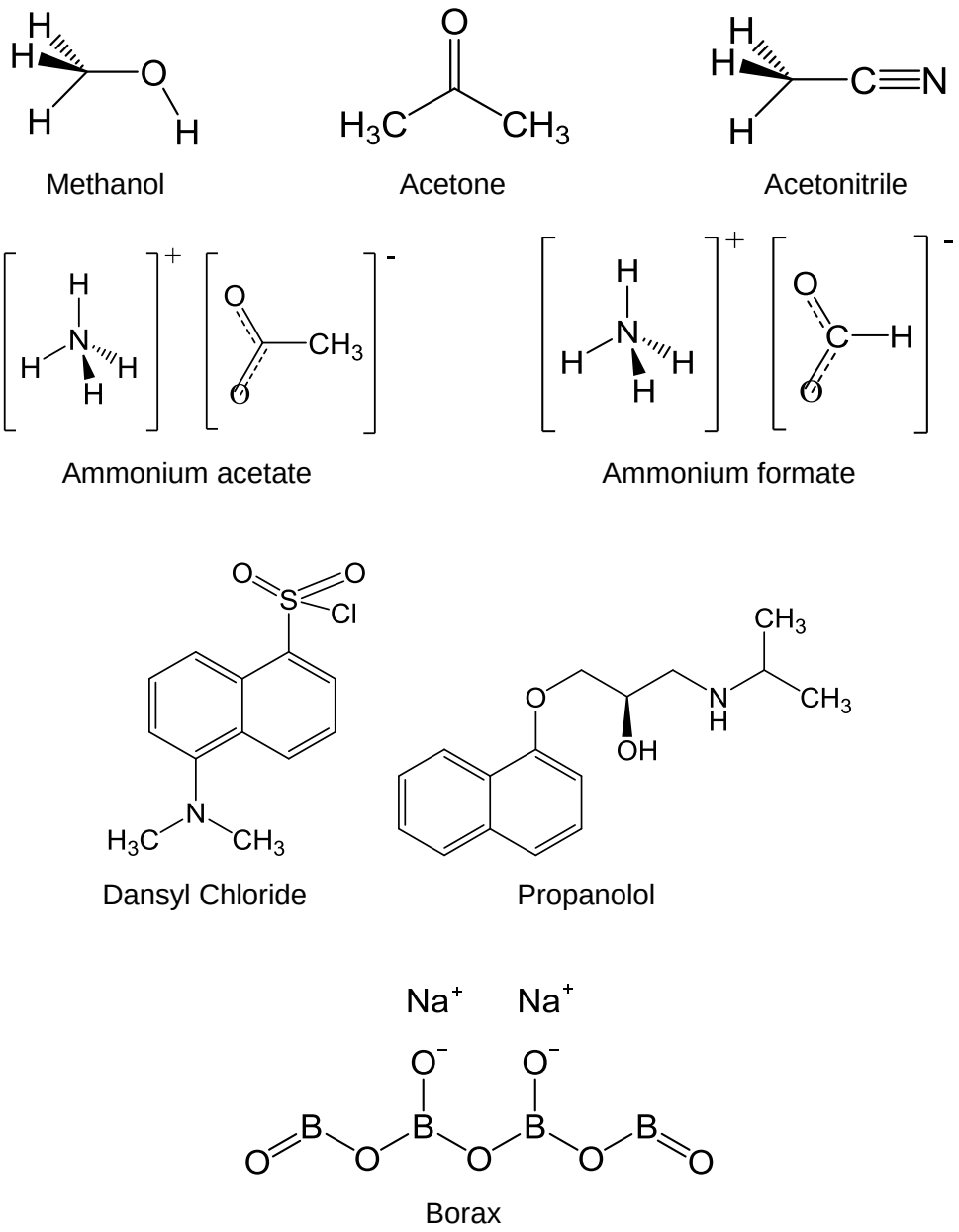


**Figure 9 – Other chemicals used in the research project.**

Table 3 – Other chemicals used.

| Chemical product | MW (DA) | Purity | CAS number | Safety & risks | Producer | Function |
|---|---|---|---|---|---|---|
| **Acetone** | 58.08 | ≥ 99.7% (for HPLC) | 67-64-1 | R: 11-36-66-67-39/23/24/25-23/24/25 S: 9-16-26-45-36/37 | J. T. BAKER | Solvent |
| **Acetonitrile** | 41.05 | ≥ 99.9% (for HPLC) | 75-05-8 | R: 11-36-20/21/22-10-36/37/38-23/24/25-41-24-20/22 S: 16-36/37-45-36/37/39-27-26-36 | VWR | Solvent |
| **Ammonium acetate** | 77.08 | ≥ 98.0% | 631-61-8 | S: 24/25 | Sigma-Aldrich | Buffer |
| **Ammonium formate** | 63.06 | ≥ 99.995% | 540-69-2 | R: 36/37/38 S: 26-36-37/39 | Sigma-Aldrich | Buffer |
| **Dansyl Chloride** | 269.75 | ≥ 99.0% | 605-65-2 | R: 34 S: 26-36/37/39-45-3 | Fluka | Derivatization |
| **Disodium tetraborate (for borate preparation)** | 201.22 | ≥ 99.5% | 1330-43-4 | R: 62-63-36/38-36/37/38 S: 36/37-24/25-26-36-23 | Merck | Buffer |
| **Methanol** | 32.04 | ≥ 99.9% (for HPLC) | 67-56-1 | R: 10-20/21/22-68/20/21/22-39/23/24/25-23/24/25-11-40-36-36/38-23/25 S: 36/37-7-45-16-24/25-23-24 | Sigma-Aldrich | Solvent |
| **Propanolol hydrochloride** | 295.8 | | 3506-09-0 | R: 22 | | Internal Standard |

## 4.2. Analytical instrumentation

The instrumentation used for analysis of amines and acids consisted in a high performance liquid chromatograph-mass spectrometer.

For acids, the HPLC instrument was a Hewlett Packard Series 1100 (USA) that consisted of an autosampler, a quaternary pump and a vacuum degasser system.

The Mass spectrometer used was a Bruker Esquire 3000plus (Bruker Daltonics®, USA) coupled with an ion trap ionization source.

Chromatographic separation was performed using a Shiseido HPLC Packed Column (Shiseido®, Japan). The length of the column was 150 mm, with an inner diameter of 4.6 mm and 5 µm of particle size.

**Figure 10 – Hewlett Packard Series 1100 HPLC and Bruker Esquire 3000plus mass spectrometer.**

In turn, for amines, the HPLC used was an Agilent 1260 Infinity (Agilent®, USA), and consisted of an autosampler, a binary pump and vacuum degasser system. Mass spectrometer used was an Agilent 6400 Series Triple Quad LC/MS (Agilent®, USA).

The chromatographic column was an Agilent Poroshell 120 EC-C18 HPLC column (Agilent®, USA), and the length of the column consisted of 50 mm, with an inner diameter of 3.0 mm and 2.7 µm of particle size. An Agilent Poroshell 120 EC-C18 UHPLC Guard pre-column (Agilent®, USA) was also used. The length was 5mm with an inner diameter of 3.0 mm and 2.7 µm of particle size.

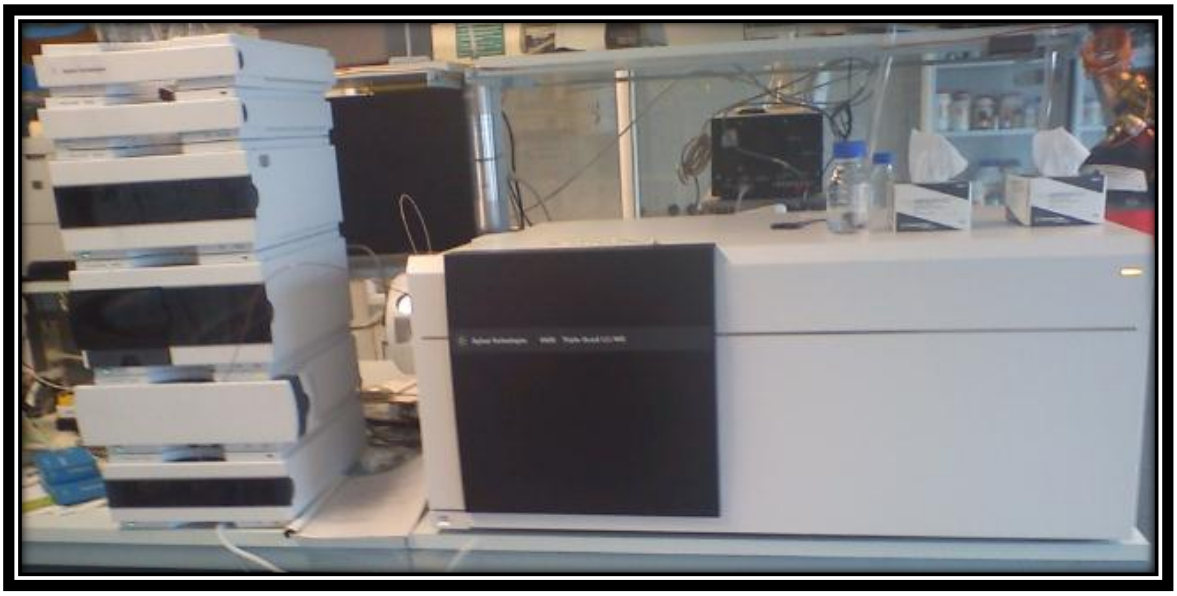

**Figure 11 – Agilent 1260 Infinity HPLC and Agilent 6400 Series Triple Quad LC/MS mass spectrometer.**

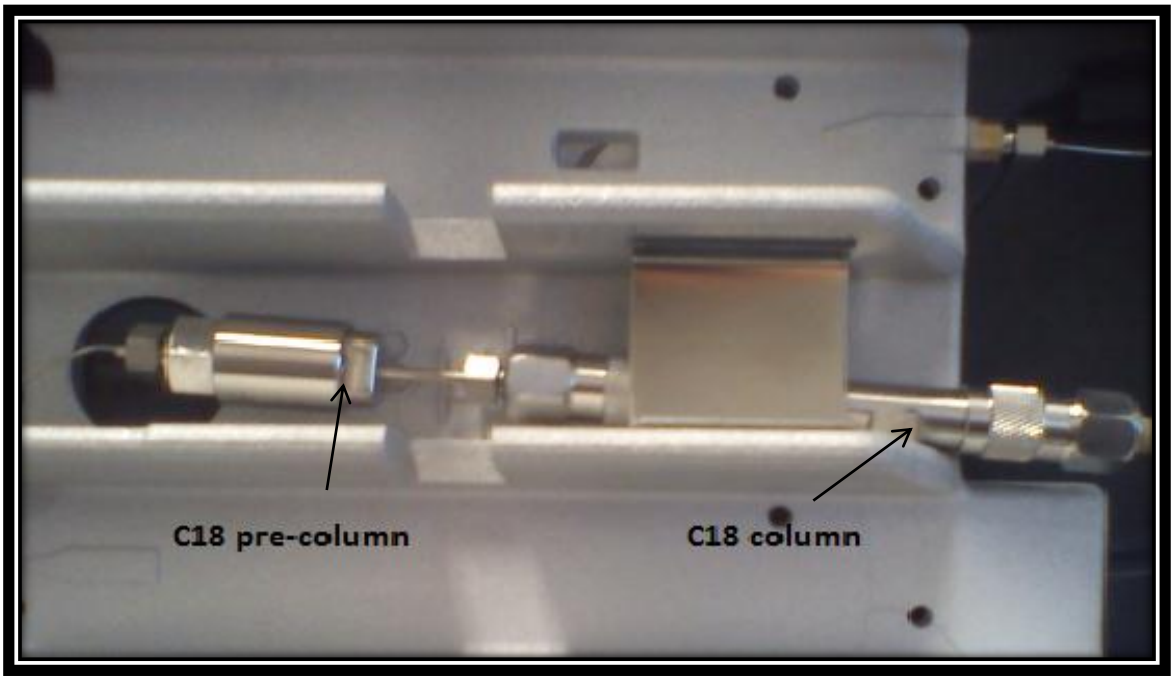


**Figure 12 – Agilent Poroshell 120 EC-C18 UHPLC Guard pre-column and Agilent Poroshell 120 EC-C18 HPLC column.**

Nitrogen was used as a nebulizing and drying gas for both techniques.

## 4.3. Sampling procedure

Samples of atmospheric aerosol particles and total suspended particles were collected from atmosphere. For this purpose were used two types of collecting devices, a vacuum pump and a DMA. The samples were collected with the purpose of being used in future investigational studies. For this study, were used samples previously collected in the boreal forest of Hyytiälä.

### 4.3.1. Vacuum pump sampling

The first step of sampling process consisted of turning on the vacuum pump, being checked if there was any air leak. Then the filter was placed on a suitable support. Sampling was made using Whatman™ GE Healthcare quartz filters with 47 mm of diameter, due to the wide diversity of possible interactions with analytes and their high surface area which allows adsorbing a greater amount of aerosols, although having also the disadvantage of adsorbing more quantity of artifacts from gas phase.

Finally, was proceeded the collection of particles during a given period of time by placing the support outside from a window of the laboratory, being the filter changed at the start of working hours and at its end.

After the corresponding collection period, the filter was removed with a tong and divided in half, one half being intended for analytical determination of aerosol particles and the other to determine the total suspended particles.

Both filters were placed in a BD Vacutainer® vacuum tube, it was registered the type, time and date of sampling, and samples were stored in the freezer to prevent degradation and contamination. Also to prevent contamination of samples, all steps were carried out using gloves.

### 4.3.2. Differential mobility analyzer sampling

Aerosols with a size of 30 nm were collected by a differential mobility analyzer. DMA sampling process consisted of placing a filter, with a properly cleaned tong, in the two DMA supports, one for collection of aerosol particles and the other to collect total suspended particles.

Filters used were Durapore® Membrane Filters of 0.45µM HV and 47mm of diameter, being the filter material consisted of PTFE. The choice of such filters is related to their good aerosol particles adsorption capacity and their perfectly fitting to the support.

After a certain time, the filters were removed and placed in distinct BD Vacutainer® vacuum tubes, using the same procedure as for the samples collected by using a vacuum pump.

## 4.4. Optimization of amine sampling process

In order to optimize the process of collecting amine compounds present in aerosol particles, it was tested two distinct types of filters, namely PTFE and Quartz, immersed in a solution of acetonitrile and Amberlite XAD-7.

Amberlite XAD-7 is an extremely absorbent nonionic polymeric resin that allows the pre-concentration of certain compounds.

It was also tested which is the number of immersions that provide the best results, by immersing both filters in the acetonitrile and XAD-7 slurry for one, three and eight times.

The procedure consisted in using an apparatus, containing a vacuum pump, a filter holder and an air flow meter. Then, the filter was placed on the filter holder, the air was blown into the air flow meter and has been registered the value obtained.

The next step consisted in measuring air flow after the connection of the meter device to the vacuum pump. The measurement was performed in the presence and absence of each type of filter, and by recording the values obtained by blowing and using a vacuum pump was possible to evaluate the existence and extent of back pressure, which must be as small as possible in order to increase the efficiency of the collecting process.

Results obtained are shown in the following Table.

**Table 4 – Air flow obtained for PTFE and Quartz Filters by blowing and with a vacuum pump.**

| Type of filter | Number of immersions | Air flow by blowing/ $L.min^{-1}$ | Air flow with vacuum pump/$L.min^{-1}$ |
|---|---|---|---|
| **Without filter** | | 6.50 | 22.00 |
| **PTFE** | Blank | 1.75 | 17.15 |
| | 1 | 1.60 | 17.50 |
| | 3 | 1.60 | 17.60 |
| | 8 | 1.50 | 16.80 |
| **Quartz** | Blank | 5.20 | 20.75 |
| | 1 | 5.20 | 21.00 |
| | 3 | 5.20 | 21.05 |
| | 8 | 5.20 | 20.90 |

As can be seen in Table 4, the air flow does not vary significantly with the number of immersions in the slurry, whereby any of the options tested can be used.

However, quartz filters showed a better performance because it allowed to obtain higher air flows, which demonstrates the existence of a reduced back pressure comparing to PTFE filters. Thus, quartz filters proved to be more suitable for collecting aerosols than PTFE filters and should be preferably used in future procedures to collect aerosol particles.

## 4.5. Extraction of analytes by dynamic ultrasound assisted extraction

The analytes retained on the filter during sampling process need to be extracted from the filter to a solution in order to make possible their instrumental analysis. In this study the technique used for this purpose was the ultrasound assisted extraction.

Firstly, the filters were placed in an extraction chamber, using sterile tongs. Next, the extraction chamber was immersed in an ultrasonic bath, and the analytes were extracted with a solution consisting of 50% methanol and 50% of another solution composed of 1% acetic acid in water, at a flow rate of extraction of 0.5 mL/min, for 20 minutes.

The use of methanol as an extraction fluid is related to the fact that was the same solvent used for preparing standard solutions, because of the high solubility of analytes in this solvent, while the use of acetic acid in water is justified by the fact that extracted samples are used not only for analysis of amines but also for the separation and analysis of acids by LC-MS using a C18 column, so it is desirable that acid analytes are in the neutral form. Because the derivatization of amine constituents was effected prior to their instrumental analysis, acetic acid has no implications in their separation and analysis.

All components of ultrasonic bath and extraction chamber liable to cause contamination of the samples were thoroughly cleaned with methanol. To evaluate the occurrence and extent of contamination were also analyzed three blanks consisting of a clean filter which was not subject to the sampling process.

After the extraction process, samples were evaporated by a stream of nitrogen at 40°C to achieve a final volume of approximately 1 mL.

