Master Thesis

Application of Multidimensional Gas Chromatography to the Analysis of Odor-active Compounds

Mehrdimensionale Gaschromatographie zur Bestimmung geruchsaktiver Verbindungen

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Abstract

Odour is ubiquitous and often it is just a small percentage of food that consists of odour-active compounds. The odour is of enormous importance, because it is a criterion in the decision about acceptance or rejection of a product. In this work, gas chromatography (GC) as the method of choice for the analysis of odour-active compounds, is in focus.

In the theoretical part of this work two-dimensional gas chromatographic methods, heart-cut gas chromatography (MDGC) and comprehensive gas chromatography (GC × GC), were compared regarding their application in the separation of aroma-relevant compounds. The question, which instrumental requirements regarding the interface and the detector are demanded, was answered. In addition to that, it was discussed when two-dimensional methods are beneficial or necessary compared to techniques with one-dimensional gas chromatographic hyphenated with mass selective detection (1D-GC/MS).

The practical work can be classified in three parts: *i*) a method development and characterisation of the mass-selective detection in 1D-GC/MS, *ii*) sample measurement in 1D-GC/MS, and *iii*) a method development for heart-cut MDGC. Samples were standard solutions, non-alcoholic elder flower extracts and beer with different hop sorts. Measurements were carried out via headspace solid phase microextraction (HS-SPME). By means of one-dimensional gas chromatography different ion sources and different ionisation methods were investigated. A higher sensitivity was observed for the pure EI source compared to the combi source that combines electron impact and chemical ionisation. The factor of the sensitivity increase was between two and 20.
In the investigation of elder flower extracts of different sorts a significant difference between the sorts regarding the investigated odour-active compounds was discovered. 21 compounds were quantified with different ionisation methods by means of an external standard method. Thereby no significant difference in the determination of the concentration was found comparing different A difference in the results were found due to variation of sample amount. The reasons for that were discussed.
Additionally, a method was developed to transfer separation problems in an one-dimensional GC system to a two-dimensional heart-cut GC and to solve them by means of this technique.

Kurzfassung

Geruchsaktive Verbindungen stellen mengenmäßig den geringsten Anteil eines Lebensmittels dar. Dennoch besitzt das Aroma eine enorme Wichtigkeit, da er ein wichtiges Kriterium in bei der Entscheidung über Akzeptanz oder Ablehnung von Produkten darstellt. Gaschromatographie (GC) als die Methode der Wahl für die Bestimmung der aroma-relevanten Verbindungen steht in dieser Arbeit im Mittelpunkt.

Im theoretischen Teil dieser Arbeit wurden zweidimensionale gaschromatographische Methoden, die Heart-Cut Gaschromatographie (MDGC) und die comprehensive Gaschromatographie (GC×GC), hinsichtlich ihrer Anwendung in der Trennung von Aromastoffen verglichen. Die Fragestellung, welche Anforderungen dabei an die Instrumentation hinsichtlich Interface und Detektor gestellt werden, wurden beantwortet. Außerdem wurde beschrieben, wann zweidimensionale gaschromatographische Methoden gegenüber Methoden mit eindimensionaler Gaschromatographie in Kombination mit massenselektiver Detektion (1D-GC/MS) von Vorteil bzw. notwendig sind. Die praktische Arbeit wurde in drei Teile gegliedert werden: *i*) eine Methodenentwicklung und Charakterisierung der massenselektiven Detektion mit 1D-GC/MS, *ii*) Messungen von Proben mit 1D-GC/MS und *iii*) einer Methodenentwicklung für Heart-Cut GC (MDGC). Proben waren hierbei Standardlösungen, nicht-alkoholische Holunderblütenextrakte und Bier mit unterschiedlichen Hopfensorten gewesen. Die Messungen wurden mittels Dampfraum-Festphasenmikroextraktion (HS-SPME) durchgeführt. Mit Hilfe von eindimensionaler Gaschromatographie wurden unterschiedliche Ionenquellen und unterschiedliche Ionisationsmethoden getestet. Dabei konnte festgestellt werden, dass die reine Elektronenstoßquelle eine erheblich bessere Sensitivität im Vergleich zur Kombiquelle, mit der die Methoden Elektronenstroßionisation und chemische Ionisation möglich sind, aufwies. Der Faktor der Sensitivitätssteigerung lag dabei zwischen zwei und 20 je nach Verbindung.
Bei der Untersuchung von Holunderblütenextrakten unterschiedlicher Sorten konnte ein signifikanter Unterschied zwischen den Sorten, bezüglich der untersuchten geruchsaktiven Verbindungen, festgestellt werden. 21 Verbindungen wurden mit den unterschiedlichen Ionisationsmethoden mit Hilfe einer externen Kalibration quantifiziert. Dabei konnten keine signifikanten Unterschiede bei der Konzentrationsbestimmung zwischen den unterschiedlichen Ionisationsmethoden festgestellt werden.
Ein Unterschied in den Ergebnissen der Quantifizierung ergab sich durch der Variation der Probenmenge. Die Ursachen dafür werden in der Arbeit diskutiert.
Es wurde außerdem eine Methode entwickelt um Trennprobleme auf einer eindimensionalen gaschromatographischen Methode auf eine zweidimensionale Heart-Cut GC Methode zu übertragen und mit Hilfe dieser zu lösen.

Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Place	Date	Signature

Acknowledgements

This thesis was written at the Institute for Analytical Chemistry and Food Chemistry at the Graz University of Technology from September 2011 until July 2012.
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Graz, July 2012 Dian Fedl

Abbreviations

^{1}D	First Dimensional
^{2}D	Second Dimensional
1D	One-dimensional
2D	Two-dimensional
^1t_R	Retention Time of the first Dimension
^2t_R	Retention Time of the second Dimension
AED	Atomic Emission Detector
DC	Direct Current
ECD	Electron Capture Detector
EI	Electron Impact
FPD	Flame Photometric Detector
GC	Gas Chromatography
GC-O	Gas Chromatography Olfactometry
GC×GC	comprehensive two-dimensional gas chromatography
LMCS	Longitudinally Moving Cryogenic System
LOQ	Limit of Quantification
LOD	Limit of Detection
FID	Flame Ionisation Detector
HS	Headspace
m/z	Mass-to-Charge Ratio
MDGC	Multidimensional Gas Chromatography
μ-EDC	Micro-Electron Capture Detector
MS	Mass Spectrometry
MSD	Mass-Selective Detector
n.d.	not detected
NPD	Nitrogen Phosphorus Detector
qMS	Quadrupole Mass Spectrometer
RF	Radio Frequency
S/N	Signal-to-Noise Ratio
SEI	Semi Electron Impact
SCI	Semi Chemical Ionisation
SIM	Single/Selected Ion Monitoring
TCD	Thermal Conductivity Detector
TD	Thermal Desorption
TOF	Time of Flight
VOC	Volatile Organic Compound

Contents

Part I.

Introduction

1. General Aspects and Motivation

The aroma of natural products consists of a huge amount of compounds having different structures, volatilities, reactivities and occurring in small concentrations. Odour-active substances play an important role in our daily life and are decisive whether we like or dislike a product especially in the food sector.
Analysis of aroma-relevant compounds is an important way to evaluate the quality of products. Potent compounds with a low odour threshold can reduce the quality even at very low concentrations. An example for that are trichloranisoles which are responsible for cork-taint in wine.
But the analysis of odour-active compounds is not only a part of the evaluation of product qualities. It can also be beneficial for understanding biological pathways and thermal reactions in which their formation occurs. There are various sources that provide aroma-relevant compounds. In fruit and vegetable odour formation is a process of catabolism, i.e. an enzymatic degradation process. In the Maillard reaction the formation of aroma-relevant substances is based on reactions of amino acids with reducing sugars by application of heat. There is also flavour formation by microorganisms as part of their primary metabolism or by enzymatic activity after their lysis. [1]
All these processes generate a variety of volatile odour-active compounds that influence the aroma in a positive or negative way. To get an impression of the complexity of aroma a number has to be mentioned: 3500 volatile compounds are known to be formed in the Maillard reaction, and numerous of them have a low odour threshold and therefore contribute to the aroma of the heated product. [1]
Due to their volatility gas chromatography is the method of choice to separate mixtures odour-active compounds and to analyse these substances qualitatively and quantitatively. However, mixtures with a high complexity are often challenging to separate and can not easily be resolved by GC analysis. Therefore, development regarding two-dimensional GC separation systems was carried out resulting in two general 2D GC techniques that are discussed and compared in this work. The benefit of two-dimensional gas chromatography lies in subjecting a sample to two different separation columns which vary in their selectivity, resulting in an increase of peak capacity, sensitivity and selectivity.
But food analysis in not the only sector where limited peak capacity of one-dimensional GC is an issue. Also in petrochemistry, in environmental analysis and in fragrance industry people often deal with samples of high complexity. The analysis of these also require multi-dimensional separation methods.

As aforementioned, there are two different techniques: *i*) the heart-cut two dimensional GC technique and *ii*) the comprehensive two-dimensional GC technique, which are both

described in the theoretical part of this work.
These techniques are compared and evaluated in respect to the analysis of odour-active compounds. The analysis was executed via HS-SPME and subsequent GC-analysis with mass-selective detection.
The questions, which are discussed in this work, are

(i) for what kind of analysis are two dimensional gas chromatographic techniques beneficial or even necessary,

(ii) in terms of what do these two two-dimensional GC techniques differ, and

(iii) when does one-dimensional GC analyses give sufficient and satisfactory results.

In chapter 2 a short overview is given about the principles of gas chromatography with a focus on headspace analysis and detection methods regarding the analysis of odour-active compounds. The following chapter describes the principle of multi-dimensional gas chromatography (chapter 3) and later both techniques are specified, heart-cut gas chromatography in chapter 4 and comprehensive gas chromatography in chapter 5. A comparison of these techniques and a subsequent comparison of multidimensional GC techniques with one-dimensional GC in chapter 6 complete the theoretical part.
In the subsequent part the practical work is described. First the analysed samples are specified (chapter 7), then the analysis carried out with one-dimensional GC and finally multi-dimensional GC analysis are described in chapter 8, respectively in chapter 9.

Part II.

Theoretical Part

2. Gas Chromatography

Gas chromatography is an analytical method to separate mixtures of compounds for a subsequent identification and quantification [2]. It is a powerful separation technique for volatile and semi-volatile compounds. Furthermore, there is the possibility to analyse less volatile compounds by transforming them into volatile derivatives, e.g. analysing fatty acids via their fatty acid methyl esters. The compounds suitable for GC analysis must have an appreciable volatility below 350 °C to 400 °C without degradation or reacting with other compounds [3]. Compound structure and molecular weight can be indicators for the suitability for GC analysis [2].

2.1. Principles of Gas Chromatography

The separation principle of gas chromatography is based on a combination of partition and adsorption chromatography, depending on the stationary phase. In general a gas chromatographic system consists of six basic components:

- gas supply and flow controllers
- injector
- detector
- oven
- column
- data processing system

In the block diagram in figure 2.1 gas flow and path of the information in a gas chromatograph is shown.
The sample is injected and transferred through the separation column by a carrier gas. The sample compounds interact with the inner coating of the column and elute after a certain time, the retention time. The effluent of the column in analysed constantly by a detector that provides a signal to the data processing system.
Due to the focus of this work on two-dimensional GC techniques basic principles is not discussed into detail.
In this chapter different techniques of headspace analysis and common detectors for GC analysis are described. A short overview of the GC columns is given in section 4.2.

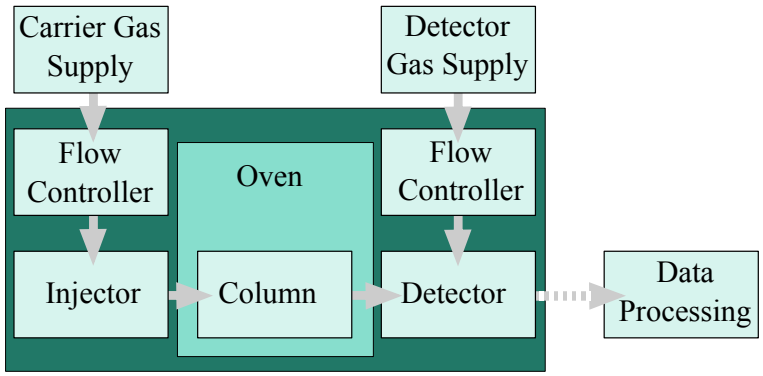

Figure 2.1.: Scheme of a gas chromatographic analysis system is displayed. The solid arrows show the gas flow, the interrupted arrow electronic signal flow.

2.2. Headspace Analysis of Aroma-relevant Compounds

Headspace analysis is a suitable method for the determination of volatile organic compounds (VOCs) including odour-active compounds. The challenge is to extract the analytes reproducibly from matrices, like food, cosmetics, polymers, or pharmaceutical raw materials. By headspace analysis the analytes are transferred into the gas phase which is subsequently analysed by an in instrument, like a gas chromatograph. [4]
Headspace analysis is a gentle way to extract volatile compounds of a sample and introduce them in a GC. It can be carried out in several ways: as a single step extraction (static headspace), by stepwise repeating of the extraction (multiple headspace extraction), and by stripping the volatiles by a continuous flow of an inert purge gas (dynamic headspace) [5]. These methods are described in the following sections and a closer look at solid phase microextraction (SPME) technique is taken.

2.2.1. Static Headspace Methods

Static headspace analysis comprises two steps. First the sample placed in a vessel that is closed. Then the vial is thermostat-controlled at a constant temperature until equilibrium between the two phases (sample and headspace) is reached. Subsequently an aliquot of the vial's gas phase is introduced into the carrier gas stream which carries it into the column. This transfer can be carried out by a number of ways [5]:

- manually, for example by using a gas-tight syringe, or
- automatically, by means of pressurization of the sample vial and a time or volume controlled transfer of an aliquot into the column or by adsorption traps, like by solid phase microextraction (SPME).

2.2.2. Dynamic Headspace Methods

The dynamic headspace technique is a continuous method of gas extraction which separated volatile sample constituents from the matrix by a continuous flow of an inert gas flow above the sample. This technique is known as *purge and trap*. A high sample surface is advantageous. If a aqueous sample is to be analysed a sintered glass disk where the gas flows through provides bubbles which increase the gas-sample interface. [5]
The idea of dynamic headspace analysis is the complete removal of the volatiles of interest from the matrix in order to analyse these compounds. The purged volatiles must be focused in a trap. This process can be carried out thermally, by a cold trap, or by a cartridge packed with an adsorbent. So-trapped compounds are usually released by applying heat and are transferred by a carrier gas flow to the column. However, there is also the possibility to extract those compounds by a small amount of solvent. This general set-up is modified and various variants emerged. [5]

2.2.3. Multiple Headspace Analysis

Multiple headspace analysis is also an exhaustive extraction. It is performed by consecutive analyses on the same sample vial. With each removal of an aliquot of the sample's headspace the analyte in the sample is reduced and chromatogram of each injection show declining peak areas. When the peak area is zero, the amount of analyte in the sample is exhausted. The total amount of the analyte is calculated by the addition of the peak areas, deriving from the target analyte, in the chromatograms. The advantage of this technique is that matrix effects are eliminated, because the total amount of the analyte is extracted. [4]

2.2.4. Headspace Solid Phase Microextraction (HS-SPME)

Headspace solid phase microextraction is a static headspace method in which the sample compounds are trapped by an adsorption process. It was developed in 1989 and is commercially available since 1993. [4] SPME technique is a two-step procedure. The liquid or solid sample is placed in a vial which is close by an aluminium cap with a septum the same way it is carried out for other static head space analysis. A fiber coated with a film of stationary phase is housed within a syringe and can be exposed. The syringe protects the fiber and allows easy penetration of the vial and inlet septa.
For extracting the compounds of the headspace the needle penetrates the septum and the fiber is exposed to the sample headspace. This process is shown in figure 2.2. It can be seen that the extraction is dependent on two distribution equilibria (sample – headspace and headspace – fiber). After sample enrichment by adsorption of the compounds on the coating, the fiber retracted again and the syringe is introduced into the injection port where desorption is carried out thermally by exposing the fiber. [5]
SPME can also be carried out by exposing the fiber into a sample but this is not discussed further, here.

The aforementioned distribution equilibria have to be considered and this includes,

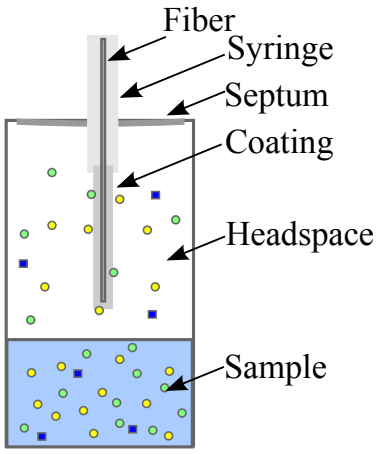

Figure 2.2.: Principle of HS-SPME is shown. The two distributions to be considered (sample – headspace and headspace – fiber) are visible here.

that the amount of adsorbed compounds by the fiber coating increases with increasing solubility in the fiber coating. Another issue to be remarked is that volatility can be enhanced by increasing the temperature but this also lowers the coating/headspace distribution coefficient, $K_{F/G}$. [5]
Each compound will behave differently depending on its polarity, its volatility and its organic/water partition coefficient. Further parameters affecting the distribution are the volume of the sample, the volume of the headspace, respectively the volume/surface ratio, rate of agitation, pH of the solution and the temperature of the sample. [6]

HS-SPME has several benefits in comparison to extraction methods with solvents, e.g. solid phase extraction (SPE), which are:

- it is solvent-free, i.e. eliminates solvent disposal,
- it is simple and straightforward (only sorption and desorption steps) ,
- it can easily be automated,
- it has a high concentration ability, and
- phases can be chosen according to the separation problem (here are a wide range of selective fiber coations available)
- it is non-exhaustive.

The fact that this technique is non-exhaustive means that less of the sample is extracted in comparison to SPE (about 2 to 20 % of the compounds in the sample) but all of the extracted compounds are injected into the GC. In SPE about 90 % of analyte is extracted from the sample and only 1 to 2 % is injected. This means that this sample preparation method can be applied in "living" or dynamic systems with minimal disturbance. [7]
But there are some disadvantages to be considered. A change in the matrix have an effect on quantitative results due to changes in the value of the distribution constants. [7]

In this work a coating of DVB/Carboxen/PDMS is used. This is a multiple-component bipolar phase that contains a combination of DVB-PDMS (50 µm) layered over Carboxen-PDMS (30 µm). This composition expands the molecular weight of the extracted compounds due to meso- and macropores of the outer layer and micropores in the inner layer. This coating is used for extraction of odour compounds and volatile flavour compounds. [7]

Quantification with SPME

Quantification is possible with SPME, but the conditions have to chosen carefully. The concentration of the analyte has to be within the linear range of the method. For quantification kinetic considerations have to be made referring to the relationship between the analyte concentration and the extraction time [7]:

- The extraction time is independent of the analyte's concentration in the sample
- The relative amount extracted at a defined period of time is independent of analyte concentration
- The absolute amount extracted at a defined period of time is linearly proportional to the concentration of the analyte in the sample.

2.3. Detectors for Gas Chromatography

A compound eluting from the chromatographic column interacts with the detector. An electrical signal in generated and the size of the signal is related to the amount of the eluting compound making quantitative analysis possible. There are several detectors in GC analysis which differ in technique and in fields of application. In this section the detectors in GC analysis are classified and the most common ones are specified later. [2] [3]

2.3.1. Characteristics

In gas chromatography the detector should give constantly an electrical signal over the whole retention period. Desirable characteristics are a high sensitivity, linearity, and detectivity. Furthermore it should be relatively simple and inexpensive. [3]
Most detectors used for gas chromatographic analysis where especially designed for this use. Exceptions are the thermal conductivity detector and the mass selective detector. The flame ionisation detector (FID) is the most popular detector. [3]

2.3.2. Detector Classification

There are some classification to categorize a detector. The most important ones are mentioned in this section.

Concentration vs mass flow range

It has to be distinguished between detectors that measure the concentration of the analyte in the carrier gas compared to those detectors that directly measure the amount of analyte irrespective of the volume of the carrier gas. The peak areas and peak heights are affected in a different way by changing the gas flow rate. [3]

Table 2.1.: Examples of concentration and mass flow detectors

Concentration	Mass Flow
TCD, ECD	FID, NPD

Destructive vs non-destructive

Non-destructive detectors are necessary if the separated compounds are used for further analysis. In cases where the compound can only be analysed when destroyed, there is the possibility to split the column effluent and guide one part of the flow to the destructive detector and the other part is available for further analysis. This is done when analysing with GC-olfactometry and applying a chemical detector, e.g. FID or MS, in parallel. [3]

Table 2.2.: Examples of destructive and non-destructive detectors

Destructive	Non-destructive
EI-MS, FID, FPD, NPD	TCD

Selective vs universal

The selectivity is a further classification of detectors. A universal detector detects all solutes whereas a selective one detects particular types or classes of compounds. For example FID is not very selective and detects all organic compounds while ECD is very selective and detects only electronegative species, like halogen-containing molecules.
Universal detectors are advantageous for screening, if all solutes should be detected. [3]

Table 2.3.: Examples of selective and universal detectors

Selective	Universal
NPD, FPD, AED	TCD

2.3.3. Detector Characteristics

Besides the signal, that is provided by the detector and is specified later, there are further more characteristics of the detector. The most important ones are discussed in the following paragraphs.