Ultrasonic bath and nitrogen evaporator devices are represented in figure 13.

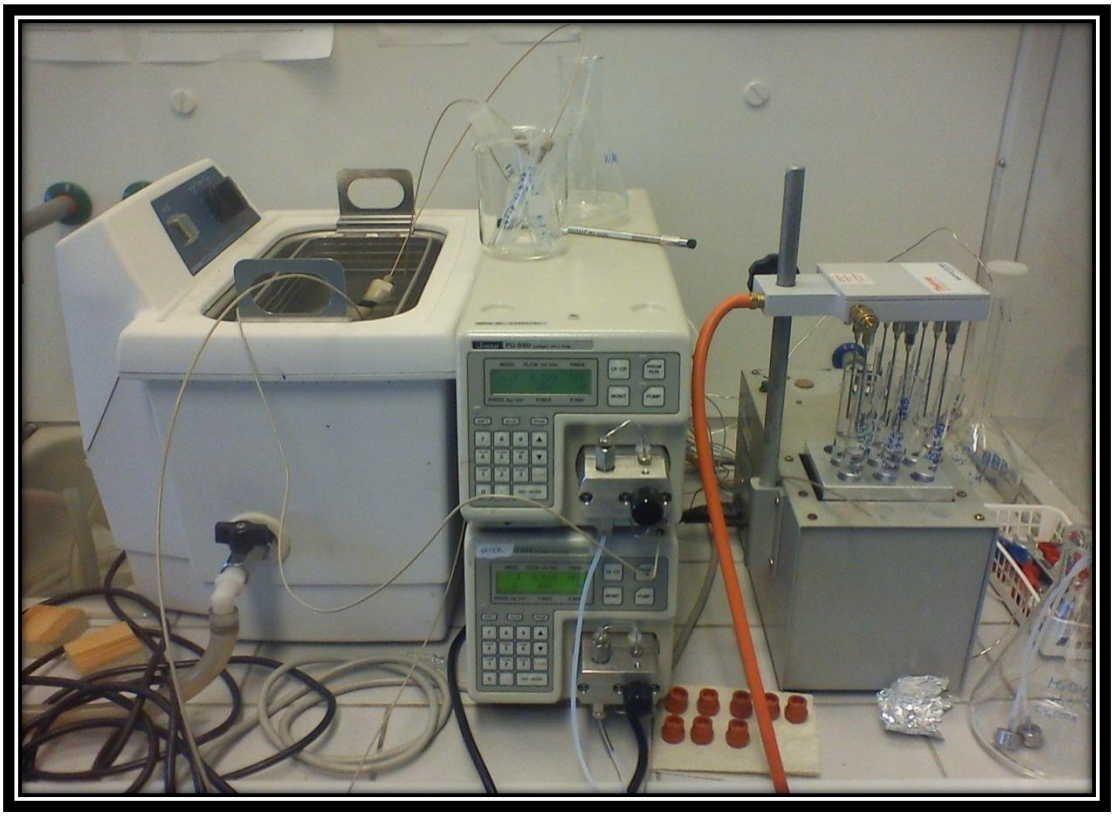

**Figure 13 – Ultrasonic bath and nitrogen evaporator.**

## 4.6. Pre-analytical procedure

Before starting the process of instrumental analysis, must be undertaken the maintenance of analytical instruments. To this purpose, ESI source was properly cleaned before analysis of the samples with a solution of 50% Milli-Q water and 50% isoprene.

The volume of solvent must be also checked, as well as all analytical parameters.

Instrumental components must be verified to detect irregularities such as the presence of bubbles in the tubes or an inappropriate nebulizer gas flow rate.

After the change of solvents, is necessary to perform a purge process. Then, is proceeded the conditioning of the column, which can take between 15 to 45 minutes depending on the flow rate used.

Before starting the analysis, it is also necessary that solvent flow rate and pressure stabilize, after which it is possible to ensure a greater reproducibility of the results.

When the experimental conditions are well defined and stable, can then be initiated the process of analysis.

## 4.7. Data Acquisition

Data acquisition is an important step to the analysis of compounds under investigation. For that purpose, different softwares were used for acids and amines.

To analyze acids were used two softwares, namely the HY-STAR® and the EsquireControl®.

HY-STAR® controls the operation of chromatograph, allowing to define the analytical and experimental conditions such as the volume injected, the flow rate of the mobile phase, the type of elution (isocratic or gradient), the total time of chromatographic separation, the maximum and minimum pressures, the temperature of the oven, among others.

EsquireControl®, in turn, controls the operation of mass spectrometer, since it allows to perform functions such as tune, optimization of operating parameters of spectrometer, definition of the polarity (positive or negative), the scan time, the pressure of nebulizer and drying gas, the choice of the analysis method, among others.

In turn, in case of amines, was used for data acquisition the Agilent Mass Hunter Workstation Data Acquisition® software to control the operation of the chromatograph and mass spectrometer. This software allows setting and controlling the most important experimental parameters and operating conditions cited above for acids.

## 4.8. Data processing

In order to process the data obtained from instrumental analysis of acids in study was used the Bruker Daltonics Data Analysis software®.

Mass spectrum is obtained by recording the signal intensity as a function of time. From the program is then possible to integrate the spectral peaks for a given value of m/z, being the values obtained for samples subtracted with the value obtained for the blank, which allows to remove the signal obtained for interferents and thus increasing the accuracy of the analytical process.

In case of amines are used two softwares for processing data, the Agilent MassHunter Qualitative Analysis® and the Agilent MassHunter Quantitative Analysis®. In qualitative analysis is possible to observe and analyze the mass spectrum, allowing to verify which compounds are detected and to proceed to the integration of individual peaks, in order to check the value of their area and the retention times, among other features. In turn, the software Agilent MassHunter Quantitative Analysis® permit to select the method of analysis, automatically determine the concentration, retention time,

analytical response and accuracy, set and adjust the calibration curve, among other options for more specific functional convenience.

# 5. Results

## 5.1. Analysis of acid compounds in aerosol samples

During the development of a separation and determination method to analyze acid compounds in samples of atmospheric aerosol particles by LC-MS, was proceeded firstly the optimization of analytical parameters.

### 5.1.1. Mass spectrometer parameters

The first step before using LC-MS analytical instrument is calibration. Calibration was performed automatically in EsquireControl® software.

Then, it was proceeded the optimization of mass spectrometer parameters. To this end, 100 mL of each analyte was directly injected by a Cole-Parmer® syringe pump into the mass spectrometer and the optimal values of the parameters automatically determined from the optimization menu of EsquireControl® software.

Then, in order to set values of conformity to allow an optimized fragmentation for most acid compounds studied, is normally used the average of the obtained values for a group of compounds within a defined retention time range, thus obtaining the values of parameters to be applied during the process of instrumental analysis in MRM mode.

The optimized values obtained for each compound are shown in Table 5.

### 5.1.2. Optimization of separation method

Chromatographic separation of the compounds of analytical interest is affected by a variety of factors and experimental conditions.

In this study was developed a separation method with the use of an HILIC stationary phase type column, by optimizing various factors, in particular the composition of buffer solution, pH and flow rate in order to find the most effectively experimental conditions.

For that purpose, was used a standard solution containing the compounds to be analytically determined from aerosol samples. Some of the acid solutions used were already prepared in methanol, the remainder having been prepared in acetonitrile. Then, from acid solutions, it was subsequently prepared a solution in acetonitrile containing all compounds of analytical interest at a final concentration of 20 µg/mL.

**Table 5 – Optimized mass spectrometer parameters.**

| Analyte | Molecular ion/ (m/z) | Skimmer/ V | Cap exit/ V | Oct1 DC/ V | Oct2 DC/ V | Trap drive | OCT RF/ Vpp | Lens 1/ V | Lens 2/ V |
|---|---|---|---|---|---|---|---|---|---|
| **Adipic** | 145.14 | -23.5 | -91.7 | -4.60 | -0.46 | 33.9 | 87.5 | 4.3 | 56.5 |
| **Azelaic** | 187.22 | -30.6 | -100.0 | -8.90 | 0.00 | 23.5 | 116.7 | 4.0 | 55.0 |
| **Benzoic** | 121.12 | -15.0 | -83.3 | -4.60 | 0.00 | 38.5 | 50.0 | 5.5 | 85.0 |
| **Caprylic** | 143.21 | -40.5 | -100.0 | -10.25 | 0.00 | 20.4 | 300.0 | 2.0 | 40.0 |
| **Maleic** | 115.07 | -57.5 | -154.2 | -12.30 | -1.46 | 20.4 | 58.3 | 0.0 | 20.5 |
| **Malic** | 133.09 | -30.6 | -50.0 | -4.60 | -0.54 | 41.3 | 50.0 | 3.5 | 64.0 |
| **Malonic** | 103.06 | -41.9 | -66.7 | -11.27 | -4.60 | 24.5 | 50.0 | 1.5 | 100 |
| **Mandelic** | 151.15 | -26.3 | -75.0 | -6.91 | -0.38 | 33.6 | 79.2 | 6.5 | 61.0 |
| **Oleic** | 281.46 | -19.3 | -87.5 | -8.19 | -1.07 | 44.1 | 287.5 | 3.3 | 73.0 |
| **Palmitic** | 255.42 | -15.0 | -170.8 | -7.94 | -1.00 | 29.4 | 50.0 | 3.8 | 52.0 |
| **Pinic** | 185.20 | -33.4 | -116.7 | -4.60 | 0.00 | 19.9 | 79.2 | 2.5 | 59.5 |
| ***cis*-Pinonic** | 183.23 | -27.8 | -125.0 | -4.60 | -0.69 | 23.1 | 62.5 | 3.0 | 49.0 |
| **Sebacic** | 201.25 | -15.0 | -87.5 | -8.19 | 0.00 | 28.0 | 120.8 | 9.5 | 100 |
| **Stearic** | 283.48 | -27.8 | -162.5 | -20.00 | -1.69 | 39.3 | 233.3 | 0.0 | 43.0 |
| **Tartaric** | 149.09 | -22.1 | -87.5 | -10.76 | -4.60 | 30.2 | 91,7 | 2.8 | 100 |
| **Vanillic** | 167.14 | -23.5 | -91.7 | -4.60 | -0.46 | 33.9 | 87.5 | 4.3 | 56.5 |

Sample consisted of 50 µL of standard solution and 50 µL of mobile phase in the same proportion used during the analytical process, being the sample volume injected of 25 µL.

A solvent, namely acetonitrile, and a buffer solution constituted the mobile phase. Buffer solutions used were ammonium acetate and ammonium formate, being the choice of these solutions related with the fact that they are volatile compounds, facilitating their vaporization process.

Nebulizer gas pressure was set to 40 psi, and the drying gas flow rate was 8 L/min at a drying temperature of 320°C.

All the defined parameters are represented in Table 6.

Table 6 – Analysis parameters.

| ESI | |
|---|---|
| **Polarity** | Positive |
| **Scan range (m/z)** | 50-400 |
| **Averages** | 2 |
| **Max. accumulation time (ms)** | 20.00 |
| **ICC Target** | 8000 |
| **Source** | |
| **HV Capillary (V)** | +3800 |
| **HV End Plate Offset (V)** | -500 |
| **Nebulizer (psi)** | 50.0 |
| **Dry Gas (L/min)** | 4.0 |
| **Dry Temp (ºC)** | 320 |
| **Expert Parameter setting** | |
| **Skimmer (V)** | -40.0 |
| **Cap exit (V)** | -101.0 |
| **Oct 1 DC (V)** | -12.00 |
| **Oct 2 DC (V)** | -1.70 |
| **Oct RF (Vpp)** | 122.9 |
| **Trap Drive** | 37.5 |
| **Lens 1 (V)** | 5.0 |
| **Lens 2 (V)** | 60.0 |

Initially, it was possible to obtain a separation method by gradient elution according to the Table 7. The buffer solution used initially consisted of ammonium formate with a concentration of 0.006 M and pH 5.0, and the flow rate of mobile phase was 0.7 mL/min.

Table 7 – Gradient elution for acids separation using ammonium formate buffer.

| Initial time/ min | Final time/ min | % Acetonitrile | % Ammonium formate buffer solution (pH 5.0) |
|---|---|---|---|
| 0 | 4 | 100% | 0% |
| 4 | 10 | 90% | 10% |
| 10 | 14 | 80% | 20% |
| 14 | 35 | 80% | 20% |

However, during the analysis of the sample, occurred problems related with the use of a mixture consisted of ammonium formate and formic acid as an additive for pH adjustment, due to the occurrence of precipitation in the system tubes.

Therefore, was proceeded to the replacement of ammonium formate by ammonium acetate, with a concentration of 0.020 M and pH 4.5, being the flow rate of mobile phase 0.5 mL/min.

. It was then developed a separation method from a standard solution containing all the acid compounds to be determined analytically from samples of atmospheric aerosol particles, at a concentration of 5 µg/mL for each compound.

The method consisted of a gradient elution according to the following Table.

**Table 8 – Gradient elution for acids separation using ammonium acetate buffer.**

| Inicial time/ min | Final time/ min | % Acetonitrile | % Ammonium acetate buffer solution (pH 4.5) |
|---|---|---|---|
| 0 | | 95% | 5% |
| 0 | 12 | 60% | 40% |
| 12 | 13 | 95% | 5% |
| 13 | 16 | 95% | 5% |

Eleven acids were detected and most of them well separated with the method developed. The exception were Oleic, Palmitic and Stearic acids that, due having very similar and large structures, and consequently similar and high pKa values, didn't provide a good separation. Mandelic and Vanillic acids also have quite similar structures and didn't show a good separation but, because pKa values are fairly different and their peaks were narrow and well-defined, the separation obtained was probably enough to permit the unequivocally analysis of these compounds.

Chromatogram obtained is represented in Figure 14.

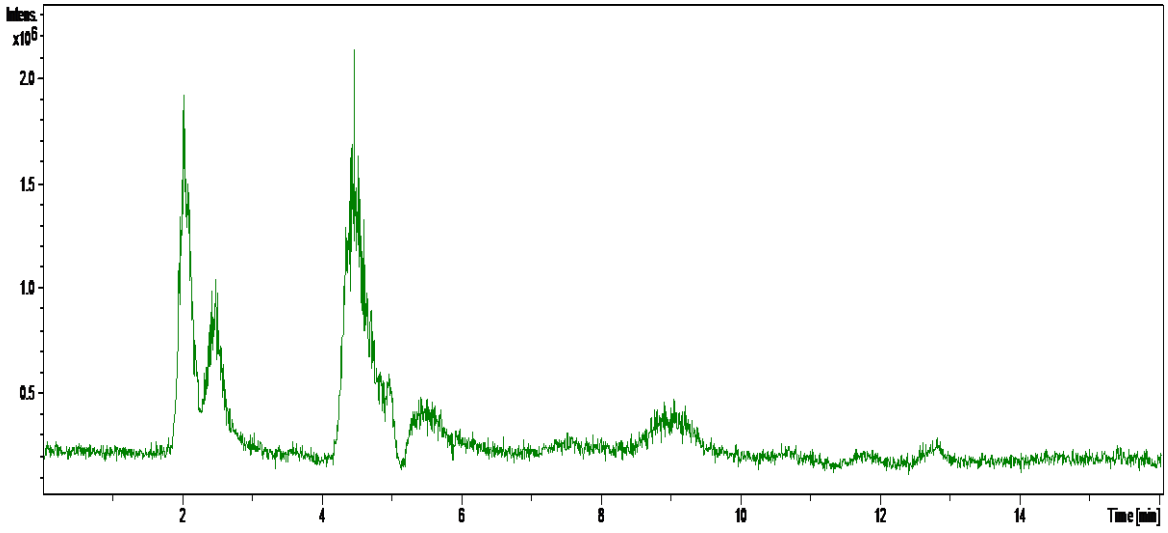


**Figure 14 – Chromatogram obtained for acids.**

Standard deviation and relative standard deviation were calculated from ten samples.

Although separation has been quite satisfactory as well as the repeatability obtained for retention times of analytes, as shown by the standard deviation and the relative standard deviation values, the same did not happen to the peak area values as presented in the following Table.

**Table 9 – Repeatability of the method. Number of samples analyzed in brackets.**

| | | Average (10) | | | σ | | Rsd | |
|---|---|---|---|---|---|---|---|---|
| **Compound** | **Acidity ($pK_a$)** | **Molecular ion/ $g.mol^{-1}$** | **RT/ s** | **Area** | **RT/ s** | **Area** | **RT/ %** | **Area/ %** |
| **Azelaic acid** | 4.6; 5.5 [1] | 187.22 | 11.8 | 967148 | 0.2 | 172607 | 1.75 | 17.85 |
| **Benzoic acid** | 4.2 [2] | 121.12 | 8.6 | 1176748 | 0.2 | 126428 | 1.97 | 10.74 |
| **Maleic acid** | 1.9; 6.1 [3] | 115.07 | 2.5 | 5847198 | 0.0 | 1492936 | 0.97 | 25.53 |
| **Mandelic acid** | 3.4 [4] | 151.15 | 9.1 | 2144739 | 0.1 | 59516 | 0.95 | 2.77 |
| **Oleic acid** | 9.8 [5] | 281.12 | 4.3 | 4047261 | 0.1 | 959963 | 1.75 | 23.72 |
| **Palmitic acid** | 9.7 [6] | 255.42 | 4.5 | 2776333 | 0.1 | 548677 | 1.68 | 19.76 |
| **Pinic acid** | 4.6 [7] | 185.00 | 12.7 | 1461552 | 0.3 | 195583 | 2.12 | 13.38 |
| ***cis*-Pinonic acid** | 4.6 [8] | 183.23 | 7.7 | 5739035 | 0.3 | 639630 | 4.38 | 11.15 |
| **Sebacic acid** | 4.7; 5.4 [1] | 201.25 | 10.7 | 523930 | 0.2 | 89123 | 1.85 | 17.01 |
| **Stearic acid** | 10.2 [5] | 283.49 | 4.3 | 1538728 | 0.1 | 369556 | 1.51 | 24.02 |
| **Vanillic acid** | 4.5 [9] | 167.14 | 8.9 | 2122300 | 0.2 | 260631 | 1.89 | 12.28 |

However, because the column used had already some lifetime, the study would have to be continued with a new column as the verified poor repeatability of the area can be associated with a possible degradation of the column. Furthermore, the column used for separation can have smaller particle size to increase the retention efficiency of analytes, and an internal standard can be added to the sample in order to improve the repeatability.

Also, the study of fragment ions resulting from MRM analysis of analytes would have to be developed, due to enabling compounds with the same ratio m/z to be identified and quantified, and to decreasing the uncertainty associated with the identification of the compounds studied because each analyte has a characteristic spectral fragmentation pattern.

## 5.2. Amine analysis

### 5.2.1. Characterization of the method

For analysis of amines by LC-MS has been used a previously developed method and a standard solution containing all the amines of analytic interest possibly present in aerosol samples, total samples, and gas-phase samples to define a calibration curve. Samples were analyzed in Dynamic MrM mode.

The volume of sample injected was 50 µL and the flow rate was set to 0.3 mL/min.

A column consisted of a C18 type stationary phase was used as well as a pre-column containing the same material to filtrate the solvents.

Nebulizer gas pressure was set to 30 psi and the drying gas flow rate to 8 L/min being the drying temperature 300°C. With concern to the Capillary voltage used was 4000 V in positive mode. The value for the Cell Accelerator Voltage was 7 and for the fragmentor was set to 135 for all compounds.

A gradient elution was applied, according to the Table below and analysis parameters are represented in Table 11.

**Table 10 – Gradient elution for amines separation.**

| Initial time/ min | Final time/ min | % Acetonitrile | % Acetic acid (1% in water) |
|---|---|---|---|
| 0 | | 50% | 50% |
| 0 | 10 | 90% | 10% |
| 10 | 11 | 50% | 50% |
| 11 | 15 | 50% | 50% |

Table 11 – Analysis parameters.

| Compound | Retention time/min | Mw (DA) | $Mw_{Dansyl\ derivates}$ (DA) | Percursor ion $[M+H]^+$ (m/z) | Product ion (m/z) | Collision energy |
|---|---|---|---|---|---|---|
| **Cadaverine** | 7.6 | 102.18 | 568 | 569 | 170 | 30 |
| **Dibutylamine** | 10.4 | 129.24 | 362 | 363 | 157 | 20 |
| **Diethylamine** | 6.1 | 111.15 | 306 | 307 | 157 | 20 |
| **Dimethylamine** | 3.9 | 73.14 | 278 | 279 | 157 | 20 |
| **Dipropylamine** | 8.5 | 45.08 | 334 | 335 | 157 | 20 |
| **Ethanolamine** | 1.6 | 101.19 | 294 | 295 | 157 | 20 |
| **Ethylenediamine** | 6.4 | 61.08 | 526 | 527 | 293 | 15 |
| **Histamine** | 7.9 | 60.10 | 344 | 345 | 170 | 20 |
| **Isobutylamine** | 5.4 | 73.14 | 306 | 307 | 157 | 20 |
| **Methylamine** | 2.4 | 31.06 | 264 | 265 | 157 | 20 |
| **3-phenylpropylamine** | 7.1 | 135.21 | 368 | 369 | 157 | 25 |
| **Propanolol** | 9.2 | 259.34 | 492 | 493 | 170 | 25 |
| ***sec*-Butylamine** | 5.3 | 73.14 | 306 | 307 | 157 | 20 |
| **Spermidine** | 10.2 | 145.25 | 611 | 612 | 193 | 35 |
| **Tryptamine** | 5.4 | 160.22 | 393 | 394 | 144 | 10 |
| **Tyramine** | 9.8 | 137.18 | 603 | 604 | 170 | 30 |

### 5.2.2. Quantification of analytes

In order to obtain a calibration curve that allows the quantitative determination of analytes under study was prepared a solution of amines in methanol, containing all the compounds to be determined analytically in a final concentration of 100 µg/mL.

From the mentioned solution, standards were then prepared in a concentration range corresponding to the expected concentrations for the samples. The concentrations of standards were 10 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL, 400 ng/mL e 500 ng/mL.

All standards contained borate buffer, at pH 9.32, to promote the protonation of amines and, thereby, improve its retention in the chromatographic column and subsequent separation.

Was also added dansyl chloride, from a solution prepared with 25.0 mg of compound in 5 mL acetone, for derivatization of analytes. Derivatization enabled the increase of molecular mass, which is determinant for the analytical detection of the compounds because the instrument can only detect analytes whose mass is in the

order of 50 m/z. In addition, this analytical step reduces the polarity of molecules, improving its retention in the column. Derivatization process occurs in an ultrasonic bath during 30 minutes at 35ºC.

Dansyl chloride was prepared every day of sample preparation due to be photosensitive and rapidly degraded. For the same reason was also protected from the light using aluminum foil and stored in the refrigerator.

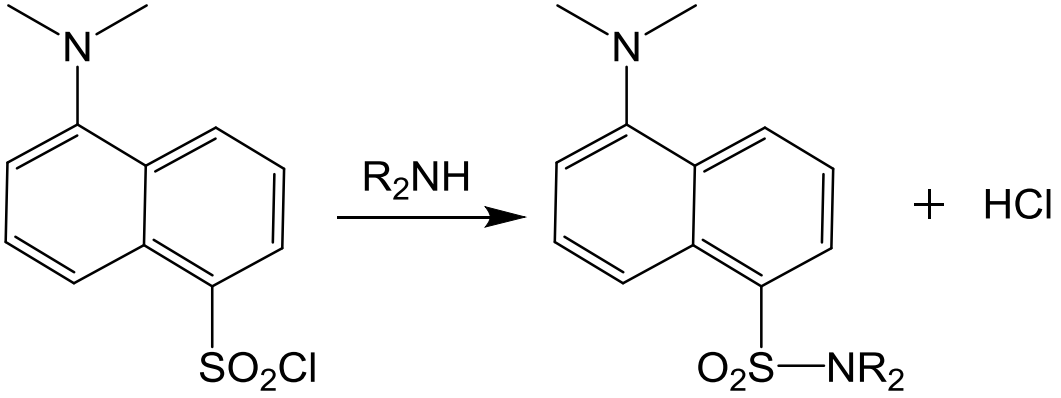


**Figure 15 – Derivatization reaction.**

Finally, was added as an internal standard propanolol from a methanol solution with concentration of 640 µg/mL. This step allows increasing the accuracy of the analytical determination by reducing uncertainties associated with the processes of preparation and sample introduction. Various final concentrations of propranolol were tested and was evidenced that the concentration of 5 µg/mL was the most appropriate, due to having the best response intensity.

Standards and samples were analyzed in the same conditions, and prepared containing 125 µL of standard or sample obtained by extraction of compounds from the respective filter, 125 µL of internal standard, 100 µL of borax buffer solution, 50 µL of dansyl chloride and 100 µL of methanol to make up a final volume of 0.5 mL.

Calibration curve was obtained by plotting the amount of analyte area divided by the area value obtained for the internal standard against concentration.

The obtained calibration curves are summarized in Table 12. An example of a calibration curve is shown in figure 16, and the remaining graphs are attached in Annexes.

However, the calibration curve obtained for spermidine was not satisfactory because the analytical response was too small. A reason for this evidence could be the fact that spermidine has three possible places for the occurrence of derivatization which can produce different products, making it difficult to determine analytically. For that reason the compound was not analyzed.

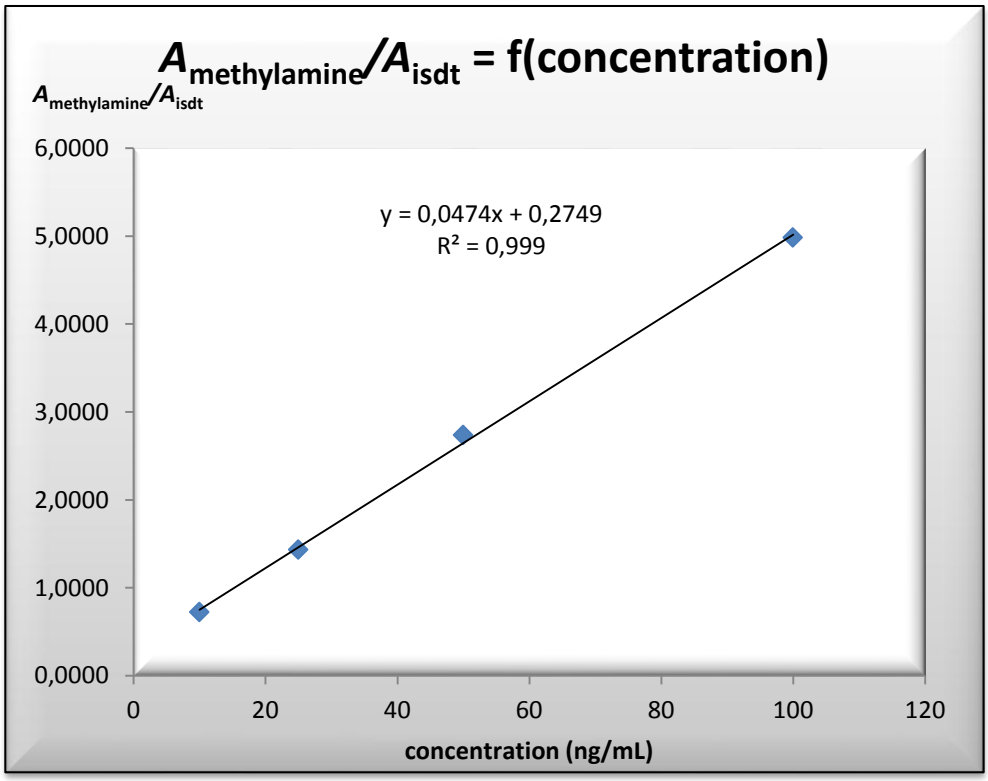


**Figure 16 – Representative graph of Area for methylamine/Area for isdt *vs.* concentration.**

**Table 12 – Calibration curve obtained for amine analysis.**

| Analyte | Equation | $R^2$ | N | Points/ µg.L$^{-1}$ | LOQ |
|---|---|---|---|---|---|
| **Cadaverine** | y = 0.00182x + 0.009549 | 0.998 | 5 | 10, 25, 50,100, 300 | 0.0470 |
| **Dibutylamine** | y = 0.00577x + 0.1225 | 0.997 | 5 | 10, 25, 50, 100, 200 | 0.0148 |
| **Diethylamine** | y = 0.00453x + 0.05432 | 1.00 | 4 | 10, 25, 50, 100 | 0.0189 |
| **Dimethylamine** | y = 0.0771x + 0.7705 | 1.00 | 5 | 10, 25, 50, 100, 300 | 0.00111 |
| **Dipropylamine** | y = 0.00777x + 0.1160 | 0.997 | 4 | 10, 25, 50, 100 | 0.0110 |
| **Ethanolamine** | y = 0.0088x + 0.0956 | 0.998 | 4 | 10, 25, 50, 100 | 0.0097 |
| **Ethylenediamine** | y = 0.000644x + 0.0159 | 0.983 | 4 | 10, 25, 50, 100 | 0.133 |
| **Histamine** | y = 0.000227x + 0.007145 | 0.998 | 5 | 10, 25, 50, 100, 500 | 0.377 |
| **Isobutylamine** | y = 0.0241x + 0.2119 | 0.992 | 4 | 10, 25, 50, 100 | 0.00355 |
| **Methylamine** | y = 0.0474x + 0.2749 | 0.999 | 4 | 10, 25, 50, 100 | 0.00181 |
| **3-phenylpropylamine** | y = 0.0204x + 0.1505 | 0.995 | 4 | 10, 25, 50, 100 | 0.00420 |
| ***sec*-Butylamine** | y = 0.00690x + 0.1442 | 0.997 | 4 | 10, 25, 50, 100 | 0.0124 |
| **Tryptamine** | y = 0.0144x – 0.1221 | 0.998 | 5 | 10, 25, 50, 100, 500 | 0.00595 |
| **Tyramine** | y = 0.00198x + 0.02884 | 0.999 | 3 | 10, 25, 50 | 0.0432 |

Repeatability was determined from the standard deviation and the relative standard deviation of propranolol peak area obtained in 27 samples, as represented in the following Table. Results demonstrated the good repeatability of the method for the analysis of amines by LC-MS.

**Table 13 – Repeatability of the method. Number of samples analyzed in brackets.**

| Repeatability | | |
|---|---|---|
| **Average (27)** | **σ** | **Rsd** |
| 24723 | 2060 | 8.33% |

Repeatability was also determined from 3 standard samples with a 10 ng/mL concentration for all compounds. Results were very satisfactory as shown in the Table 14.

**Table 14 – Repeatability obtained for all compounds in study (10ng/mL). Number of samples analyzed in brackets.**

| | Average (3) | | σ | | Rsd | |
|---|---|---|---|---|---|---|
| **Compound** | **RT/ s** | **Area** | **RT/ s** | **Area** | **RT/ %** | **Area/ %** |
| **Cadaverine** | 7.6 | 562 | 0.0 | 30 | 0.42 | 5.25 |
| **Dibutylamine** | 10.4 | 8941 | 0.0 | 330 | 0.14 | 3.69 |
| **Diethylamine** | 6.1 | 38569 | 0.1 | 1497 | 0.83 | 3.88 |
| **Dimethylamine** | 3.9 | 86035 | 0.1 | 1500 | 2.04 | 1.74 |
| **Dipropylamine** | 8.5 | 8384 | 0.0 | 324 | 0.28 | 3.86 |
| **Ethanolamine** | 1.5 | 29057 | 0.0 | 928 | 1.91 | 3.20 |
| **Ethylenediamine** | 6.5 | 1833 | 0.1 | 94 | 0.81 | 5.12 |
| **Histamine** | 8.0 | 35 | 0.0 | 14 | 0.40 | 38.43 |
| **Isobutylamine** | 5.4 | 58706 | 0.1 | 4847 | 1.20 | 8.26 |
| **Methylamine** | 2.3 | 188907 | 0.0 | 113 | 0.23 | 0.06 |
| **3-phenylpropylamine** | 7.1 | 38653 | 0.0 | 2361 | 0.60 | 6.11 |
| ***sec*-Butylamine** | 5.3 | 16627 | 0.1 | 1401 | 1.28 | 8.43 |
| **Tryptamine** | 5.4 | 22687 | 0.1 | 1716 | 1.25 | 7.56 |
| **Tyramine** | 9.9 | 352 | 0.0 | 27 | 0.15 | 7.58 |

## 5.2.3. Discussion of results

Results obtained upon analysis of aerosol samples and total suspended particles are shown in Table 15.

Were analyzed a total of 3 samples of total suspended particles and 7 of aerosols from a size corresponding to 30nm, after separation of the remaining particles by DMA. Samples were collected in the boreal forest of Hyytiälä, Finland, for a period of six months which elapsed between March and August 2012.

Results suggest that the compounds present in higher quantities in aerosol particles are respectively dimethylamine, tryptamine, isobutylamine, dipropylamine, methylamine, *sec*-Butylamine and diethylamine. These compounds may be responsible for the growth process of atmospheric aerosol particles and consequently for the direct and indirect effects on the environment associated with aerosols.

**Table 15 – Average concentration and concentration range of the target analytes. Number of samples analyzed in brackets. All concentration values are expressed in ng.m$^{-3}$.**

| | TS (3) | | Aerosol, 30nm (7) | |
|---|---|---|---|---|
| **Compound** | **Mean** | **Range** | **Mean** | **Range** |
| **Cadaverine** | ND | | ND | |
| **Dibutylamine** | ND | | ND | |
| **Diethylamine** | 0.0247 | ND-0.0472 | 0.412 | ND-0.914 |
| **Dimethylamine** | 11.9 | ND-35.7 | 29.5 | ND-98.0 |
| **Dipropylamine** | 0.243 | ND-0.730 | 2.31 | ND-39.8 |
| **Ethanolamine** | ND | | 0.0345 | ND-0.207 |
| **Ethylenediamine** | ND | | 0.325 | ND-1.95 |
| **Histamine** | ND | | ND | |
| **Isobutylamine** | 1.33 | ND-3.40 | 2.37 | ND-6.12 |
| **Methylamine** | 0.121 | ND-0.363 | 1.19 | ND-5.88 |
| **3-phenylpropylamine** | ND | | ND | |
| ***sec*-Butylamine** | 0.328 | ND-0.983 | 1.06 | ND-6.12 |
| **Tryptamine** | 0.959 | ND-2.87 | 2.70 | ND-6.80 |
| **Tyramine** | ND | | ND | |

[a] ND, No detectable.

Although the existence of detailed results on the chemical composition of ultrafine particles is very limited, the values obtained for ethylenediamine and dipropylamine were in agreement with those reported in the literature, albeit the ranges of values obtained have been quite superior [10, 11].

Some limitations of the results include the fact that it was assumed a 100% efficiency of collection and extraction, and values obtained for gas phase were used as

a blank which, although the quantities of particles is theoretically minimum or even probably under de limit of detection, may contain residual amounts of particles.