Noise

Noise is the signal which is produced by the detector in absence of a sample [3]. It is also called background and it appears on the baseline. The noise arises from different sources. One of them is from the electronic components generating random fluctuations. This kind of noise can be kept low by an intelligent circuit design. Especially, effective shielding and grounding reduce the noise to certain extend.
Another type of noise occurs from the chromatographic gases and is related to their purity.
The definition of the noise by the American Society for Testing and Materials, ASTM, is two parallel lines drawn between the peak-to-peak maxima and minima enclose the noise. The ratio of signal to noise is characteristic for the performance of the detector. Commonly, the smallest signal, attributed to the analyte, has a signal-to-noise ratio (S/N) of 2. [3]

Time Constant

The time constant is a measure of the response speed of a detector. It is the time the detector takes to respond to 63 % of a sudden change in signal. The full response (actually 98 % of full response) takes four time constants and is referred to as the *response time*. Increasingly longer time constants effect the shape of a chromatographic peak resulting in a change in retention time and in peak width. The retention time gets larger and the peaks get broader when the time constant increases. The peak area is not affected. Quantitative analysis based on the area stay accurate but those based on peak height will be incorrect. The time constant should be less than 63 % of the peak width at half height, w_h. The overall time constant for the entire system is limited by the largest value for any of the individual compounds. Large time constants have the advantage of decreasing the short-term noise from a detector. This effect is called *damping* and can be advantageous to a certain extend. However, there is the risk of loosing valuable chromatographic information by exhausting damping. [3]

Cell Volume

The cell volume is also called detector dead volume and is the space between the end of the column and the point of detection. This delay in reaching the detector can have an effect on the shape of the peaks. Is this volume large peaks tend to tailing and broadening. [8]

Signal

The signal is the detector output which is of interest in an analysis process. The magnitude is proportional to the amount of analyte. It is the basis for quantitative analysis, an important application for GC. The signal specifications discussed in the following paragraphs are sensitivity, minimum detectability, linear range, and dynamic range. [3]

Sensitivity

The sensitivity of the detector, S, is equal to the signal output per unit concentration or per unit mass of an analyte in the carrier gas. The units of sensitivity are based on area measurements and differ for the two main detector classifications, concentration and mass flow rate. [3]
In a diagram plotting *detector signal vs. concentration* (*mass* respectively) of analyte the slope displays the sensitivity. The more sensitive a detector is the steeper is the slope and ice versa. [3]

Minimum Detectability

The lowest point in a diagram plotting detector *signal* vs. *concentration* that represents the lower detectable limit has several names such as *minimum detectable quantity* (MDQ), *limit of detection* (LOD), and *detectivity* [3]. The IUPAC definition of the *minimum detectability*, D, as

$$D = \frac{2N}{S} \tag{2.1}$$

where N is the noise level and S is the sensitivity defined above. The noise is multiplied with the factor 2 because as mentioned before the detectable signal should be twice the noise level. [3] The units for this measure are $\mathrm{mg\,mL^{-1}}$ for a concentration type and $\mathrm{mg\,s^{-1}}$ for a mass flow rate type detector.
The limit of quantification (LOQ) is related to the detectability and should be above the LOD. There are different guidelines to define the LOD and the LOQ. The American Chemical Society (ACS) guidlines specify that S/N should be three for the LOD and 10 for the LOQ. But there is another definition which state that the LOQ should not be less than two times the LOD. [3]

Linear Range

The diagram plotting the *detector signal* as a function of the *concentration of the analyte* becomes non-linear for high concentrations. Hence it is necessary to establish the upper limit of linearity. This can also be seen by plotting the *sensitivity* vs. *concentration*. According to the American Society for Testing and Materials (ASTM) specification, the upper limit of the linear range is the concentration corresponding to a sensitivity equal to 95 % of the maximal measured sensitivity. [3]
The lower level of the linear range is the minimum detectivity, discussed before. The linear range is defined as the quotient of the both ends:

$$\text{Linear range} = \frac{\text{Upper limit}}{\text{Lower limit}} \tag{2.2}$$

A large value is desired for this parameter. The unit is for the both limits the same and due to that the linear range is dimensionless. [3]
The linear range should not be confused with the *dynamic range*. The upper limit of the dynamic range is the concentration at which the signal does not increase with increasing concentration, hence it is at a higher concentration than the upper limit of the linear range. [3]

2.4. Common GC Detectors

In this section detectors commonly used in GC analysis are specified and their application in the field of odour-active compounds are discussed. Schemes of common detectors, which are not based on mass separation, are shown in figure 2.3.

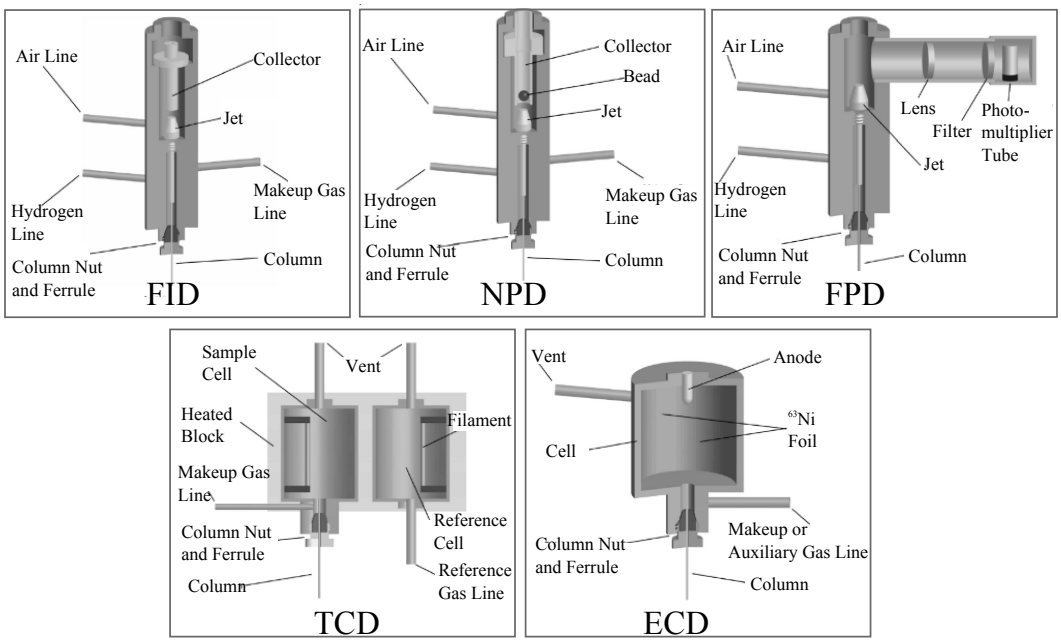

Figure 2.3.: Schemes of common non-mass selective GC detectors [8]

2.4.1. Flame Ionisation Detector (FID)

It is an ionization detector especially invented for GC analysis [3]. A voltage is applied to the flame jet and the collector and a hydrogen and oxygen (air) flame is maintained at the tip of the jet. The carrier gas exiting from the column is guided into the flame and a combustion process of the organic compounds eluting from the columns is started. The ionic species generated in this process move to the charged collector and produce a small current that is measured. [2]
The FID is a detector with a characteristic high sensitivity and it has the advantage of

negligible internal volumes and relatively high data acquisition rate of 50 Hz to 300 Hz. In this process water is produced and therefore the detector has to be heated to at least 125 °C to prevent the condensation of water in the detector. Most FIDs operate at a temperature of 250 °C or more. [3] [9]
This detector responds to all organic compounds that burn in an oxy-hydrogen flame. The signal is approximately proportional to carbon content. However, when a oxygen or a nitrogen atom is present the signal decreases. Relative response values are often tabulated as *effective carbon numbers*, ECN. For example ethane has a ECN of 2.0 and Ethanol has a value of 1.48. [3]
The gases have to be pure and free from organic material because this would increase the detector noise. Compounds that do no burn in the flame are not detected [3]. Parameters which influences the detector signal are the flow rate of hydrogen, air and makeup gas flow rate as well as the temperature. Especially the flow rate of hydrogen affects the detector response. It has a maximum for each carrier gas and the optimum is at about the column flow rate. The air flow rate is not so critical. Nitrogen or helium are used as makeup gas whereby nitrogen increases the detector sensitivity by 10 to 20 % over helium. FID is a mass flow detector that means that changes in the carrier gas and the makeup gas flow rated do not significantly effect the sensitivity. [3]
With a linear range of 10^6, which is the largest among all GC detectors, FID is an optimal detector for screening a sample and for mixtures of compounds which occur in a wide concentration range. [3]

2.4.2. Electron Capture Detector (ECD)

The electron capture detector was invented in 1961 by Lovelock and it is a selective detector that detects compounds with an electronegative heteroatom, like a halogen [3]. The ECD is an ionisation-type detector. Electronegative molecules eluting from the column cause a decrease in the level of ionisation unlike most detectors of this class [3].

Radioactive ^{63}Ni emits beta particles and if no analyte is present the negative charge is detected. These negatively charged particles collide with the nitrogen molecules which form the carrier gas and produce more electrons according to equation 2.3.

$$\beta^- + \mathrm{N_2} \longrightarrow 2\,\mathrm{e^-} + \mathrm{N_2^+} \tag{2.3}$$

The electrons formed in this process effect a high standing current of about 10^{-8} A when collected by a positive electrode. In the presence of electronegative molecules, A, some of the free electrons are captured by A and thereby decrease the standing current (see equation 2.4).

$$\mathrm{A} + \mathrm{e^-} \longrightarrow \mathrm{A^-} \tag{2.4}$$

The negative ions have lower mobilities than the free electrons and are not collected by the anode. Mathematical relationship are similar to Beer's Law, which describes absorption processes for electromagnetic radiation. The extent of the capture of electrons in proportional to the concentration of the analyte. [3]

Applicaton in the field of the analysis of odour-active compounds
ECD is sensitive regarding electronegative atoms in a molecule and, therefore, is used for halogenated odour-active compounds like trichloranisole and tribromanisole. These are compounds which cause malodour in wine like the cork taint. [10]

2.4.3. Nitrogen Phosphorus Detector (NPD)

The nitrogen phosphorus detector was invented in 1964 by Karmen and Giuffrida as *alkali flame ionisation detector*. It consists of a FID to which a bead of an alkali metal salt. Karmen found that the FID shows selectively higher sensitivity when alkali metal salt is present near the flame. [3]
A voltage is applied to the jet and the collector and a bead usually made of rubidium silicate is located above the jet. The bead is electrically heated and a plasma is sustained around the bead by adding hydrogen and air. But the hydrogen flow is too low to support a flame. Nitrogen and phosphorus containing compounds react in this plasma around the bead and produce ions. These ions move to the charged collector and a small current is generated and measured. No hydrocarbon ionisation takes place and this results a high selectivity of nitrogen and phosphorus containing compounds. It has an ionisation efficiency which is about 10 000 times better for nitrogen and phosphorus compounds in comparison to FID and that means that also the sensitivity for these compounds are increased by this factor. [2] [3]
The mechanism is not well understood. But it shows enhanced detectivity for phosphorous-, nitrogen- and some halogen-containing substances. The range of selectivity of nitrogen containing compounds over carbon containing compounds in common NPDs is 10^3 to 10^5. The value for phosphorus containing compounds is 10^4 to $10^{5.5}$. [3]

Applicaton in analysis of Odour-active Compounds
Nitrogen containing odour-active compound groups like pyrazines are formed during Maillard reaction which is also referred to as non-enzymatic browning. In this reaction which occurs at higher temperatures during cooking and roasting processes amino acids and reducing sugars react and flavour compounds, e.g. thiols, thiazoles, furans, pyrrols, and pyrazines, are generated. [11]
Pyrazines are compounds of high odour intensity and low threshold values. They can be produced by microorganisms and are also present in wines like in *Cabernet Sauvignon* and *Cabernet Franc*. Pyrazines are also present in paprika and chilli. [10] [12] There also pyrroles and pyridines which are odour-active. Some of them are listed in table 2.4.
Amines are also aroma-relevant compounds. Their odour thresholds are pH-dependent. [12] NPD is beneficial for detecting these compounds because in FID and MS the signal of the nitrogen containing compounds are often overlaid by other non-nitrogen containing compounds eluting at the same time.