The response of internal standard also showed a significant variation between different batches of samples, although it has been constant during the analysis of each batch. For that reason, new studies must be made in order to improve the internal standard response.

Furthermore, new methods should be tested to provide a mean for determination of the used method reproducibility.

Despite of the associated uncertainties, this study allowed to obtain more qualitative knowledge about the amines present in aerosol particles, and possibly responsible for their growth events.

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# 6. Conclusion

The development of novel analysis methods is essential to allow the analytical determination of atmospheric aerosol particles as well as to understand its effects on the environment.

In this investigational study was developed a new analytical method for determining aerosols by LC-MS based on HILIC. For this purpose, various experimental parameters and conditions were optimized. The method allowed the successful separation of 11 acid compounds present in aerosol particles.

Further studies should however be performed to improve the repeatability of the method, thereby making possible their effective application to aerosol samples.

Concerning the amines, were tested two different filters made of PTFE and Quartz immersed in a slurry composed of acetonitrile and XAD-7 for sampling process, being also investigated the optimum number of immersions to determine which gives a lower backpressure. Results showed the greatest ability of quartz filters for collecting aerosols because back pressure was well below to that obtained for PTFE filters.

The number of immersions did not cause a significant variation in the said parameter, and it was concluded that the number of immersions of the filter in the slurry is not a relevant experimental condition for collection of amines.

Finally, was proceeded the analysis of amines using a previously developed method, which permit to analyze 14 aliphatic and aromatic amines. Aerosol particles with a size corresponding to 30nm were collected in the boreal forest of Hyytiälä, Finland, by DMA, and the compounds were subsequently extracted by dynamic ultrasound-assisted extraction.

For the analytical process was tested propranolol as an internal standard, the reason being related with the good repeatability demonstrated by this compound when obtaining a calibration curve. However, a good repeatability was not evidenced during the analysis of different batches of samples, and should be carried out studies to understand this occurrence. Though, the method showed a good repeatability between samples of the same batch, which allowed to perform the analysis of aerosol and total suspended particle samples.

The method enabled to identify and quantify nine amines, being the results obtained for ethylenediamine, diethylamine and dipropylamine in agreement with previous studies on 30nm aerosol particles, whereby the method of analysis proved to be promising.

Further investigational studies must be made in order to obtain new knowledge about the complicated set of processes comprising the formation of aerosol particles

that govern the local and global effects of atmospheric aerosols. The development of novel analytical determination methods which allows the analysis of trace compounds with high accuracy is essential to achieve this aim and to make possible the concerted action against the adverse effects associated with aerosols.

# 7. ANNEXES

**Table 16 – Samples collected.**

| Sample number | Date | Time | Sample number | Date | Time |
|---|---|---|---|---|---|
| **1** | 14/09/12 | 09:05 | **27** | 08/10/12 | 09:00 |
| **2** | 18/09/12 | 09:20 | **28** | 08/10/12 | 17:00 |
| **3** | 18/09/12 | 16:00 | **29** | 09/10/12 | 09:00 |
| **4** | 19/09/12 | 10:00 | **30** | 09/10/12 | 16:30 |
| **5** | 20/09/12 | 10:00 | **31** | 10/10/12 | 09:15 |
| **6** | 21/09/12 | 10:30 | **32** | 10/10/12 | 16:15 |
| **7** | 24/09/12 | 09:10 | **33** | 11/10/12 | 09:00 |
| **8** | 24/09/12 | 16:00 | **34** | 11/10/12 | 16:30 |
| **9** | 25/09/12 | 09:10 | **35** | 12/10/12 | 09:00 |
| **10** | 25/09/12 | 17:00 | **36** | 15/10/12 | 09:30 |
| **11** | 26/09/12 | 09:00 | **37** | 16/10/12 | 09:30 |
| **12** | 26/09/12 | 16:30 | **38** | 16/10/12 | 16:30 |
| **13** | 27/09/12 | 09:05 | **39** | 17/10/12 | 09:00 |
| **14** | 27/09/12 | 16:30 | **40** | 17/10/12 | 17:00 |
| **15** | 28/09/12 | 09:00 | **41** | 18/10/12 | 09:00 |
| **16** | 28/09/12 | 15:35 | **42** | 18/10/12 | 17:05 |
| **17** | 31/09/12 | 09:10 | **43** | 19/10/12 | 09:30 |
| **18** | 31/09/12 | 17:00 | **44** | 19/10/12 | 16:00 |
| **19** | 02/10/12 | 09:05 | **45** | 22/10/12 | 09:00 |
| **20** | 02/10/12 | 17:00 | **46** | 22/10/12 | 17:00 |
| **21** | 03/10/12 | 09:10 | **47** | 23/10/12 | 09:30 |
| **22** | 03/10/12 | 17:00 | **48** | 24/10/12 | 09:00 |
| **23** | 04/10/12 | 09:00 | **49** | 24/10/12 | 17:00 |
| **24** | 04/10/12 | 17:00 | **50** | 25/10/12 | 09:00 |
| **25** | 05/10/12 | 10:30 | **51** | 25/10/12 | 17:00 |
| **26** | 05/10/12 | 16:40 | **52** | 26/10/12 | 12:30 |

**Table 17 – Preparation of a standard solution containing all the acid compounds to be determined in a final concentration of 100µg/mL.**

| Acids | mass/ mg | Solution Volume/ mL | Initial Concentration/ µg/mL | Added Volume/ µL | Final Volume/ mL | Final Concentration/ µg/mL |
|---|---|---|---|---|---|---|
| **Adipic** | 4.9 | 5 | 980 | 100 | 5 | 20 |
| **Azelaic** | 10.3 | | 2060 | 48.5 | | |
| **Benzoic** | 11.2 | | 2240 | 44.6 | | |
| **Caprylic** | 5.0 | | 1000 | 100 | | |
| **Maleic** | 4.6 | | 920 | 109 | | |
| **Malic** | 11.0 | | 2200 | 45.4 | | |
| **Malonic** | 5.0 | | 1000 | 100 | | |
| **Mandelic** | 5.3 | | 1060 | 94 | | |
| **Oleic** | 35.4 | | 7080 | 14.1 | | |
| **Palmitic** | 7.3 | | 1460 | 68 | | |
| **Pinic** | 5 | | 1000 | 100 | | |
| ***cis*-Pinonic** | 8.2 | | 1640 | 61 | | |
| **Sebacic** | 5.2 | | 1040 | 96 | | |
| **Stearic** | 4.8 | | 960 | 104 | | |
| **Tartaric** | 10.5 | | 2100 | 47.6 | | |
| **Vanlillic** | 10.7 | | 2140 | 46.7 | | |

• Example of calculation for adipic acid:

$$c_{initial} \times v_{initial} = c_{final} \times v_{added}$$

$$v_{added} = \frac{5\ mL \times 20\ ppm}{980\ ppm} = 0.10\ mL = 100\ \mu L$$

**Table 18 – Preparation of a standard solution containing all the amine compounds to be determined in a final concentration of 100µg/mL.**

| Amines | mass/ mg | $V_{solution}$/ mL | $C_{standard\ initial}$/ µg/mL | $V_{standard\ added}$/ µL | $V_{final}$/ mL | $C_{standard\ final}$/ µg/mL |
|---|---|---|---|---|---|---|
| **Cadaverine** | 9.6 | 10 | 960 | 2600 | 25 | 100 |
| **Dibutylamine** | 19.9 | | 1990 | 1256 | | |
| **Diethylamine** | 10.1 | | 1010 | 2475 | | |
| **Dimethylamine** | 10.1 | | 1010 | 2475 | | |
| **Dipropylamine** | 27.4 | | 904 | 2760 | | |
| **Ethanolamine** | 29.4 | | 2940 | 850 | | |
| **Ethylenediamine** | 71.3 | | 7130 | 351 | | |
| **Histamine dihydrochloride** | 29.7 | | 2970 | 842 | | |
| **Isobutylamine** | 15.1 | | 1510 | 1656 | | |
| **Methylamine** | 40.7 | | 1628 | 1536 | | |
| **3-phenylpropylamine** | 37.9 | | 3790 | 660 | | |
| ***sec*-Butylamine** | 24.1 | | 2410 | 1037 | | |
| **Spermidine** | 29.2 | | 2920 | 856 | | |
| **Tryptamine** | 10.7 | | 1070 | 2336 | | |
| **Tyramine** | 15.4 | | 1540 | 1623 | | |

• Example of calculation for cadaverine:

$$c_{initial} \times v_{initial} = c_{final} \times v_{added}$$

$$v_{added} = \frac{25\ mL \times 100\ ppm}{960\ ppm} = 2.60\ mL = 2600\ \mu L$$

**Table 19 – Preparation of a 1µg/mL and 10µg/mL standard solutions.**

| Initial concentration of standard solution/ µg/mL | Final Volume/ mL | Final concentration of standard solution/ µg/mL | Added Volume/ µL |
|---|---|---|---|
| 100 | 10 | 10 | 1000 |
| 100 | 10 | 1 | 100 |

• Example of calculation for preparation of a 10 µg/mL standard solution:

$$c_{initial} \times v_{initial} = c_{final} \times v_{added}$$

$$v_{added} = \frac{10\ mL \times 10\ ppm}{1000\ ppm} = 0.10\ mL = 100\ \mu L$$

**Table 20 – Preparation of a 20µg/mL and 5µg/mL propranolol solutions.**

| Initial concentration propanolol/ µg/mL | Final Volume/ mL | Final concentration propanolol/ µg/mL | Added Volume/ µL |
|---|---|---|---|
| 640 | 10 | 20 | 310 |
| 20 | 0.5 | 5 | 125 |

• Example of calculation for preparation of a 20 µg/mL propranolol solution:

$$c_{initial} \times v_{initial} = c_{final} \times v_{added}$$

$$v_{added} = \frac{10\ mL \times 20\ ppm}{640\ ppm} = 0.31\ mL = 310\ \mu L$$

**Table 21 – Standard samples preparation for definition of a calibration curve.**

| $C_{standard\ final}$/ ng/mL | $V_{standard\ final}$/ ml | $C_{standard\ initial}$/ µg/mL | $V_{standard\ added}$/ µL | $V_{propanolol}$/ µL | $V_{borate\ buffer}$/ µL | $V_{dansyl\ chloride}$/ µL | $V_{MeOH}$/ ml |
|---|---|---|---|---|---|---|---|
| **10** | 0.5 | 1 | 5 | 125 | 100 | 50 | 220 |
| **25** | | | 12,5 | | | | 212.5 |
| **50** | | | 25 | | | | 200 |
| **100** | | | 50 | | | | 175 |
| **200** | | | 100 | | | | 125 |
| **300** | | | 150 | | | | 75 |
| **400** | | | 200 | | | | 25 |
| **500** | | 10 | 25 | | | | 200 |

• Example of calculation for preparation of a 10 ng/mL standard sample:

$$c_{initial} \times v_{initial} = c_{final} \times v_{added}$$

$$v_{added} = \frac{0.5\ mL \times 0.01\ ppm}{1\ ppm} = 0.005\ mL = 5\ \mu L$$

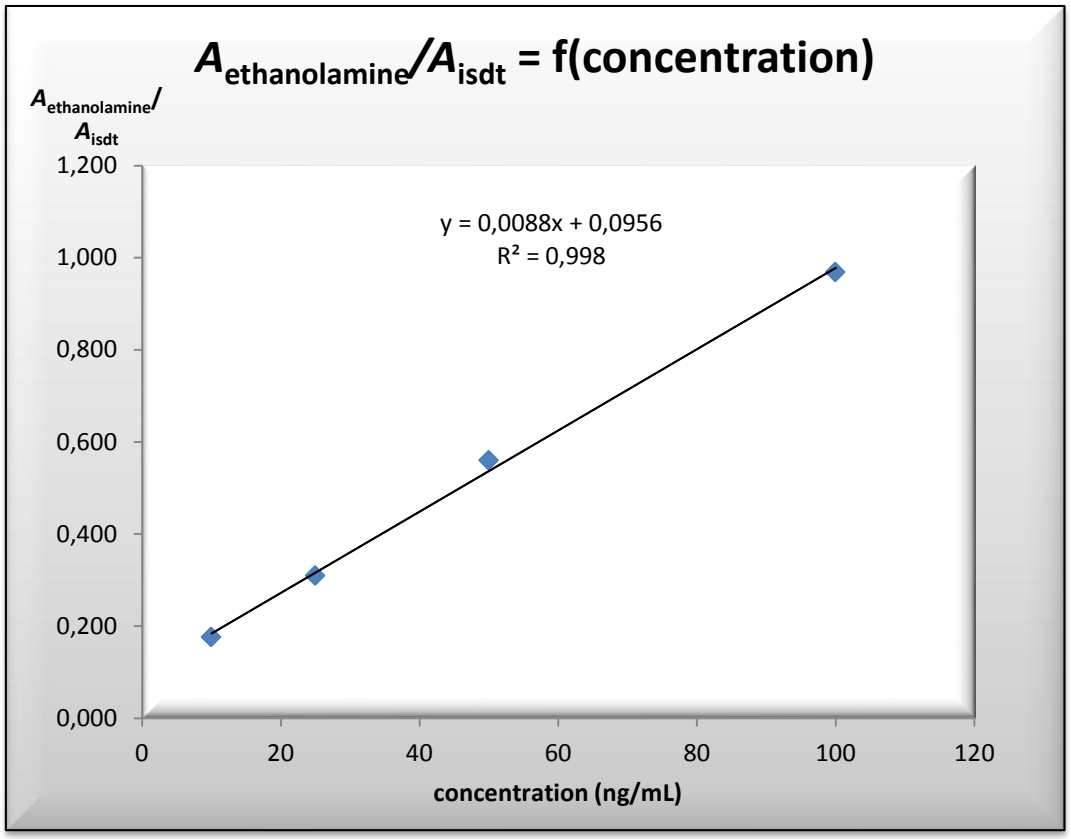


Figure 17 – Representative graph of Area for ethanolamine/Area for isdt *vs.* concentration.

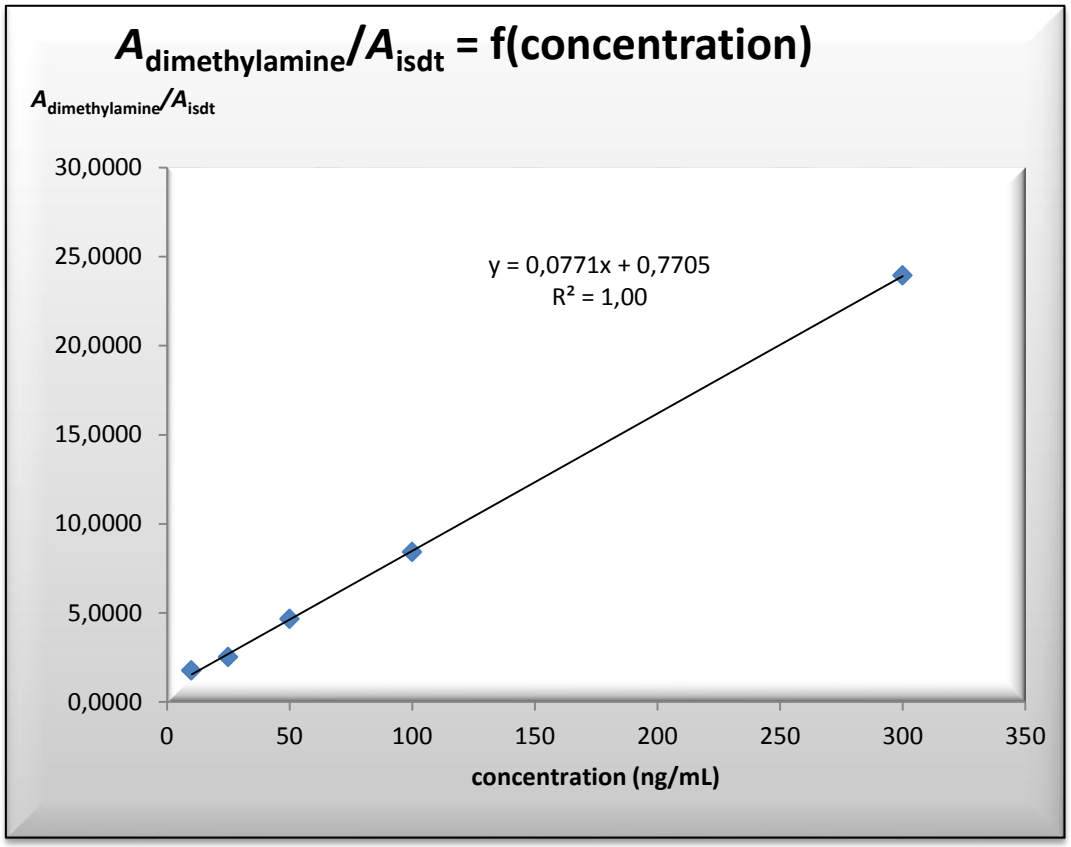


Figure 18 – Representative graph of Area for dimethylamine/Area for isdt *vs.* concentration.