Table 2.4.: Sensoric properties of some volatile nitrogen compounds [12]

Pyrrol and pyridin derivates	
Compound	**Odour**
2-acetyl-1-pyrroline	rice, crust of white bread, popcorn
2-propionyl-1-pyrroline	popcorn, heated meat
2-acetylpyridine	crust of white bread
Pyrazine derivates	
Compound	**Odour**
2,3,6-trimethyl-pyridine	earthy
2-ethyl-3,5-dimethyl-pyridine	earthy, roasted
2-acetyl-pyrazine	roasted corn
2-isoproyl-3-methoxy-pyrazine	potatoe
2-isobutyl-3-methoxy-pyrazine	paprika

2.4.4. Flame Photometric Detector (FPD)

In flame photometric detection carrier gas exiting the column is mixed with air and introduced into a oxy-hydrogen flame. Organic compounds are burned in this flame. Sulfur containing compounds form S_2 and phosphorus containing compounds form HPO. These compounds are excited and fall back to the ground state emitting light. S_2 has its emission maximum at around 394 nm and HPO has its maximum at around 526 nm. Only light of these wavelengths are filtered out of the emission spectrum and reach the photomultiplier tube where it is detected. The range of selectivity of sulfur and phosphorus containing compounds over hydrocarbons with this detector is 10^4 to 10^5. [2] [3]

Application in the Analysis of odour-active compounds

As mentioned above, when specifying the field of application for NPD, also sulfur containing volatile compounds are generated in the Maillard process while heating proteins and reducing sugars. Reaction partners are cysteine, cystine, monosaccarides, thiamine and methionine. [12]

Also onion plants (onions, leek, garlic) contain a lot of sulfur compounds which are characteristic for their flavour.

Volatile sulfur compounds can also be a product of the metabolism of microorganisms and can also be found in wine, e.g. simple alkyl thiols and mercapatanes and also more complex structures like thioacetones and terpenthioles. Some sulfur compounds are associated with tropical and citrus fruit flavour like passion fruit or grape fruit at low concentrations. [10]

Ternary thiols have an extremely low odour threshold and have the property that they are associated with fruity odour at low concentrations and at higher concentration the

odour description is "catty". But they make an important contribution towards the odour of many products, like fruits, olive oil, wine and roasted coffee. Some sulfur containing compounds and their odour is listed in table 2.5. [12]

Table 2.5.: Sensoric properties of some volatile sulfur compounds [12]

Compound	**Odour**
hydrogen sulfide	sulfureous, foul-smelling
methan thiole	sulfureous, foul-smelling
dimethylsulfide	asparagus, boiled
dimethyldisulfide	cabbage-like
methional	boild potatoe
methionol	sulfureous
3-mercapto-2-butanone	sulfureous
2-furfuryl alcohole	roasted, coffee
2-methyl-3-furanthiol	boiled meet
3-mercapto-2-methylpentan-1-ol	meaty, onion-like

2.4.5. Thermal Conductivity Detector (TCD)

A thermal conductivity detector is a differential detector and measures the thermal conductivity of the analyte in carrier gas compared to the thermal conductivity of the pure carrier gas. At least two measurement cavities, which are drilled into a metal block, are required but most common are four cavities. Each contains a resistance wire or filament. DC current is passed through the wire to heat it above the temperature of the cell block, creating a temperature difference. A small volume of the cavity is desirable for faithful reproduction of peak shapes and higher sensitivity. The carrier gas used with TCD must have a thermal conductivity that is very different from the sample to be analysed. The most commonly used gases are helium and hydrogen which have the highest thermal conductivity values. TCD response does not correlate directly with values of the thermal conductivity. [3]
Thermal conductivity detectors were applied in nearly all early GC instruments and still they remain popular, particularly for packed columns and inorganic analytes. [3]

2.4.6. Atomic Emission Detector (AED)

In the atomic emission detector molecules eluting from the column are broken down to elements by a microwave powered plasma. They are excited and their emission irradiation is measured. Different atoms have different emission wavelength which is detected by a photomultiplier after being separated by a diffraction granting. Usually argon plasmas is used for metal excitation but provides a poor excitation of non-metallic compounds. In

1989 the first instrument was developed using an atmospheric pressure helium plasma. The main advantage is that the atomic emission of many elements is detected simultaneously and chromatograms, made up of peaks from eluants, that contain only one specific element, are produced. But it is rather complex and the response depends on many parameters.

2.4.7. Olfactometric Detection

Gas chromatography-olfactometry (GC-O) is a technique based on human sensory evaluation of the eluate from a chromatographic column. The first detection with a sniffing port was reported in 1964. Further developments were made and in 1971 the first GC-O configuration was reported where humid air is added to the GC effluent. This detection technique takes advantage of the sensitivity and selectivity of the human nose. In principle the effluent of the GC is split and one part of the effluent is directed to a chemical detector, e.g. FID or MS, and the other part is guided to a so-called sniffing port where it is sniffed by a human nose. The person on the sniffing port describes the odour of the effluent and this is recorded. After the run the detector signal can be overlaid with the olfactometric signal. This type of analysis is beneficial for odour-active compounds because large GC peak areas generated by a chemical detector do not necessarily correspond to high odour intensities of a compound in the sample [13]. The relationship between concentration of a substance and intensity of the perception is given by odour threshold values which are already determined for many compounds. These values are dependent on the matrix of the sample.
It has also to be considered that there can be interactions of odour-active compounds with each other or with constituents of the matrix that can have amplifying or masking effects.
The sensory importance of an compound is difficult to predict and depends on many parameters like *i*) its concentration in the matrix, *ii*) its human nose limit of detection, *iii*) volatility, and *iv*) interaction of flavour molecules with each other and with other constituents. [13]

2.4.8. Mass Selective Detector (MSD)

Nowadays hyphenate mass spectrometry with gas chromatography (GC/MS) is an essential part of most analytical laboratories. GC/MS plays a major role in environmental, foods and flavours, aromas, petrochemical, and pharmaceutical industries.

Mass selective detectors consist of a *ion source*, where the molecules eluting from the chromatographic column (or other samples sources) are ionized and in some cases are fragmented (see figure 2.4). Aligned to the source is the *mass analyser* which separates the ions. The signal of these ions is generated by a *ion detector*, e.g. an electron multiplier. Different types of mass analysers are specified to understand which one can fulfil the requirements which are given by multidimensional GC. The combination of GC and MS is powerful because of following characteristics [3]: *i*) its high speed of analysis

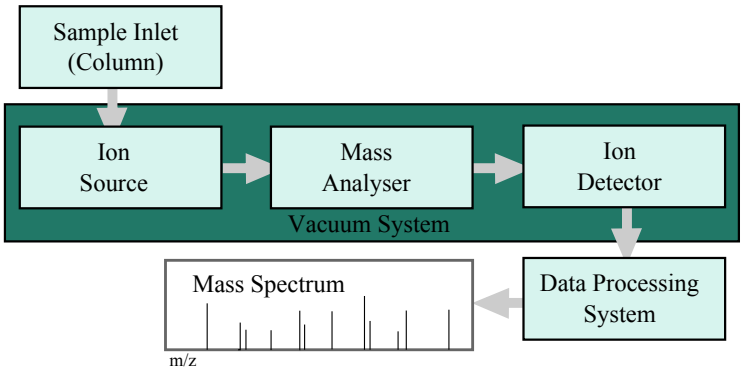

Figure 2.4.: Scheme of a mass selective detector is shown. In GC/MS the sample inlet is the separation column. Ion source, mass analyser and ion detector are embedded in a vacuum system. After data processing a mass spectrum is obtained.

ii) its high resolution *iii*) the ease of operation *iv*) its excellent quantitative results, and *v*) its moderate costs.

Pure GC analysis does not provide any structural information. It offers retention times that are related to the partition coefficients of the molecules between mobile phase and stationary phase. The retention times are characteristic, but they are not unique, i.e. various compounds can have the same retention time. In combination with mass spectrometry GC provides more information: besides the signal intensity mass spectra are provided. [3]
The ion source and the mass analyser are specified in the following sections.

Ionisation Methods

The ionisation of molecules for subsequent mass analysis can be carried out in many different ways. In the following sections electron impact ionisation (EI) and chemical ionisation (CI) are specified. There are many more ionisation methods but they are not suitable for GC measurements or not commonly used in combination with GC for other reasons and therefore, are not described in this work.

Electron Impact (EI)

Electron impact ionisation is the most common ionisation method GC is coupled with MS and therefore, it is discussed in more detail. This method can just be applied on samples which are volatile, hence it is a suitable method for GC analysis.

Mechanism

A filament, which is the cathode, is heated and thus and causes electrons to be emitted into an ionisation chamber. These electrons are attracted by an anode on the opposite side of the chamber. The energy of the electrons is controlled by the potential difference between anode and cathode. Usually a potential difference of 70 V is chosen so that the electrons have an energy of 70 eV. The GC effluent

is directed into the ionisation chamber and the eluting molecules collide with the electrons which are on their way to the anode. In order to increase the possibility of the collision a magnetic field is established so that the electrons have a helical flight path, which leads to a larger effective flight path and, therefore, to an higher impact probability. The collision of an electron with the gaseous molecule, *ABC*, often leads to a loss of an electron and a generation of a positively charged radical ion $ABC^{+}\cdot$ (equation 2.5).

$$ABC + e^{-}_{initial} \longrightarrow ABC^{+}\cdot + e^{-}_{initial} + e^{-}_{fromABC} \tag{2.5}$$

The energy of 70 eV is much higher than it would be needed to ionise a molecule. Most organic molecules ionise in the range of 8 eV to 15 eV. Therefore, further reactions during the collision of the electrons with the molecule *ABC* take place and can be summarized as followed:

Dissoziative Ionisation

$$ABC + e^{-} \longrightarrow AB^{+}\cdot + C + 2\,e^{-} \tag{2.6}$$

$$ABC + e^{-} \longrightarrow AC^{+}\cdot + B + 2\,e^{-} \tag{2.7}$$

Ion-Pair Formation

$$ABC + e^{-} \longrightarrow AB^{+} + C^{-} + 1\,e^{-} \tag{2.8}$$

Electron Capture

$$ABC + e^{-} \longrightarrow ABC^{-} \tag{2.9}$$

Dissociative Electron Capture

$$ABC + e^{-} \longrightarrow ABC^{-} + C\cdot \tag{2.10}$$

Because of the formation of fragment ions EI is referred to as *hard ionisation*. After the ionisation process the ion repeller forces the ions to move into the mass analyser where molecules with different m/z-values are separated. [14]

Advantages

This technique is perfect suitable for GC analysis, because by GC analysis volatile compounds are separated and volatility is also a requirement for EI ionisation. Another advantage is that molecules ionized with EI show a constant fragmentation profile. Compounds can be identified by the ratio of the fragments. Hence, data bases are applied, where mass spectra of many substances can be recalled.

Chemical Ionisation (CI)

By chemical ionisation the analyte molecules are not ionised directly by electrons but via a ionisation gas, e.g. methane or isobutane. Chemical ionisation is dependent on more parameters than electron impact ionisation, for example on the ionisation gas, the ionisation gas pressure, and the temperature of the ion source.

In general there are two different types of chemical ionisation, positive and negative chemical ionisation.

In the CI plasma both positive and negative ions are formed it is just a matter of the polarity of the acceleration voltage which ions are extracted from the ion source and get into the mass analyser.

Positive Chemical Ionisation

The first step, an interaction between gas molecules and electrons, is similar to electron impact ionisation. The difference to EI is that not the analyte molecules get ionized by the electrons but the molecules of the reaction gas. In the next step charge is transferred from the reaction gas ion to the sample molecule by a proton or by the ion itself.

Because of the loss of energy in the first step this method is more gentle than EI hence less fragmentation takes place (*soft ionisation*).
The initial electrons should have an energy of about 200 eV, because otherwise the electrons would have problems to penetrate the gaseous phase. Chemical ionisation is the result of competing chemical reactions, hence the sensitivity of this method strongly depends on the conditions. Parameters are [15]:

- the primary ion energy
- the electron current
- the type reactant gas
- the reactant gas pressure, and
- the ion source temperature.

In PCI mainly pseudo-Molecule ions $M + H^+$ are generated, but also ions where the reacting ion is connected to the analyte molecule can be detected.
As aforementioned the type of reaction gas also influences the energy transfer from the initial electron to the analyte molecule. By using isobutane, instead of methane, less energy is transferred to the analyte molecule in comparison to methane as reaction gas and therefore less fragmentation occurs. This is why isobutane is a softer reaction gas than methane.

The following equations show the formation of the reacting ions of methane.

$$CH_4^+ \cdot + CH_4 \longrightarrow CH_5^+ + CH_3 \cdot \tag{2.11}$$

$$CH_4^+ \cdot \longrightarrow CH_3^+ + H \cdot \tag{2.12}$$

$$CH_3^+ \cdot + CH_4 \longrightarrow C_2H_5^+ + H_2 \tag{2.13}$$

The ratio of CH_5^+ to $C_2H_5^+$ ions are dependent on the gas pressure, the temperature and the energy of the electrons [16]. Those reacting ions act as proton donor and the sample molecule, *ABC*, is ionised according to equation 2.14.

$$CH_5^+ + ABC \longrightarrow [ABC + H]^+ + CH_4 \tag{2.14}$$

Other reaction gases and their reacting ions are given in table 2.6.
To prevent the direct ionisation of the molecule *ABC* by the electrons the concentration of the reaction gas has to be much higher than the concentration of the sample [14].
Also other ionisation reactions can occur like hydride abstraction $[ABC{-}H]^+$ or addition of cations (for methane $[ABC + C_2H_5]^+$ or $[ABC + C_3H_5]^+$).

Table 2.6.: Reaction Gases for PCI and their Reacting Ions

Reagent Gas	**Reacting Ions**	**m/z**
Methane	CH_5^+	17
	$C_2H_5^+$	29
Isobutane	$(CH_3)_3C^+$	57
Ammonia	NH_5^+	18
	$(NH_3)_2H^+$	35

Advantages and Applications

Parameters can be chosen so that fragmentation is completely suppressed and only pseudo molecule ions are formed. Thus, an analysis of molecular masses of a mixture can be done. For this application often isobutane is used as reactant gas. It is also possible to ionise specific functional groups or substance classes. For example by using NH_3 protons are just transferred to highly alkaline compounds. In general, most analytes accessible to EI can be analysed with PCI. [14]

Negative Chemical Ionisation (NCI) or Electron Capture Ionisation

NCI is also called electron capture ionisation because it can selectively analyse molecules with a high electron affinity such as halogenated compounds [17].

NCI produces negative ions by electron capture processes. Usually free electrons are created by the thermoionic emission from a heated metal filament. However, those electrons are above the thermal energy so they need to be decelerated. This can be done by buffer gases such as methane, isobutane, carbon dioxide and others. The electrons emitted from the filament lose energy by the collision with the reaction gas. Subsequently these low energetic electrons are captured by the analyte molecules and negative ions are formed.

The tendency of a compound to produce negative ions is closely related to the electron affinity. Halogenated compounds having a large electron affinity are ionized more easily which results in a high sensitivity for these compounds.

Strictly speaking, NCI is not s sub-type of CI because the analyte molecules do not react with the gas molecules but directly with freely moving electrons. The gas interacts as buffer gas but does not take part in the charge transfer process. The conditions are similar to those in PCI but it has to be considered that the ions have an inverse charge in comparison to EI and PCI. Hence the extraction voltage applied to accelerate the ions out of the source into the analyser has to be reversed. Also the source temperature has to be lower to provide lower energetic electrons. [15]

Mechanism
When neutral molecules interact with an electron of higher energy and a positive radical ion is formed (see EI). If the electrons have less energy than the ionisation energy of the molecule, EI is prohibited. When the electrons approach thermal energy electron capture occurs instead [15].

Resonance electron capture

$$\mathrm{ABC} + \mathrm{e}^- \longrightarrow \mathrm{ABC}^- \cdot \tag{2.15}$$

Dissociative electron capture

$$\mathrm{ABC} + \mathrm{e}^- \longrightarrow [\mathrm{AB}]^- + \mathrm{C} \cdot \tag{2.16}$$

Ion-pair formation

$$\mathrm{ABC} + \mathrm{e}^- \longrightarrow [\mathrm{AB}]^- + \mathrm{C}^+ + \mathrm{e}^- \tag{2.17}$$

By *resonance electron capture* a radical molecule ion is yielded (equation 2.15). It occurs by capturing electrons of 0 eV to 2 eV. Whereas even-electron fragment ions are formed by *dissociative electron capture* and by *ion-pair formation*. These reactions take place when the electron energy 0 eV to 10 eV respectively larger than 15 eV for *ion-pair formation*. For the instrument GCMS-QP2010 Plus (Shimadzu) the NCI ion source provides a semi-EI mode (SEI) and a semi-CI mode (SCI), as well as NCI. This source was used in the practical part of this work. [17]

Application
Negative chemical reaction combined with GC-MS is applied in monitoring pollutants toxaphene, dioxins pesticides, halogenated methabolites, explosives and others.

Mass Analysers

After passing the ion source the ion beam is guided by a lens system into the mass analyser. The ionic species are separated according to their mass-to-charge ratio (m/z).

This is done either by magnetic or electrical fields. Typical mass analysers for GC-MS are quadrupoles and time-of-flight analysers, which are discussed in in detail. Other analysers, not discussed in this work, are for example magnetic sector field instruments, Fourier Transform Ion Cyclotron Resonance analysers (FT-ICR), Orbitrap, and Iontrap instruments. [3]

Quadrupole
The quadrupole analyser consists of four hyperbolic rods which are arranged at right angles to each other and arranged in parallel. A DC voltage (U) is applied to all rods (adjacent rods have opposite signs). Additionally a radio frequency ($V_{RF}cos(wt)$) is also applied to the four rods. This is shown in figure 2.5. Equations 2.18 and 2.19 describe the voltage applied on the rods.

$$U_1 = U + V cos(\omega t) \tag{2.18}$$

$$U_2 = -(U + V cos(\omega t)) = -U - V cos(\omega t) \tag{2.19}$$

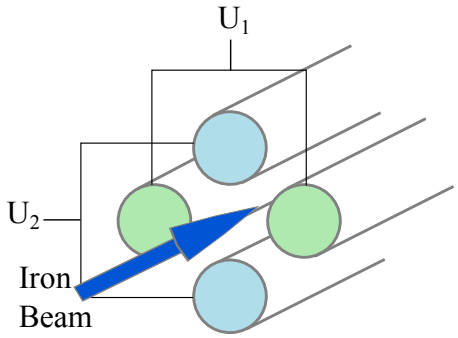

Figure 2.5.: Set-up of a quadrupole MS with the voltages U_1 and U_1 applied on the four rods. The ion beam is introduced into the mass analyser in parallel to the rods.

The ion beam accelerated out of the ion source and is introduced into the mass analyser. The direction from the source to the detector can be defined as the z-axis. The ions flying along the z-axis experience forces in x- and y-direction due to the voltages applied on the rods. The ions are rapidly attracted and then repelled from each rod. This results in a oscillating flight path of the ions. The m/z-range passing the mass analyser and reaching the detector depends on the combination of the radio frequency and the direct current. Ions, which are lighter or heavier than the m/z-range, crash into the rods. To scan a larger m/z-range of the sample the DC and the RF are varied so that the m/z-range passing the mass analyser is subsequently changed.
Due to its characteristics (simple, small, moderate in cost, and rapid scanning) it is often combined with gas chromatography analysis [3]

Time-of-Flight (TOF)
The time-of-flight analyser is a mass analyser that measures the time for ions to travel a

fixed distance very accurately. The accelerating voltage (V) and the length of the flight tube (l) are fixed for all ions. The time it takes for the ions to reach the detector depends only on their mass (m) and their charge (z). Common acceleration voltages are 10 V to 30 V and the length of the flight tube is usually 1 m to 2 m [14]
Smaller ions travel through the analyser more rapidly and larger ions more slowly. Equation 2.20 shows that the kinetic energy of the ions is dependent on the acceleration voltage.

$$E_{kin} = zeV \tag{2.20}$$

E_{kin}: kinetic energy
z: charge
e: unit charge
V: acceleration voltage

After applying this in the equation $E_{kin} = 1/2m\nu^2$ and solve it for the velocity, ν, equation 2.21 is obtained, that shows that the velocity of the ions is dependent on its mass and its charge. Via the equation $\nu = l/t$ the equation for the time in dependency of the m/z ratio is obtained.

$$\nu = \sqrt{\frac{2zeV}{m}} \tag{2.21}$$

m: mass

$$t^2 = \frac{m}{z}(\frac{l^2}{2eV}) \tag{2.22}$$

Reflectron TOF
To overcome the problem that not all ions do have exactly the same accelerating voltage at the beginning reflectron-TOF was invented where the ions are reflected by a lense system. It is also beneficial because the flight path is lengthened. Thereby, the resolution is increased dramatically.
TOF mass analysers can be used in combination with gas chromatography. Due to a

rapid separation of the ions TOF mass spectrometers are suitable for comprehensive gas chromatography where narrow peak width require a rapid detection system.

3. Fundamental Principles of Multidimensional Gas Chromatography

The main origin of multidimensional chromatography lies in planar chromatography. In 1944, the first two-dimensional thin layer chromatography has been reported. It consisted of a first dimensional separation and a second one rectangular to the first one. This was the origin of comprehensive chromatography. [18] [19]
The most important planar chromatography was the high resolution two-dimensional electrophoresis which was reported in 1975 the first time. It is still used in protein and DNA analysis [18].

In 1990, Giddings defined two requirements which have to be fulfilled to consider a multiple separation a multidimensional separation [20]:

1. The compounds of a mixture should be subjected to two (or more) separation steps in which their displacement is governed by different factors.