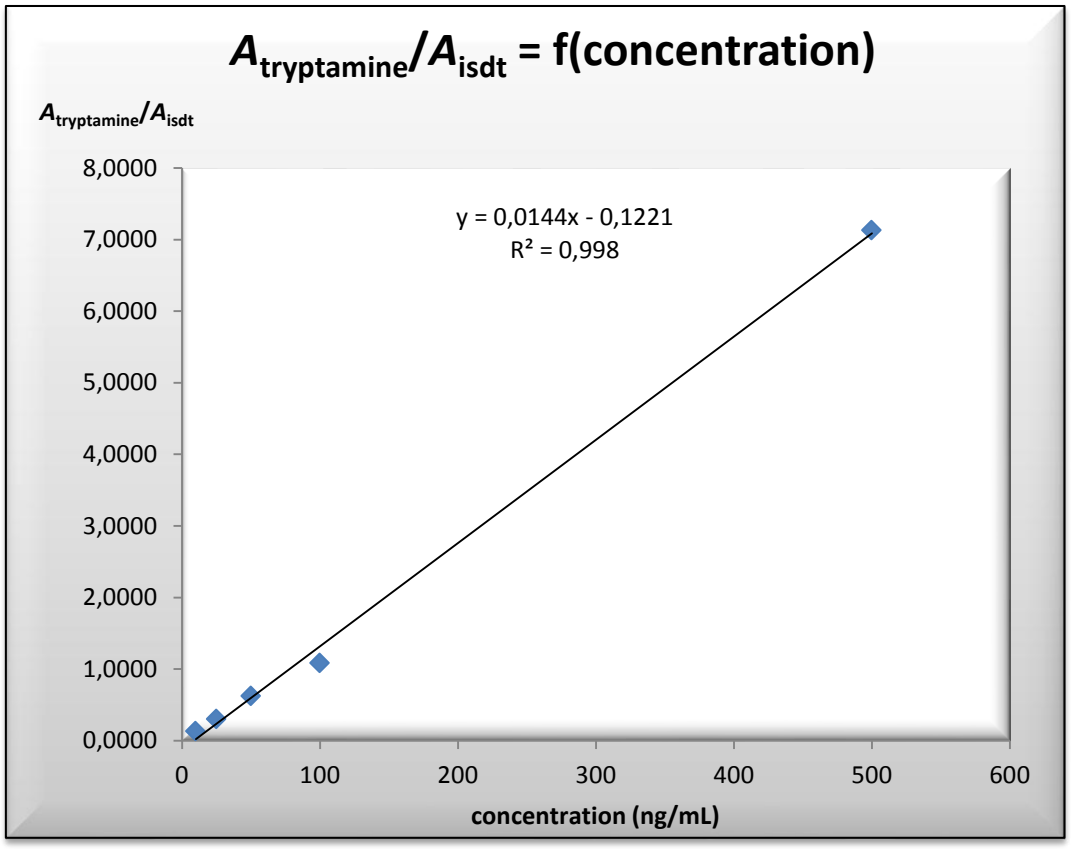


Figure 19 – Representative graph of Area for tryptamine/Area for isdt *vs.* concentration.

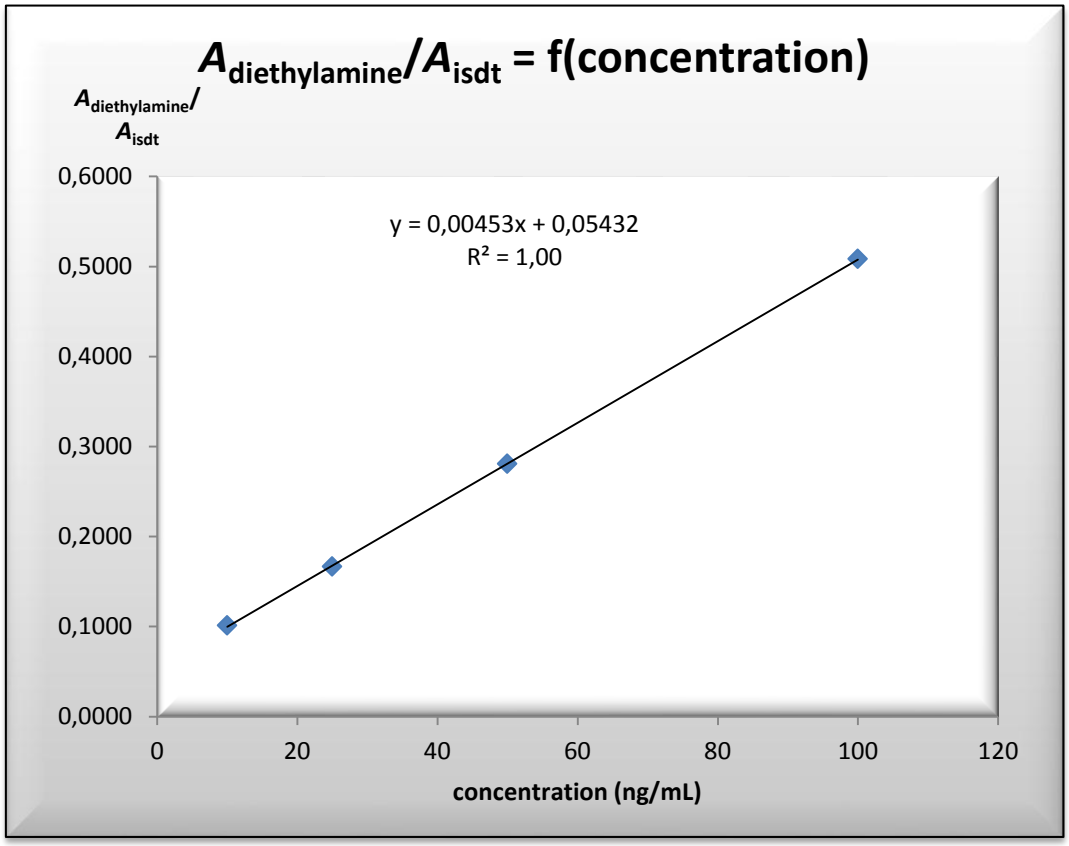


Figure 20 – Representative graph of Area for diethylamine/Area for isdt *vs.* concentration.

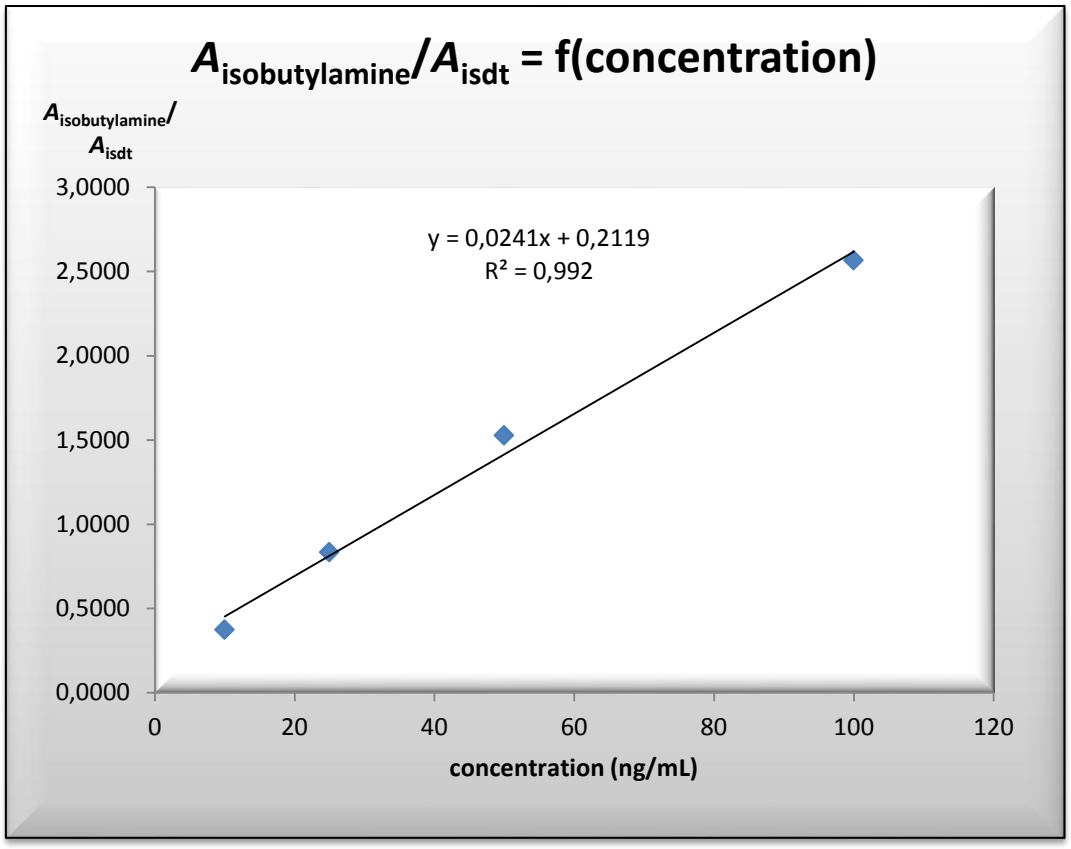


Figure 21 – Representative graph of Area for isobutylamine/Area for isdt *vs.* concentration.

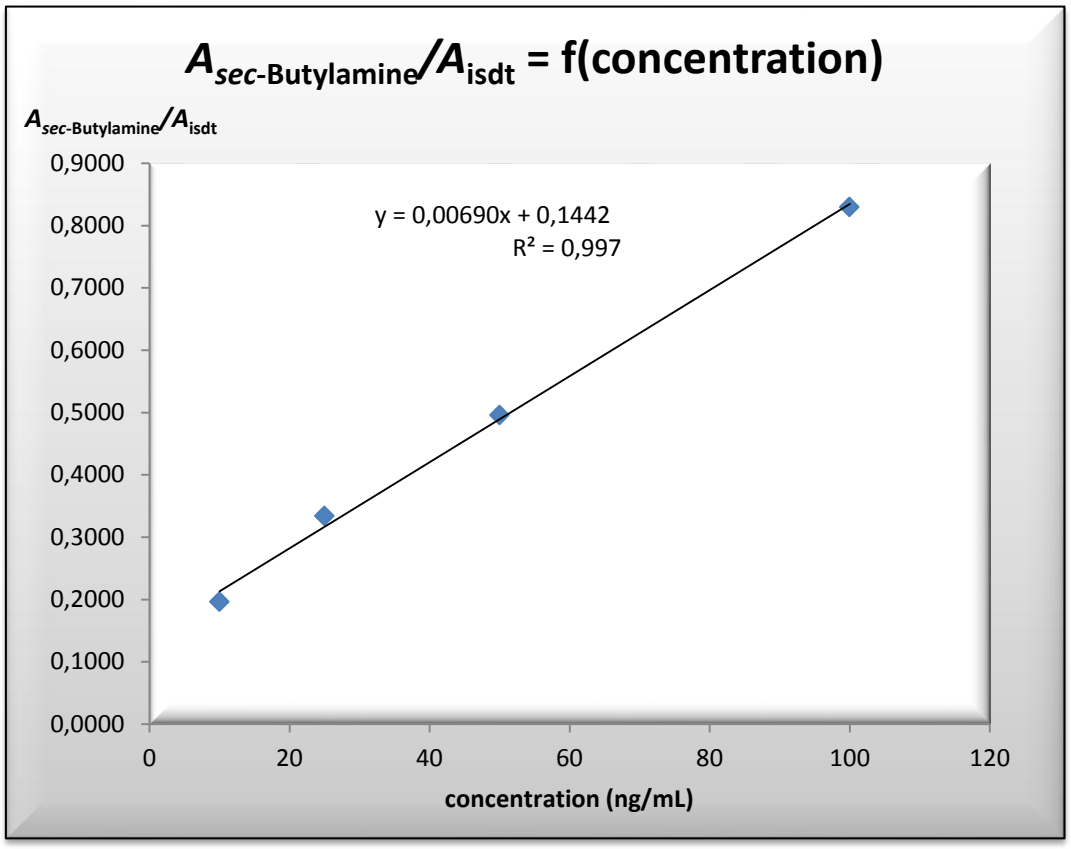


Figure 22 – Representative graph of Area for *sec*-Butylamine/Area for isdt vs. concentration.

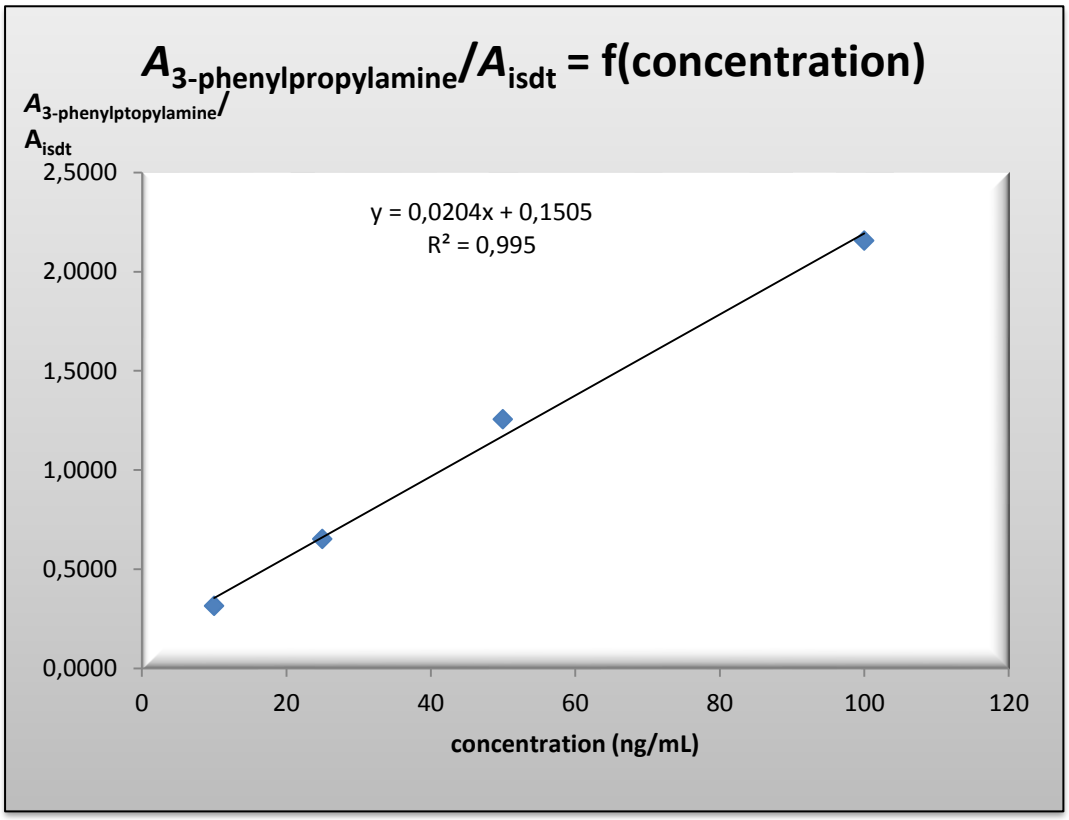


Figure 23 – Representative graph of Area for 3-phenylpropylamine/Area for isdt versus concentration.

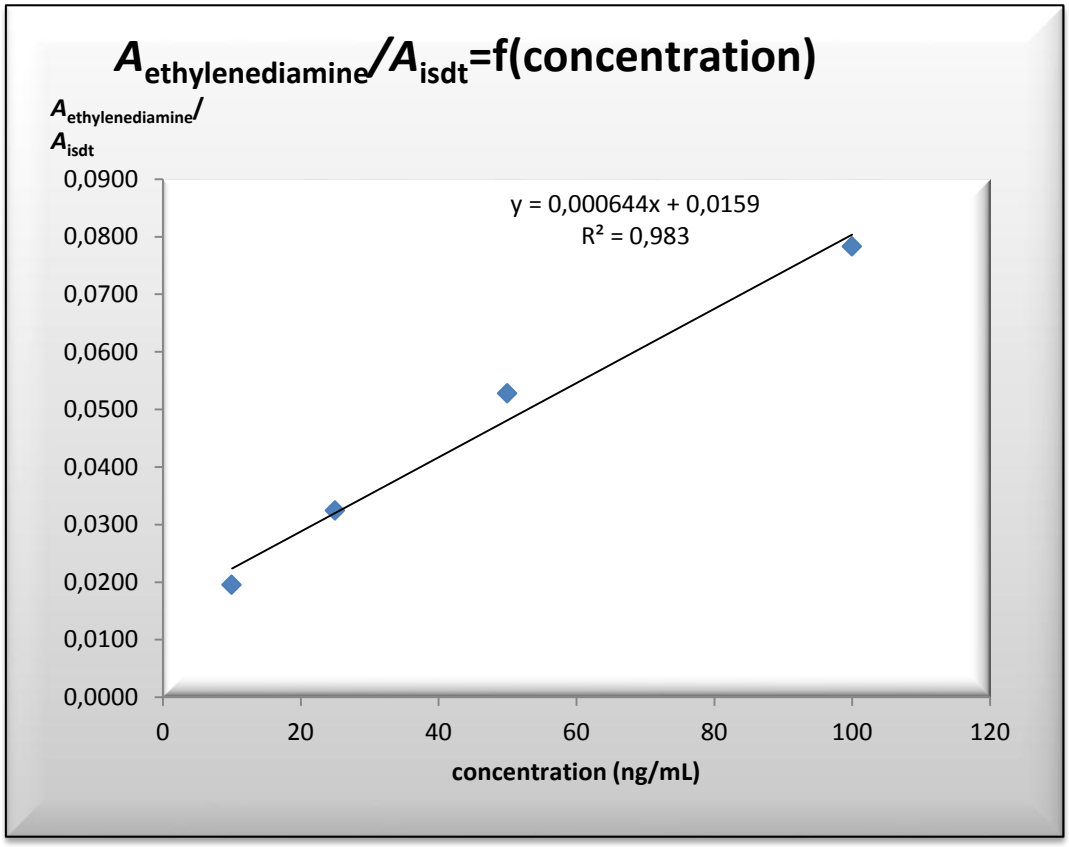


Figure 24 – Representative graph of Area for ethylenediamine/Area for isdt *vs.* concentration.