2. Analytes that have been resolved in an earlier step should remain separated until the separation process is completed.

The first topic defines that the separation steps have to be orthogonal. The orthogonality will be discussed in a section 3.3.

The principle configuration of a two-dimensional instrument is fairly simple:

first column – interface – second column,

whereby the first and the second column use different types of selectivity. [20]

In general there are two different approaches. One is heart-cutting multidimensional gas chromatography, often just called MDGC or GC-GC. With this technique one or few fractions are collected and transferred into the second dimensional column where a further separation occurs. This was also the beginning of two-dimensional gas chromatography.
The aim to increase the peak capacity for the whole chromatogram led to the development of a comprehensive two-dimensional gas chromatography (GC×GC) which is the second approach of two-dimensional GC. With this method the whole primary effluent is cut in small fractions, which collection lasts few seconds. The fractions are transferred, one after another, into a second shorter column. There, it is subjected to a further separation. In GC×GC the interface is called modulator and the modulator period defines the time how long the fractions are collected. The separation in the second dimension

column has to be at most as long as the modulation period. Otherwise the following fraction is entering the column while the previous separation is not finished. Peaks resulting because of this effect are called wrap-around peaks.
Both two-dimensional GC approaches will be discussed later in detail in the chapters 4 and 5.

Multidimensionality offers many advantages which are for example a high peak capacity, a high selectivity, and an improved sensitivity. But especially for comprehensive GC there are also challenges to be taken: need for rapid detection, customized data systems, and more complex instrumentation [21].

3.1. Advantages

3.1.1. Peak Capacity

The peak capacity is the number of peaks that fit in the space of a chromatogram, which means the number of peaks that can be resolved in a given time frame. Expressed by a formula the peak capacity is [21] [8]:

$$n = \frac{\sqrt{N}}{4R_S} \ln \frac{t_{R2}}{t_{R1}} + 1 \tag{3.1}$$

N is the plate number, n is the number of peaks, R_S is the desired resolution, and t_{R2} and t_{R1} are the maximum and minimum retention times in which the peak may elute [21]. Equation 3.1 applies to one-dimensional chromatography. The peak capacity of a comprehensive two-dimensional separation is calculated by multiplying the peak capacities of the two columns. Therefore with comprehensive GC×GC the peak capacity for the whole chromatogram is increased which is shown in figure 3.1. But this applies to the ideal case of two orthogonal columns, discussed in section 3.3. Practically the peak capacity is less than the product of the capacities of the two columns [21].
The aim of heart-cut GC is to increase the peak capacity for a fraction of the first dimensional effluent. The peak capacity in MDGC can be calculated by adding the peak capacity of both columns when cutting once, see figure 3.1. By applying more than one cut the peak capacity of the second column are multiplied with the amount of cut and afterwards it is added to the peak capacity of the first dimensional column. [18] [22]

3.1.2. Selectivity

The high selectivity of multidimensional separations is based on the combination of two columns. It can be matched specifically to the separation problem [21].
The column selection is discussed later in 4.2. Due to the application of columns with different selectivities in the two dimensions the possibility that the compounds which are not separated on one column are also not separated on a second differing column is very low.

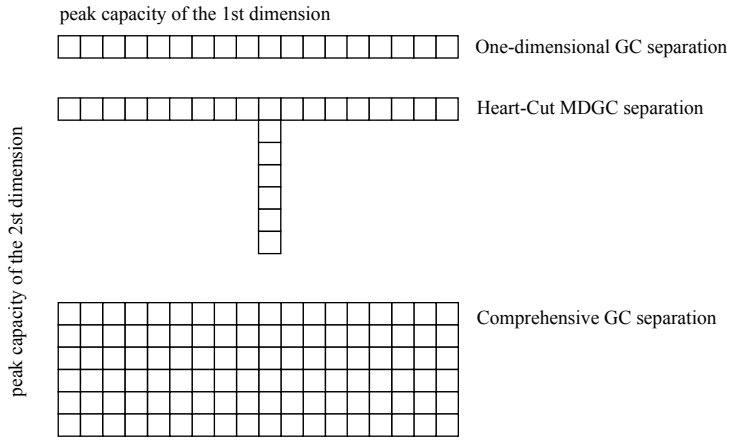

Figure 3.1.: Comparison of the peak capacity of one-dimensional GC, two-dimensional MDGC and two-dimensional comprehensive GC separation.

3.1.3. Sensitivity

In comprehensive GC the compounds which elute from the first column are focussed in the modulator (see section 5.3. This is followed by a fast separation in the second dimension due to the smaller dimensions of the ^{2}D-column, regarging the length, the inside diameter, and the film thickness. The consequence is that the peaks get sharper and narrower, and the peak height increases. It is reported that it comprehensive GC gives a 50-fold or better sensitivity than in one-dimensional GC. [22]
There is also the possibility of cryo-focussing the fraction in MDGC which increases sensitivity.

3.2. Challenges

Many challenges have to be taken especially in comprehensive GC technique. The main challenges are given by the hardware that includes the detector and the interface and by the software which carries out data processing and data visualisation.

3.2.1. Fast Detection

The challenge of a fast detection emerge for GC×GC. In general, at least 20 data points per peak are needed to define a peak properly [21]. In comprehensive GC×GC the peak widths are very narrow (about 100 ms) which is demanding for the detection system. For example, if a peak has a peak width of 100 ms, then there should be a data point at least every 5 msec or 200 data points per second. This is the maximal data acquisition rate a FID has and this is one of the fastest detectors. [21] [22]
The types of detectors that can be used for two-dimensional comprehensive are specified in chapter 5.

3.2.2. Interface

Complex instrumentation in two-dimensional gas chromatography is also one challenge. In both two-dimensional techniques chromatography requires a transfer of the first dimensional effluent to the second dimensional column. The interface between the columns traps the effluent of the first column, focusses it and transfers the fraction onto the second column. There are different interfaces in MDGC and in GC×GC that are discussed later (see section 5.3 and 4.3).

3.2.3. Data Processing

A huge amount of data are generated especially in GC × GC analysis and there is special software which can cope with that. It can transform the two-dimensional chromatogram, which is retention time as x-axis and detector signal on the y-axis, into a contour plot. The contour plot's y-axis represent the first dimensional retention time ${}^1t_R$ and on its y-axis the second dimensional retention time, ${}^2t_R$ is shown. The intensity in this plot is shown by the colour. This transformation of the gained signal is also shown in figure 5.6 in chapter 5.

3.3. Orthogonality

In analytical chemistry orthogonality in a two-dimensional system means that responses in the two dimensions are statistically independent. The separation capacity is lower when the two columns interact in a similar way with the sample components. [23]
The term orthogonality is difficult to define correctly and to measure quantitatively. Two data vectors are orthogonal if their values are statistically independent. [23]

By using comprehensive GC it is much easier to select orthogonal columns than in MDGC. Due to the fast separation in the second column and the application of one oven for both dimensions in GC×GC, the second dimension can be seen as isothermal separation because the second dimensional separation lasts few seconds at the temperature rate of about $3\,^{\circ}\mathrm{C\,min^{-1}}$ to $10\,^{\circ}\mathrm{C\,min^{-1}}$. Therefore, the boiling point component of the separation just affects the first dimension. [22]
Figure 3.2 shows the influence of the column combination on the separation of a mixture analysed via GC×GC.

In the following chapters both different approaches of two-dimensional gas chromatography, *i*) heart-cut (in chapter 4) and *ii*) comprehensive (in chapter 5) two-dimensional gas chromatography, their instrumentation, and their application are discussed in detail.

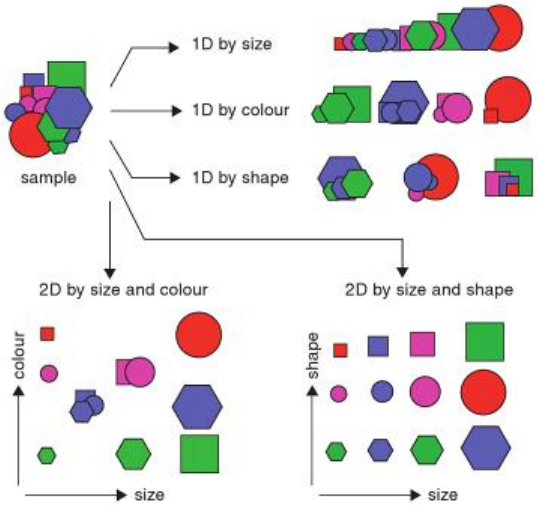

Figure 3.2.: Illustration of the separation of a mixture via 1D chromatography and GC×GC depending on the selectivity of the separation columns. [20]

4. Heart Cut Multidimensional Gas Chromatography (MDGC)

4.1. Technique

A classical Heart-Cut MDGC set-up consists of two capillary columns which have conventional dimensions and are connected in series. The columns are differing in selectivity, like a non-polar–polar or a polar–chiral column combination. A cutting and transfer device is located between the two columns and enables the passage of the first dimensional effluent into the second column. The interface is a valve which switches between the first dimension detector and the second dimension column (see figure 4.1). A cryotrap located between the two columns, which focusses the effluent thermally, is also beneficial but not necessary. The sensitivity increases by using a cryotrap through re-focussing of the analyte in the interface [24].

The columns can be located in one oven or in two separate ovens. Whereby the two ovens solution is the more flexible one and the separation in both dimension can be optimized. Therefore, columns with different temperature ranges can be used in combination, like polysiloxane and polyethylenglycol columns. This would be possible in a one-oven system but the temperature range has to be adapted to the polyethylenglycol column which is less stable at high temperatures.
Furthermore, there are no restrictions regarding the time domain, that means the duration of the second run is not coupled to the first one. This is especially advantageous for chiral separation which is very time consuming.
It has also to be considered that a two-ovens system means an additional investment in equipment [25].
But there are also further limitations in MDGC. A high increase in run time, especially for enantioselective analysis has to be considered.
Another limitation is that just a few fractions can be taken (just for few target analytes) otherwise co-elution can take place in the second dimension. For that reason, it is not the method of choice if a sample should be screened. Furthermore, it has to be considered that the method development is more challenging than for one-dimensional GC.
Finally, this technique is useful for identifying and also quantifying a few target analytes of a complex sample which can not be separated in the first dimension.

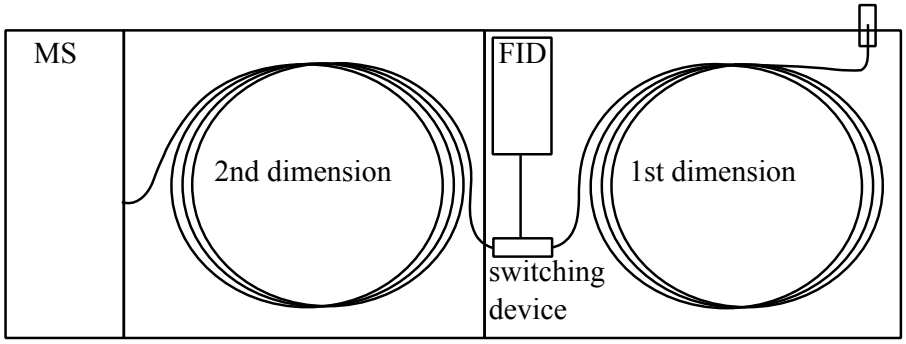

Figure 4.1.: A scheme of a multidimensional gas chromatograph with two ovens, flame ionisation detection in the first dimension, and mass selective detection in the second dimension.

4.2. Column Combinations

McNair described in his book *Basic Gas Chromatography* the column selecting process as following:

"The easiest and quickest is to ask someone who knows. That person may work in your laboratory or down the hall. If there is an experienced chromatographer nearby or otherwise accessible to you, you should not hesitate to ask." [3]

However, basic knowledge of the separation columns is necessary for the selection of a column for a specific separation problem.
Generally there are few main backbones used for the stationary phase of non-chiral columns [2]:

Polysiloxane columns are the predominant polymers used for stationary phases: substituted polysiloxanes, only a few structures used as functional groups (methyl-, phenyl-, cyanopropyl- and trifluoropropyl-) and these groups are used in various amounts and combinations. Polysiloxane stationary phases use a percentage substitution system, e.g. a 5 % phenylmethylpolysiloxane is comprised of a phenyl-group attached to 5 % of the sites of the backbone silicon atoms. The remaining 95 % of the sites have methyl-groups. The polarity of the some column types relative to each other are given in table 4.1.

Arylene modified polysiloxane columns are similar to standard polysiloxanes, but lower column bleeding and higher temperature limits.

Polyethylen glycol columns has some unique separation characteristics which can not be achieved with polysiloxane columns. Their main disadvantages are that they are sensitive towards oxygen at high temperatures and, therefore, have a narrow temperature ranges.

The stationary phases can be listed according to their polarity (see table 4.1).

Table 4.1.: Stationary phase polarity, in order of increasing polarity, based on Kovats indices [2]

Stationary Phase
100 % Methyl
5 % Phenyl-methyl
6 % Cyanopropylphenyl-methyl
20 % Phenyl-methyl
35 % Phenyl-methyl
14 % Cyanopropylphenyl-methyl
50 % Phenyl-methyl
65 % Phenyl-methyl
50 % Trifluoropropyl-methyl
Polyethylen glycol (PEG)
50 % Cyanopropyl-methyl
80 % Cyanopropyl-methyl
100 % Cyanopropyl-methyl

In MDGC analysis most common column combinations are

- polar–non-polar
- non-polar–polar
- non-chiral–chiral.

A great advantage of MDGC separation is that the two columns can be chosen according to the separation problem. There are no limitations regarding the dimensions of the column like in GC×GC.
Before choosing the columns the separation strategy has to be considered. If a group of target compounds is to be analysed there is the strategy to make the analytes elute from the first dimension within a short period of time. This retention part is then transferred to the ^{2}D-column. Subsequently the actual separation occurs in the second column. In this case the first dimension is acting like a pre-separation.
If the problem is co-elution of one or few target analytes with compounds having a similar fragmentation pattern in the first dimension these retention parts are cut and transferred to the second dimension.
Another application is the analysis of chiral compounds. A large number of organic compounds in food and beverages and also a significant number of additives (flavours, fragrances, pesticides and perservatives) are chiral molecules. For their separation the compounds have to be subjected to a chiral column which are usually cyclodextrin columns.

4.3. Switching device

The switching device is located between the first dimensional and the second dimensional column (see figure 4.2) and it has to direct the carrier gas flow either to the detector or

the ^{2}D-column. In the early MDGC systems, developed in the mid 1950's, the switching device was either a rotary or slide valve between the columns. [26] [27]
The major drawbacks of a valve system are the dead volume and the use of metal, which is in contact with the sample and can show undesirable adsorption effects. Another disadvantage is that the valves are inside the GC oven and are exposed to high temperature. [27]
Nowadays, the interface between the two columns is usually a Deans-type switch which was developed in 1968. The advantage of this switch is that there are no moving parts or valves inside the GC oven and within the sample flow. The principle is a non-intrusive manipulation of the carrier gas effluent between the two columns by means of pressure control. [27] [25]

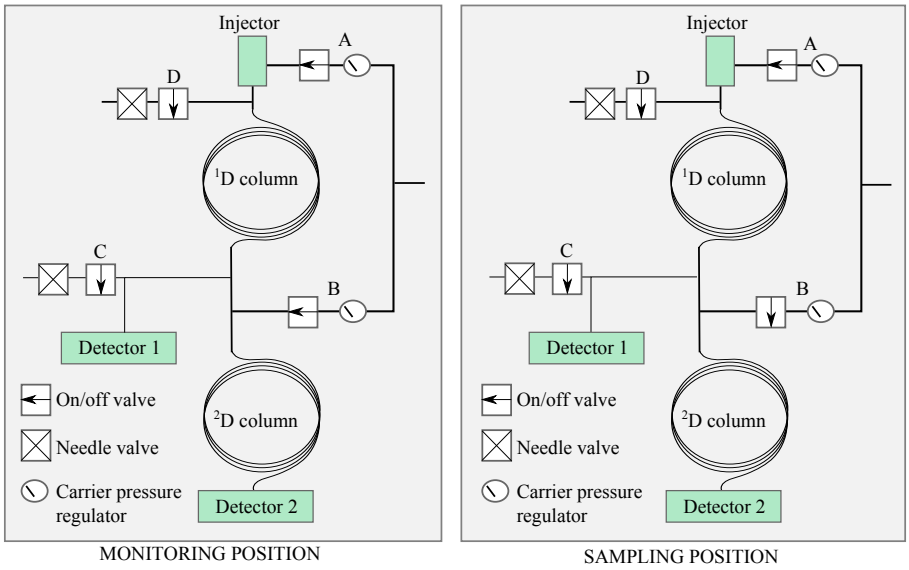

Figure 4.2.: Principle of a Deans switch in monitoring position *left* and in sample position *right*. In this positions the direction of the gas flow is changed by switching valves B and C.

By switching the valves A to D in figure 4.2 different gas flows can be achieved, which affect if the first dimensional effluent is directed to the monitor detector (detector 1) or to the second dimensional column. The positions are shortly described in the following paragraphs. [25]

Monitoring Position: The pressure is adjusted such that the flow from the primary column is diverted towards detector 1. The configuration is shown in figure 4.2. It prevents the ^{1}D-effluent to enter the ^{2}D-column by applying a slightly higher gas pressure regulated by B. This gas flow is directed to the second dimensional column and subsequently to the detector.

Sampling Position: Here, the valve between the two columns (B) is closed so that the whole carrier gas flow is provided by A. The valve behind detector, C, is also closed, so both columns are coupled in serial.

Backflush Position: This position is not shown in figure 4.2. Here, valve B and valve D are open so that the carrier gas flow is guided through the first dimensional column in direction of the injector. The pressure in the ^{2}D-column is also provided by B.

4.3.1. Limitations of the Traditional Deans Switch

A big drawback is that the Deans Switch causes retention time shifts when cutting a part out. This can be problematic when cutting more than one fraction. The reason for that is that the resistance towards the second column is higher than towards the first dimensional detector. Therefore a resistor towards the detector has to be implemented between the switch and the detector. The problem is that the resistor has to be altered when the second dimension column is changed and this can be time consuming. Invention of electronic pneumatics and flow calculator software facilities improved the system. [26]

4.3.2. Multi-Deans Switch

The multi-Deans Switch is an invention by Shimadzu and has the advantage that there is a lower probability of fluctuations and shifts in retention time by the first cut. So more cuts can be made without a further adjustment. Another advantage of a multi-Deans switch is a higher reproducability, because of the high-precision flow control. [28]

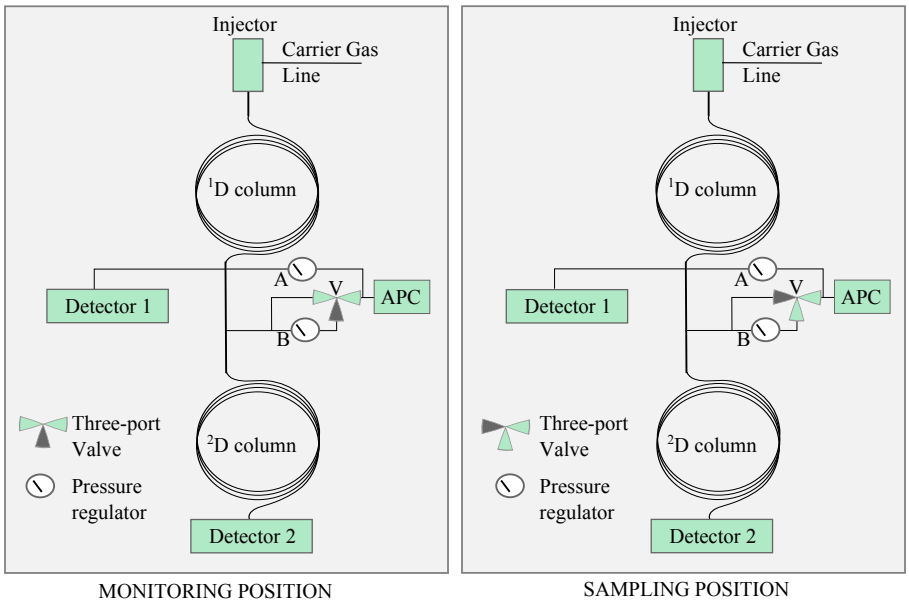

Figure 4.3.: Principle of a multi-Deans switch in monitoring position *left* and in sample position *right*. Switching between both positions is carried out by switching valve V.

In figure 4.3 the principle of the multi-Deans switch is shown.
In monitoring position (figure 4.3 *left*) the automatic pressure control (APC) system applies a pressure, P. The pressure is reduced by A to a value P_A for the pressure toward detector 1. The pressure towards the second dimensional column, P, is higher than the pressure towards the detector. The effluent from the first column is directed to the monitor detector.
For sampling (figure 4.3 *right*), valve V switches and the pressure towards the second dimensional column is reduced to P_B. $P_B < P_A$ and so the gas flow is directed to the ^{2}D-column and subsequently to detector 2.
With this switching device several heart cuts can be made without retention time shifts. [28]

4.4. Detectors

Every detector, which can be used for a one-dimensional gas chromatography, can also be used for MDGC. The detectors suitable for GC were discussed in section 2.3. Very often a combination of FID as monitor detector in the first dimension and MS as ^{2}D-detector is used. The universality of FID is beneficial for screening the first dimensional effluent and the aforementioned advantages of GC/MS can be used for target analyte detection in the second dimension.

4.5. Applications

MDGC is an useful method for separating few target analytes. This technique is also beneficial if these analytes have a similar retention index for the first column and are separated in the second column. By this way disturbing matrix effects can be removed and the first column has the function of a pre-column.
A method can be developed were the interesting compounds are co-eluting in the first dimension, so that they can be easily transferred to the second dimension. On the second dimension the actual separation takes part. This can be reached by the right column combination and a well thought-out temperature programm.
MDGC analysis is also advantageous when co-elution of few target analytes is detected by an one-dimensional technique, like GC/MS or GC-O. The separation problem can be transferred to the first dimension by choosing a similar column and the same temperature rate and the interesting parts are transferred to the second dimension where the target analytes are separated properly.
Another possibility is to do enantiomeric separation by MDGC. First a normal column and cut out the not separated chiral compounds and afterwards a chiral column like a cyclodextrin column. The enantiomeric distribution is useful for the determination of following items:

- Identifying adulterated foods and beverages
- Controlling and monitoring fermentation processes and the final product

- Evaluating age and storage effects

In this type of MDGC analysis non-chiral column are often used for pre-separation and followed by a chiral main column. [29]

An advantage of MDGC in comparison to comprehensive GC is that it is possible to apply olfactometric detection after the second dimension. The effluent can be split into two parts: one is guided to a chemical detector and the other one is released via a sniffing port.