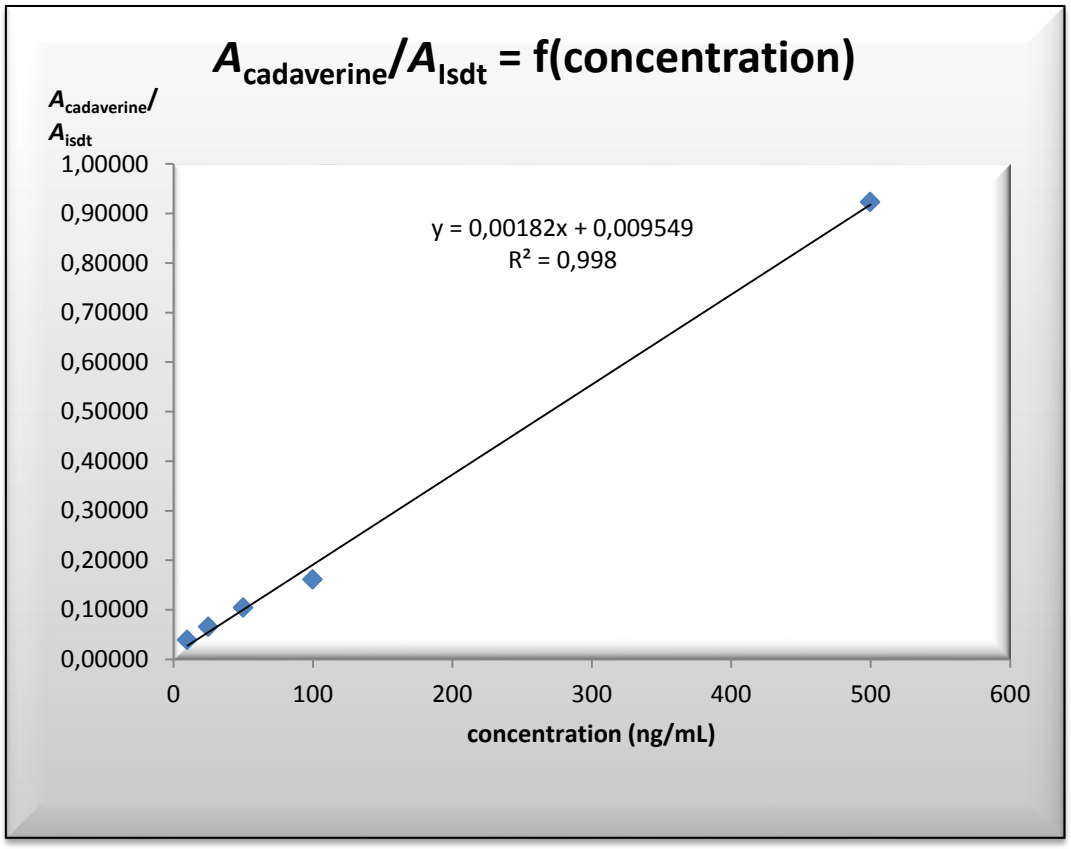


Figure 25 – Representative graph of Area for cadaverine/Area for isdt *vs.* concentration.

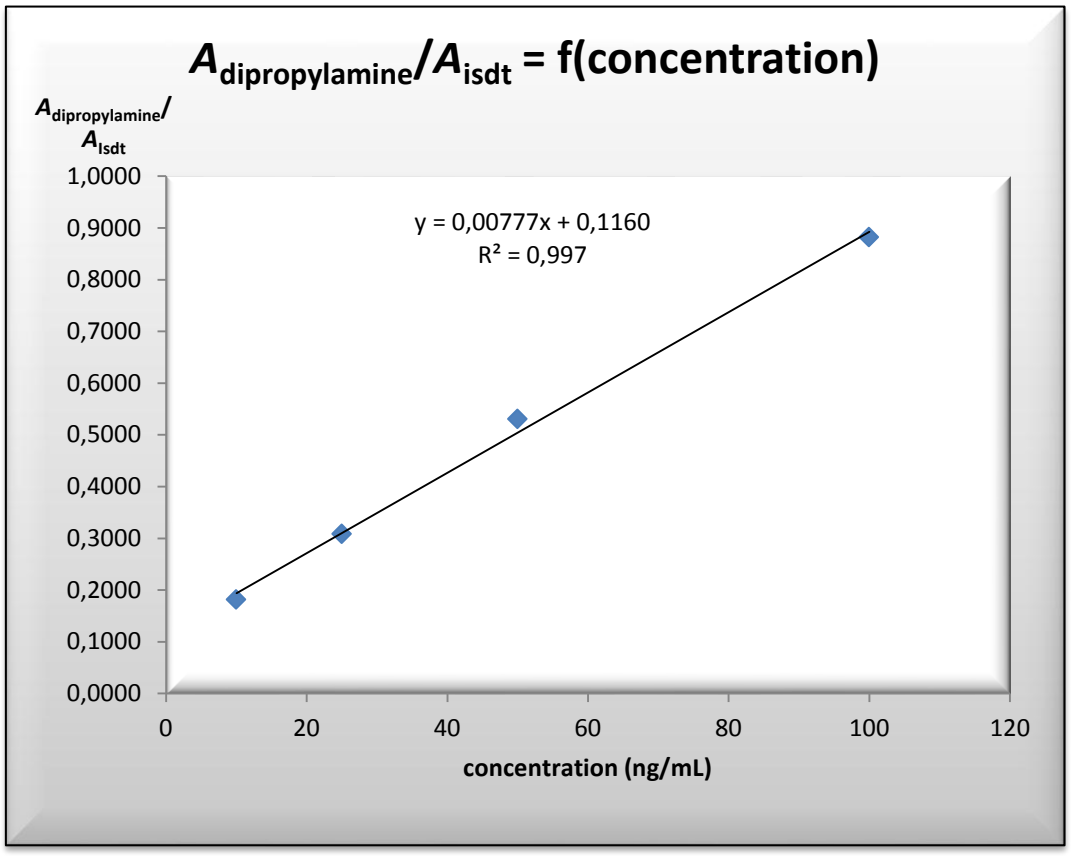


Figure 26 – Representative graph of Area for dipropylamine/Area for isdt *vs.* concentration.

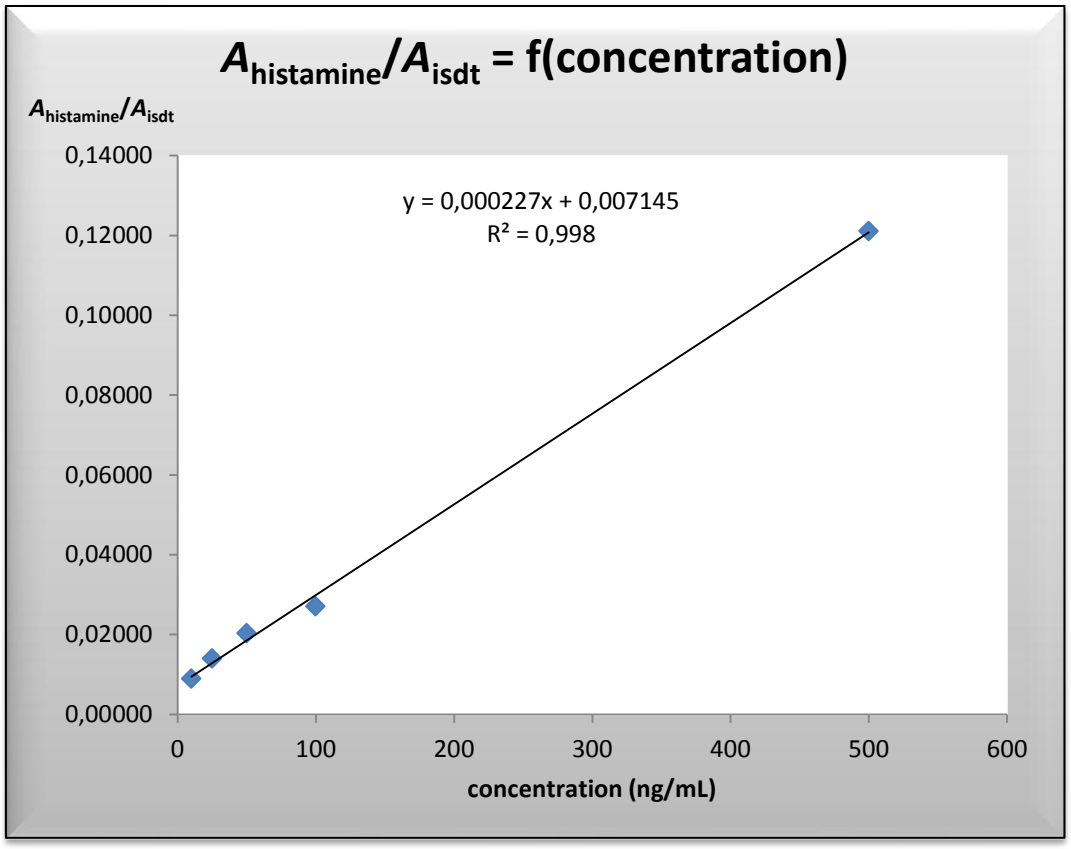


Figure 27 – Representative graph of Area for histamine/Area for isdt *vs.* concentration.

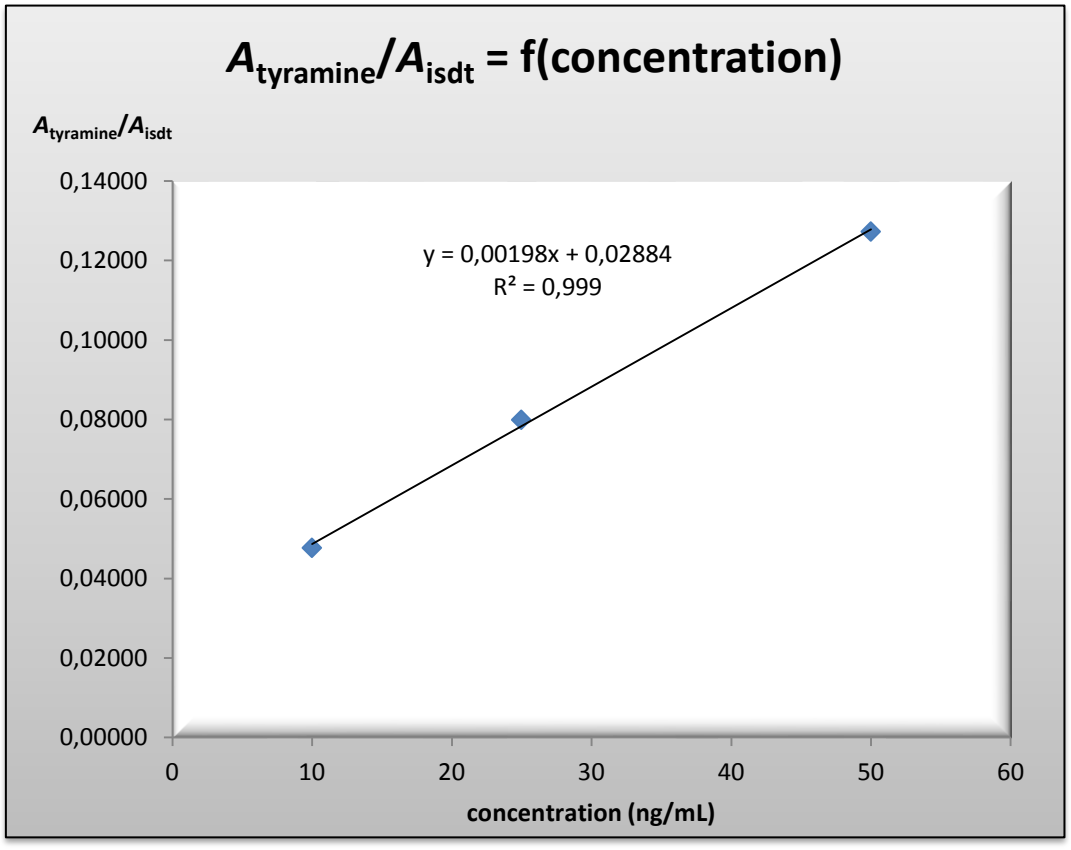


Figure 28 – Representative graph of Area for tyramine/Area for isdt *vs.* concentration.

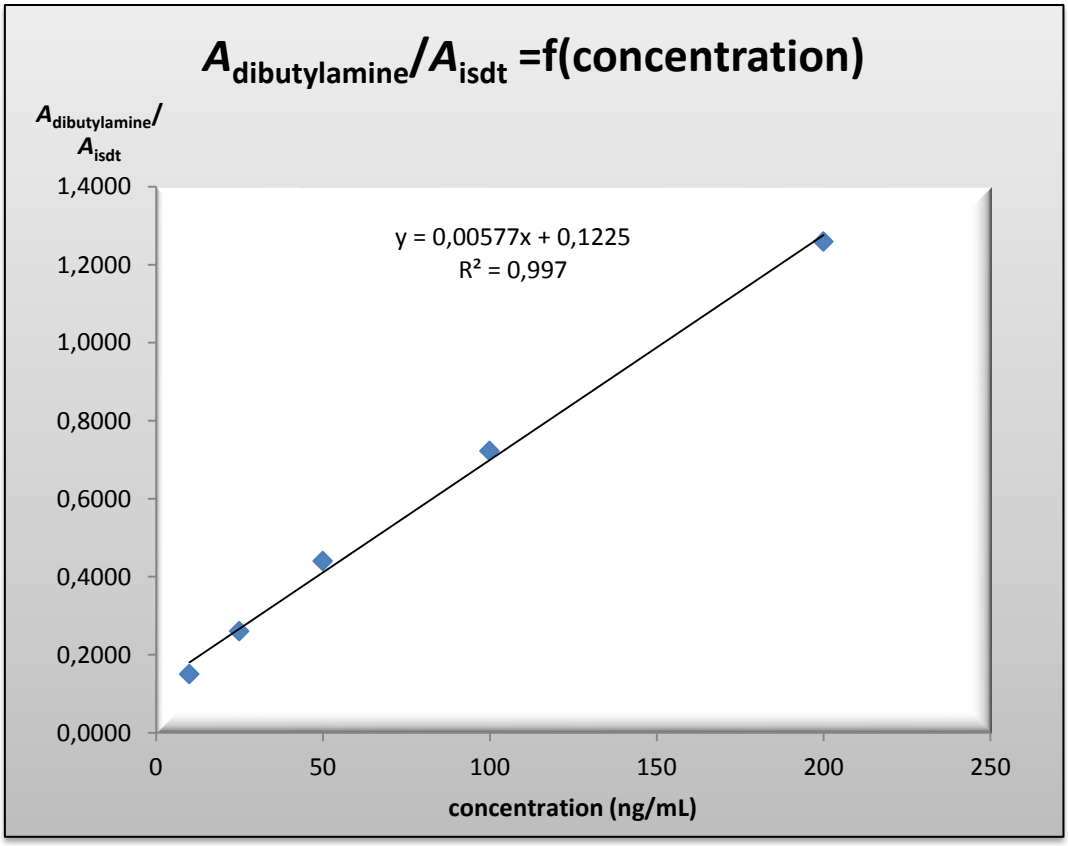


Figure 29 – Representative graph of Area for dibutylamine/Area for isdt *vs.* concentration.

## Chromatograms and mass spectrums for acids

• Adipic acid

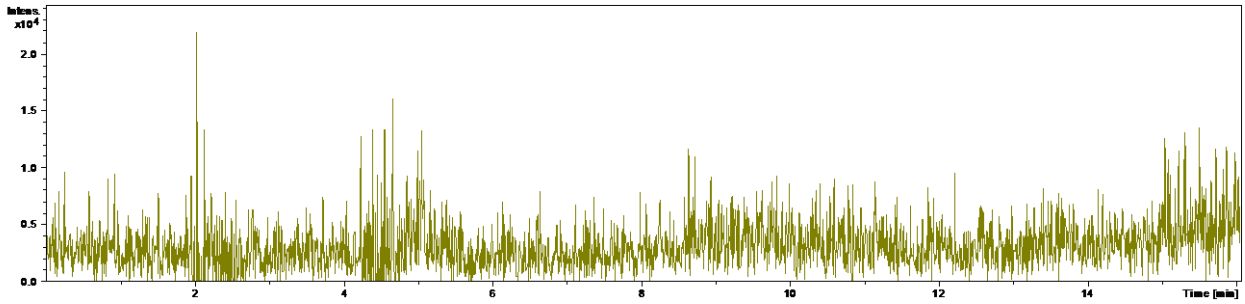

Figure 30 – Chromatogram obtained for adipic acid.

• Azelaic acid

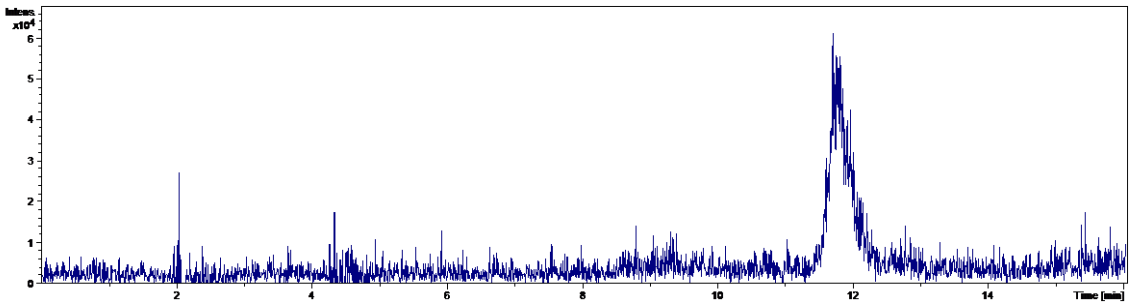

Figure 31 – Chromatogram obtained for azelaic acid.

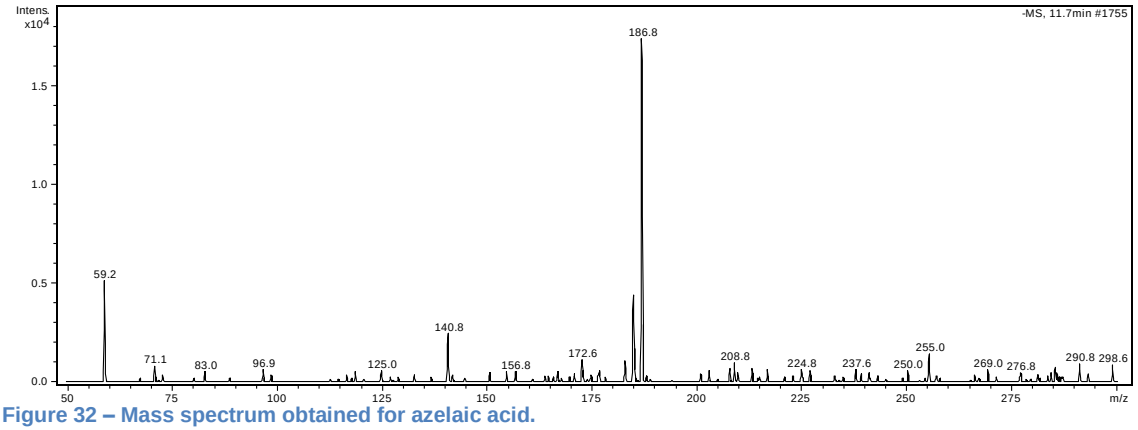


Figure 32 – Mass spectrum obtained for azelaic acid.

• Benzoic acid

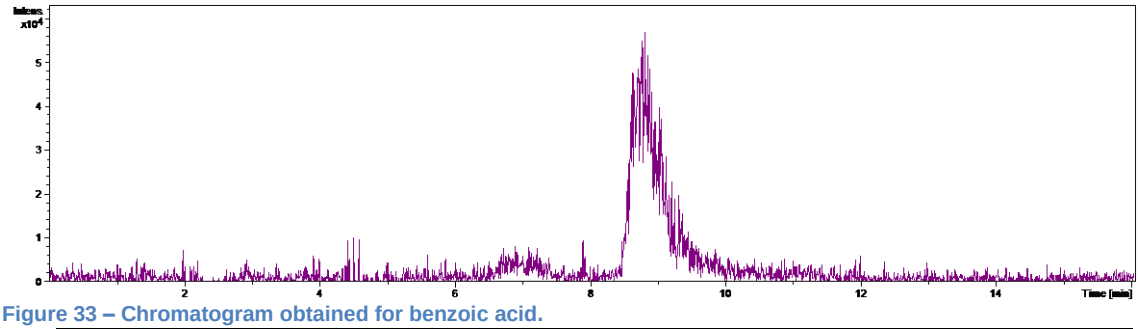


Figure 33 – Chromatogram obtained for benzoic acid.

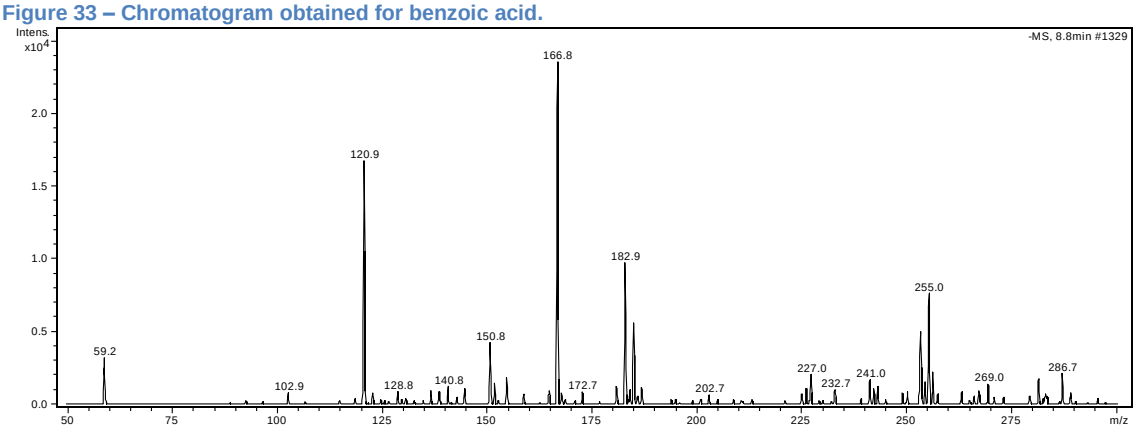


Figure 34 – Mass spectrum obtained for benzoic acid.

• Caprylic acid

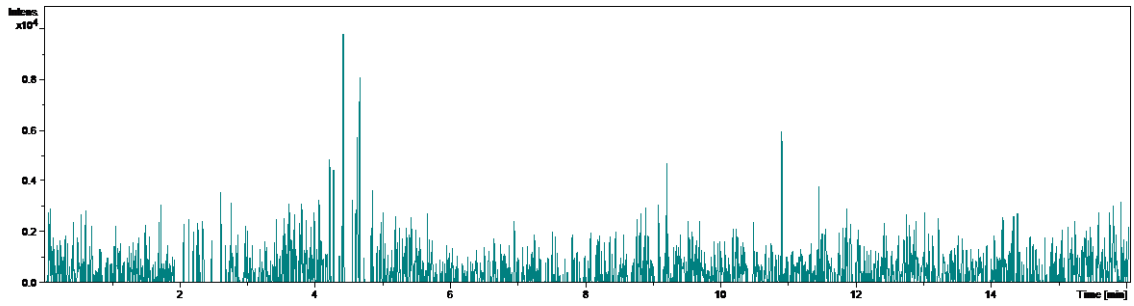


Figure 35 – Chromatogram obtained for caprylic acid.

• Maleic acid

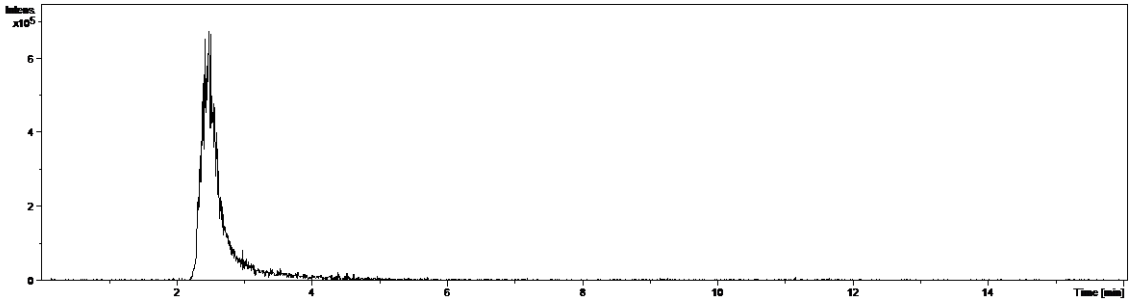


Figure 36 – Chromatogram obtained for maleic acid.

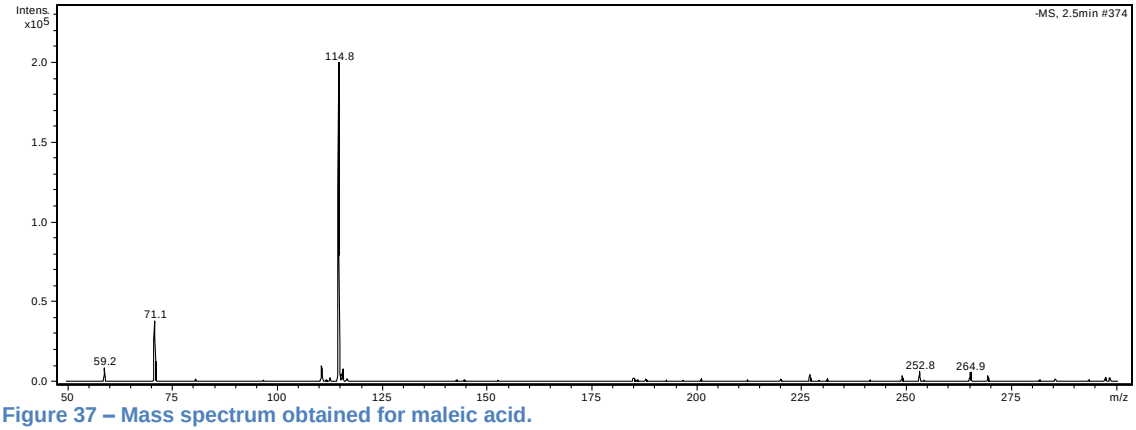


Figure 37 – Mass spectrum obtained for maleic acid.

• Malic acid

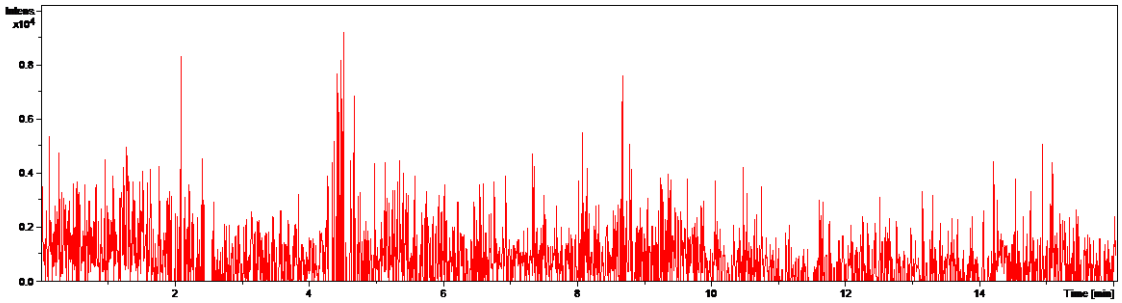


Figure 38 – Chromatogram obtained for malic acid.

• Malonic acid

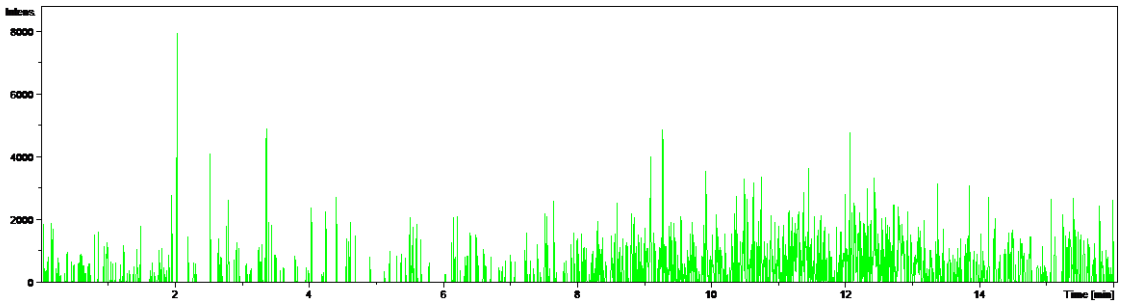


Figure 39 – Chromatogram obtained for malonic acid.

• Mandelic acid

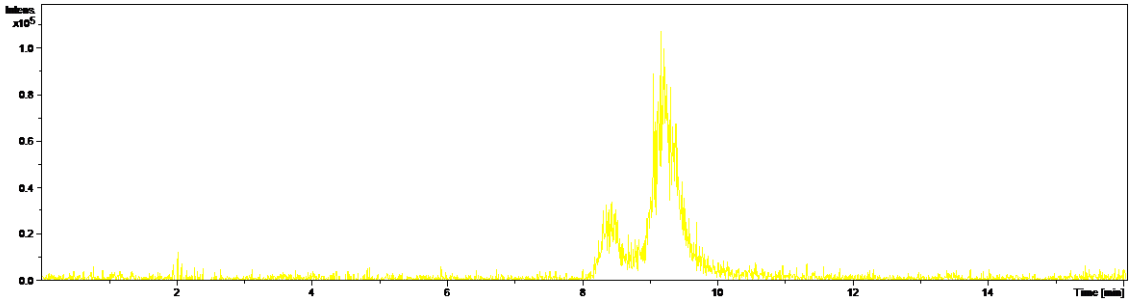


Figure 40 – Chromatogram obtained for mandelic acid.

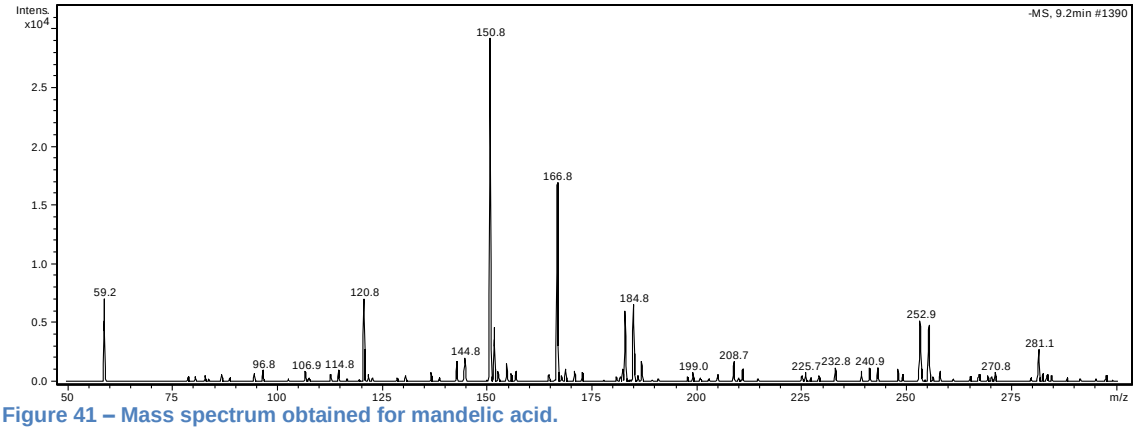


Figure 41 – Mass spectrum obtained for mandelic acid.

• Oleic acid

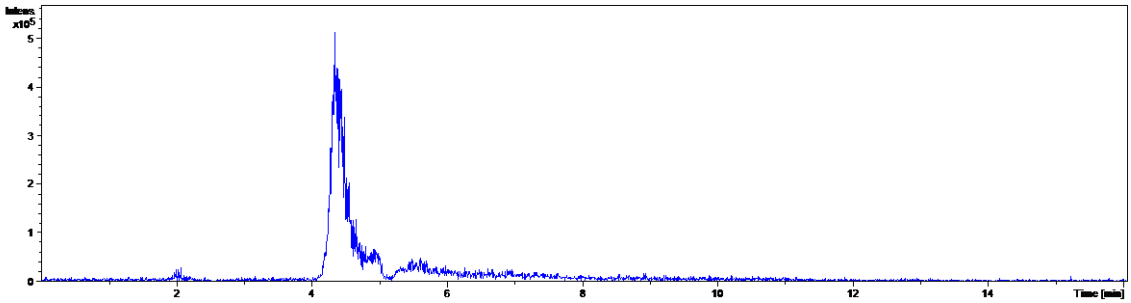


Figure 42 – Chromatogram obtained for oleic acid.

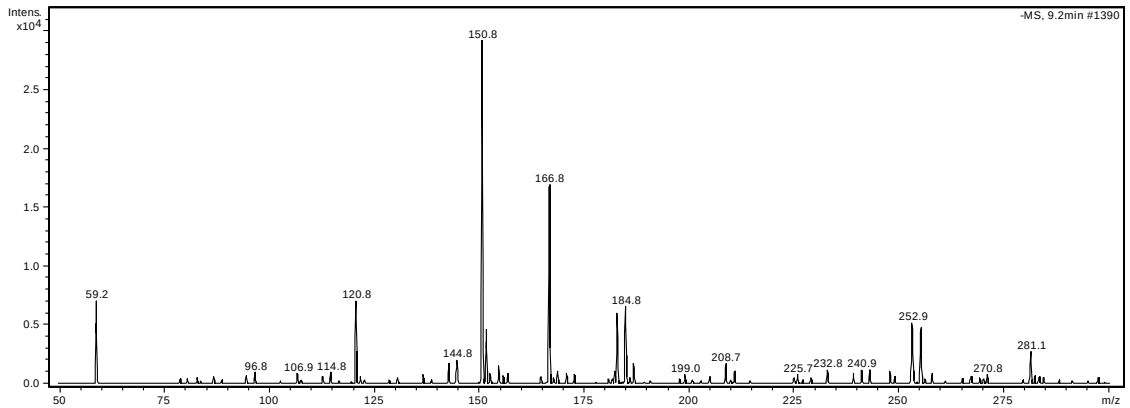


Figure 43 – Mass spectrum obtained for oleic acid.

• Palmitic acid

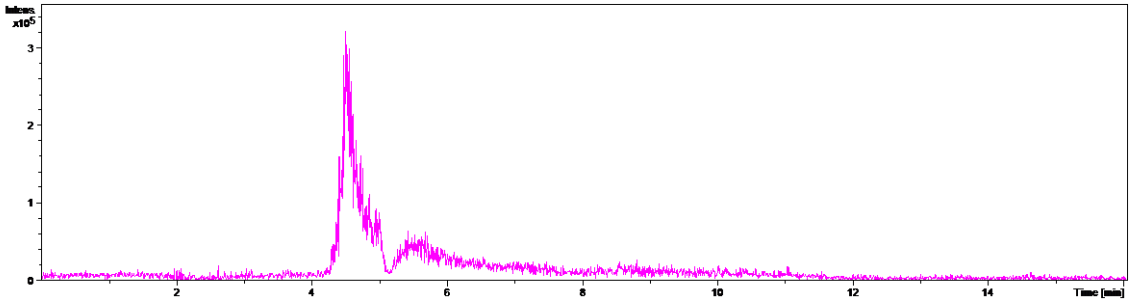

Figure 44 – Chromatogram obtained for palmitic acid.

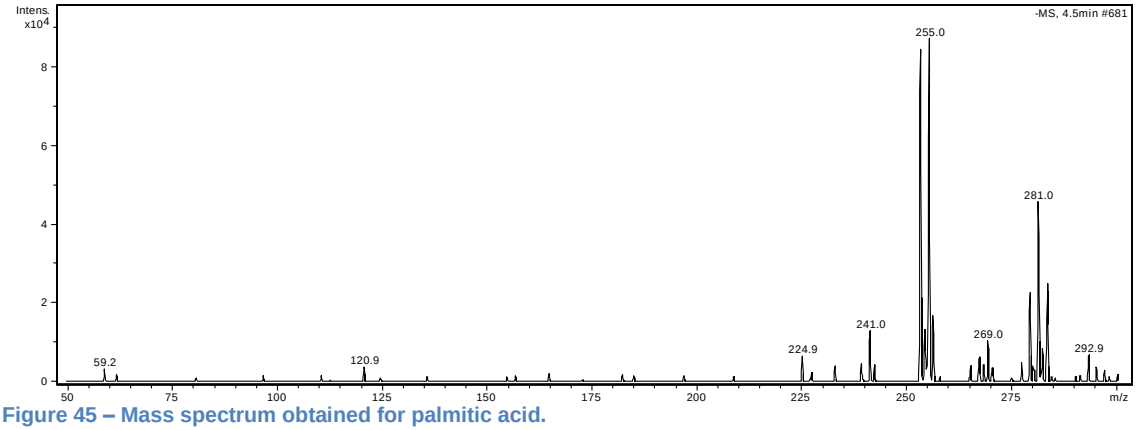


Figure 45 – Mass spectrum obtained for palmitic acid.

• Pinic acid

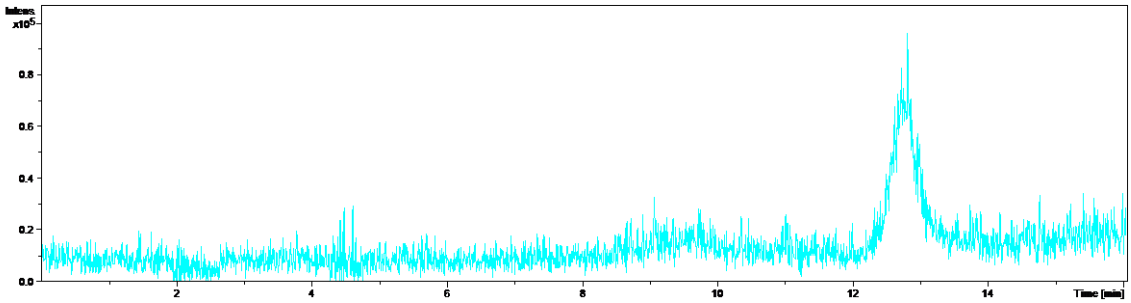

Figure 46 – Chromatogram obtained for pinic acid.

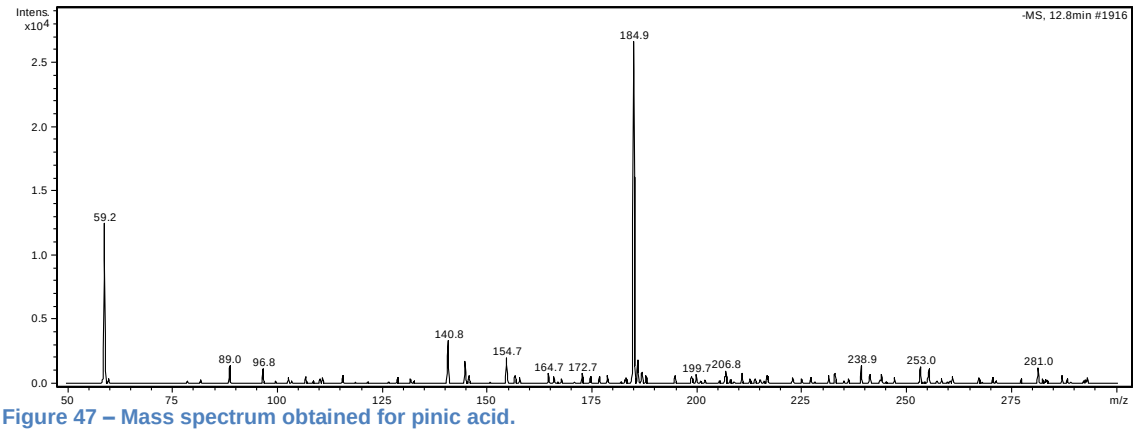


Figure 47 – Mass spectrum obtained for pinic acid.

• *cis*-Pinonic acid

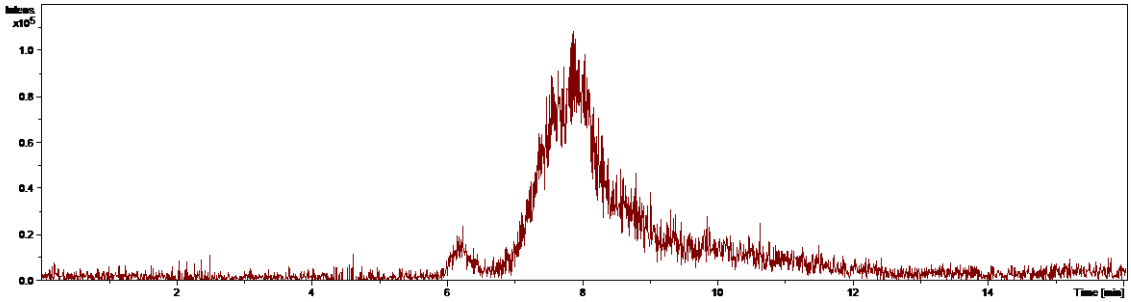

**Figure 48 – Chromatogram obtained for *cis*-Pinonic acid.**

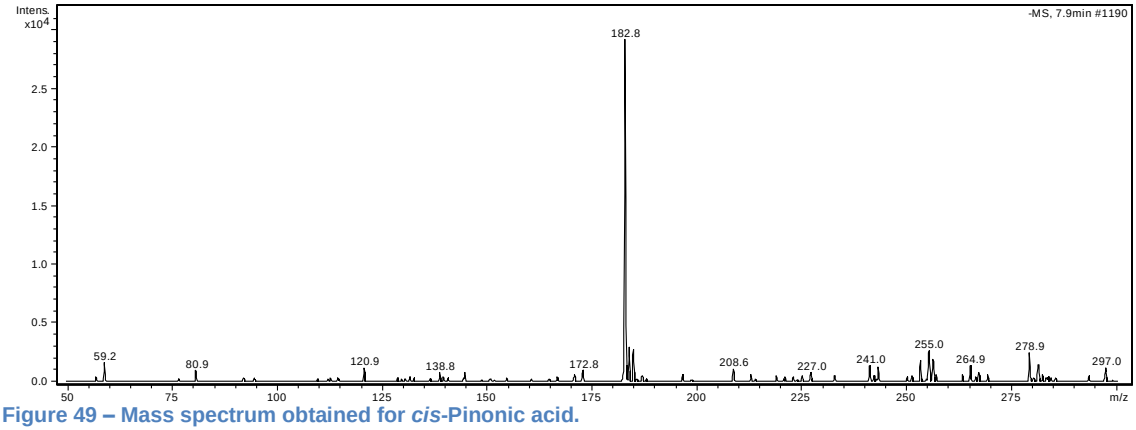


**Figure 49 – Mass spectrum obtained for *cis*-Pinonic acid.**

• Sebacic acid

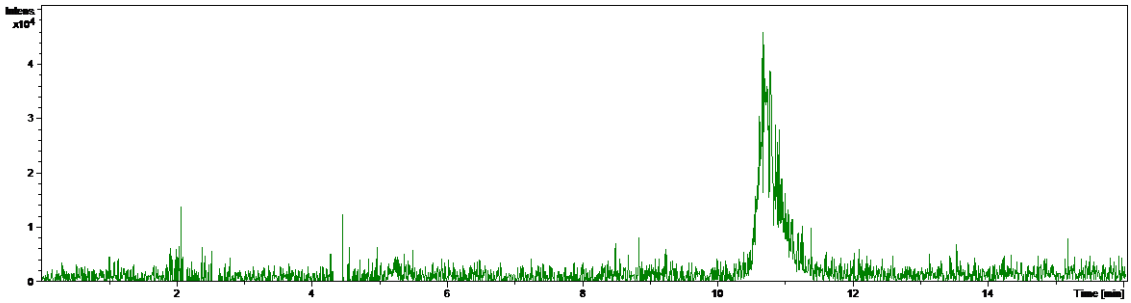

**Figure 50 – Chromatogram obtained for sebacic acid.**

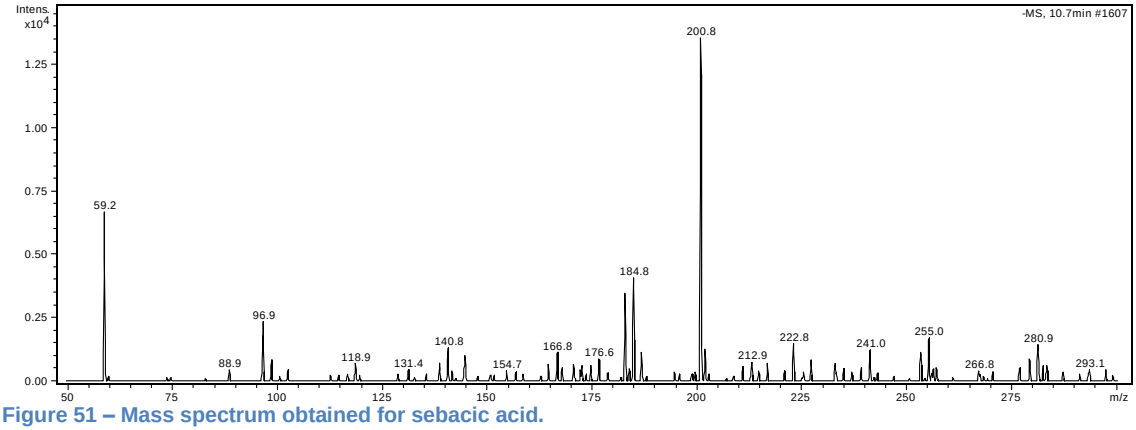


**Figure 51 – Mass spectrum obtained for sebacic acid.**

• Stearic acid

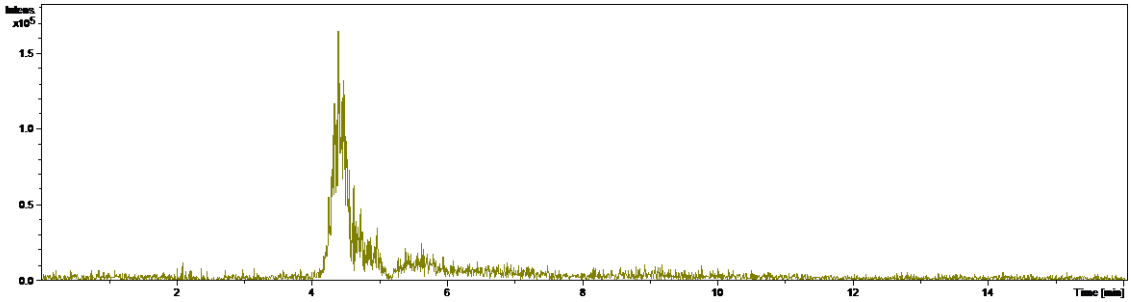


Figure 52 – Chromatogram obtained for stearic acid.

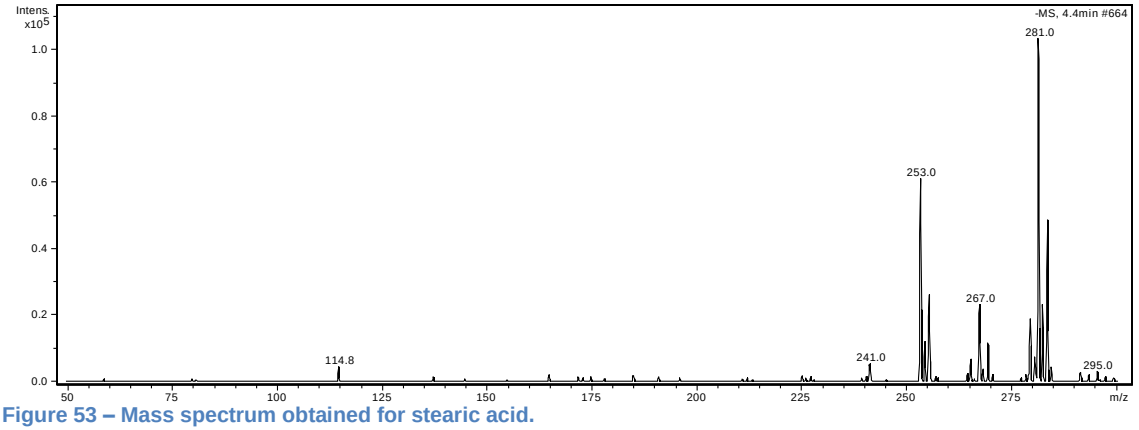


Figure 53 – Mass spectrum obtained for stearic acid.

• Tartaric acid

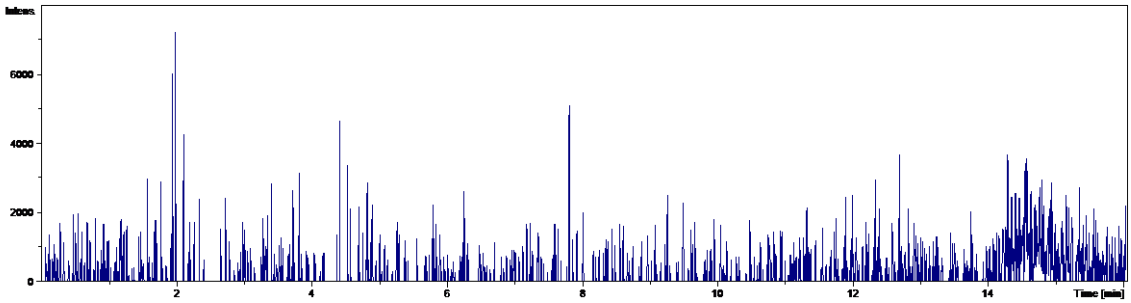


Figure 54 – Chromatogram obtained for tartaric acid.

• Vanillic acid

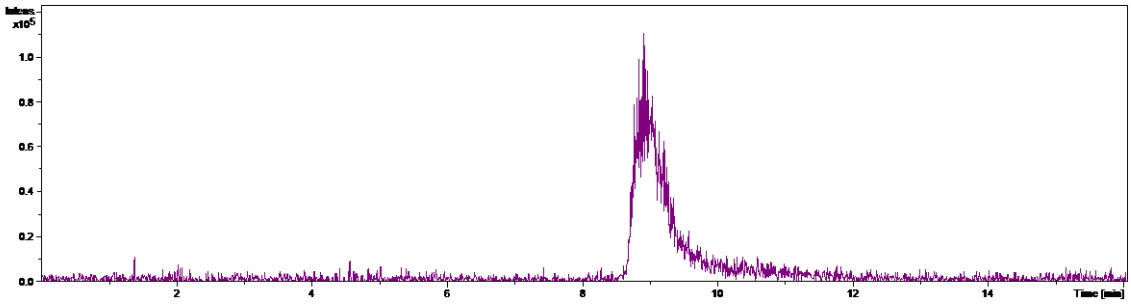


Figure 55 – Chromatogram obtained for vanillic acid.

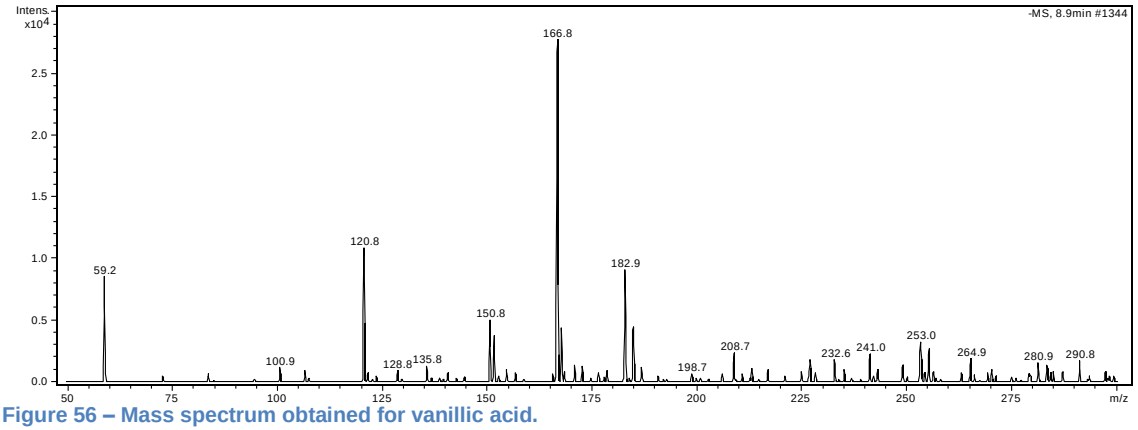


**Figure 56 – Mass spectrum obtained for vanillic acid.**