4.6. Quantification

Quantification is also possible by MDGC. But the retention time range transferred into the ^{2}D-column has to be chosen carefully. If a part of the analyte is not transferred to the second column no proper quantification is possible. This can be seen by the negative section of the calibration curve on the y-axis.

5. Comprehensive two-dimensional gas chromatography

Comprehensive two-dimensional gas chromatography was invented in the early 1990s and is probably the most promising invention in GC since the discovery of capillary columns [30].

5.1. Technique

Comprehensive GC×GC is a technique in which two distinct columns are connected in series. A scheme is given in figure 5.1. A transfer system is located between them and is called modulator. Its function is to *i*) accumulate and trap, *ii*) refocus, and *iii*) rapidly release the fractions of the primary column effluent into the second dimension column in a continuous and sequential way. The modulation period is the time required for this process. To preserve the separation achieved in the first dimension the modulation time has to be very short, usually it is in the range from 1 to 8 seconds. Different types of modulators are discussed in section 5.3. [31] [20] [9]

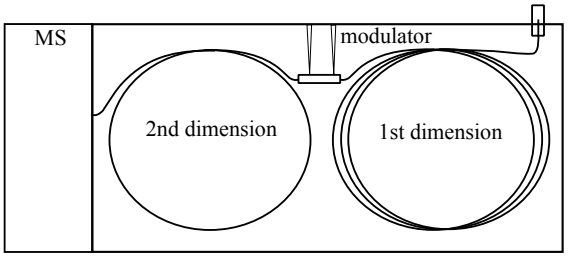

Figure 5.1.: A scheme of a comprehensive gas chromatograph with two ovens and flame ionisation detection in the first dimension and mass selective detection in the second dimension.

The compounds of one modulation have to be eluted before the next injection, therefore the secondary separations is very fast and the peak widths are narrow. Retention times of the second dimensional column have to be, at most, equal or less than the modulation time. If this is not the case then a so-called wraparound effect occurs, i.e. peaks of one modulation fraction occur in the subsequent modulation fraction. If there is no co-elution created by wraparound peaks the separation could still be considered as sufficient, but the drawbacks of a possible peak overlap and the reduction of peak capacity should not be ignored in method development. [22]
Peak eluting from the first column has to be modulated at least three to four times

otherwise the analysis is not be effective. In figure 5.2 *left* one example of the effect of the modulation can be seen regarding a single compound. The modulated peak is sliced into five parts. The interval between the peaks is the modulation time. The sum of area of the modulated peaks are equal to the area of the non-modulated peak. In figure 5.2 *right* an example of a three-component peak is shown, non-modulated and modulated. It can be seen that those compounds are not separated in the first dimension. Figure 5.2 *right* (b) shows three modulation periods in which the compounds (α, β and γ) are separated.
Comprehensive two-dimensional chromatograms are often diagrammed in a 2D plot, where on the x-axis the retention time of the first dimension, ${}^1t_R$, is printed and on the y-axis the second dimensional retention time, ${}^2t_R$, is shown. The intensity of the peaks is pictured by different colours, like in figure 5.6. Each compound is characterized by two retention time coordinates and reveals as a oval-shaped area. [19]

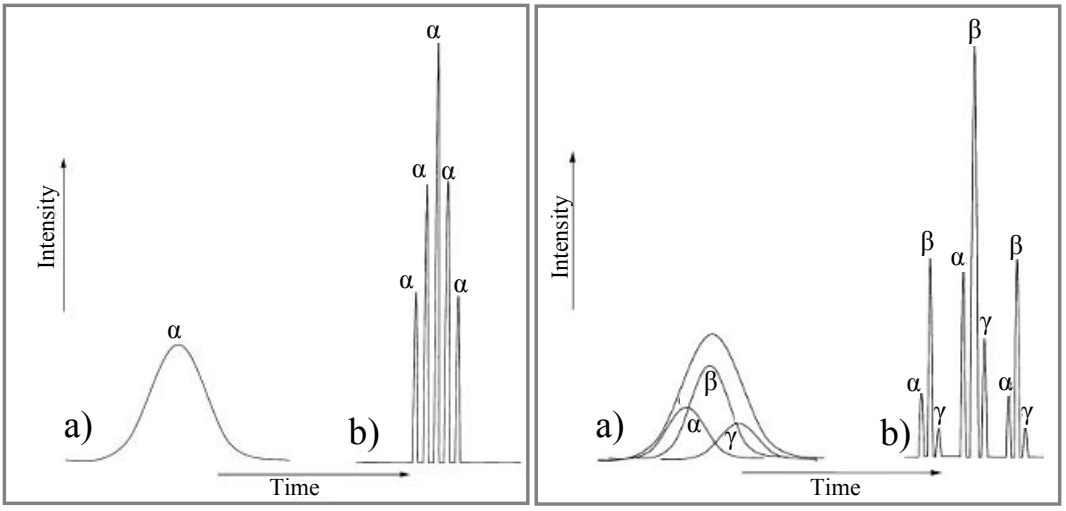

Figure 5.2.: *left*:a) unmodulated peak of a single compound α, b) peak after modulation; *right*: a) unmodulated multi-component peak α, β and γ b) peak after modulation [19]

5.2. Column Dimensions and Column Combinations

5.2.1. Column Dimensions

GC×GC instruments are usually one-oven systems. Temperature programs for the analysis are chosen to be very slow, i.e. having a low heating rate, so that the peaks eluting form the first dimension are relatively broad and are modulated more often. [9]
The first dimensional column usually has conventional dimensions while the ^{2}D-column is much smaller in its dimensions, i.e. in its length (0.5 m to 1.5 m), its inside diameter, and its film thickness (0.1 µm to 0.25 µm). It was shown that, when developing an analysis method, one column should be performed close to its optimum conditions and less optimal separation in the other dimension can be accepted. [9]

The column flow also has to be considered when choosing the column dimensions. When using a narrow ^{2}D-column, the flow rate has to be far lower than the flow used in one-dimensional GC, in general about half of it, hence the separation in GC×GC analysis takes about twice as long as in 1D GC.
A further development, making comprehensive GC more sophisticated, is the possibility to split the effluent of the first column and introduce both parts in two different ^{2}D-columns (GC×2GC). The result are two contour plots, one of each second dimensional column. [22]

5.2.2. Column Combinations

Usually any existing stationary phase used for 1D-GC can also be used in GC×GC analysis. In many studies a non-polar column is chosen as a first dimensional column. The reason for that is, that there is already much information available for a large number of compounds on this column-type for one dimensional GC analysis [9] and so the first dimensional separation can be easily optimized. [9].
A non-polar column in the first dimension is often combined with a more polar column in the second dimension. By using this combination, separation according to the boiling point occurs in the first dimension and in the second dimension the separation takes part primarily by specific interactions. The conditions in the second dimension can be seen as isothermal due to the fast separation and the contribution of the boiling point can be disregarded. Hence the two columns can be regarded as orthogonal which means the two dimensions operate independently and the entire 2D plane of the GC×GC chromatogram is available for the analysis.
Ordered structures can be found in orthogonal GC×GC separation. That means related homologues, congeners and isomers appear in continuous bands or clusters. This fact is advantageous for group-type identification. Such structured chromatograms are an important part for GC×GC analysis, especially in the petroleum industry. [22]
For more polar compounds like aldehydes, ketons, and carboxylic acids a reverse set-up (more polar ^1D column and non-polar ^{2}D-column) is more beneficial. There are also studies done with pyrazines in coffee with this type of column set-up. [9]
There is also the possibility of an enantiomeric separation by GC×GC by using cyclodextrin columns. As the separation of enantiomeric pairs requires a long runtime and, therefore, a long column, it is preferred to choose a chiral column as first dimension. It is also possible to perform the chiral separation in the second dimension, but this set-up is quite demanding. [9]

5.3. Modulators

The role of the modulator was already mentioned before and in this section it is discussed in detail. It is often said that the modulator is "the heart of a GC×GC system" [31]. The types of modulators can be divided into thermal and pneumatic (or valve-based) modulators.

5.3.1. Thermal Modulators

The term "thermal modulator" is used for devices which use either a negative or a positive temperature difference for focusing and releasing the compounds of the ^{1}D-column effluent. Generally thermal modulation improves the sensitivity because of the re-concentration of the primary column effluent. Typically these kinds of modulators are characterized by a duty cycle equal to 1. The duty cycle is the ratio of the amount of sample exiting and entering the interface. Its value can be ≤ 1.

In 1991 the first conventional comprehensive two-dimensional gas chromatograph was reported that used a thermal-desorption (TD) modulator. [31]

Thermal Sweeper

In 1996 the thermal sweeper was first described. It is a rotating device which was one of the most significant modulators, because it was the first to be commercialized [31]. After some further developments the final version was released in 1999. This type of modulator consists of a heated sweeper with a slot which rotates by means of a shaft in the direction from the primary column to the secondary column (see figure 5.3. The entrapment and the re-concentration was achieved by a capillary coated with a thick stationary-phase material. Releasing the compounds was done by the temperature of the sweeper.

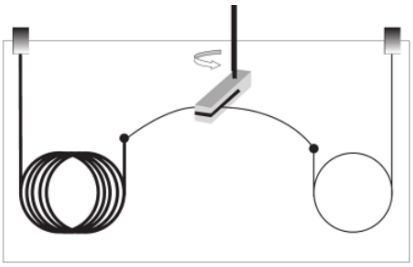

Figure 5.3.: Scheme of a thermal sweeper [22]

The thermal sweeper was also the type of modulator used in the first tree-dimensional GC×GC×GC instrument. [31]
The disadvantage of this type of modulation is that it is not suitable for focussing more volatile compounds. The temperature for trapping should be about 100 °C lower than the elution temperature of a compound to focus it properly, at most 100 °C lower than the maximal operation temperature of the stationary phase. Therefore, the development of cryogenic modulation followed.

Longitudinally moving cryogenic system (LMCS)

The first modulator of this type was the longitudinally-modulated cryogenic system (LMCS). It was reported the first time in 1999-2000 [22]. The technique is based on a cryogenic trap which moves longitudinally along the column. As cooling agent liquid

CO_2 is used. A zone of the column is cooled by a jet of CO_2 trapping compounds of the primary column effluent. In the next step the trap moves along the column and the cooled zone is heated by oven temperature. The trapped analytes are then released onto the secondary column. The LMCS device is the first reliable cryogenic modulator for routine use. [22]
The advantages of the LMCS method compared to a thermal sweeper are [31]:

- a more efficient entrapment
- no GC-oven-temperature limitation caused by the modulator, and
- a less complex construction.

A negative aspect, however, was the consumption of CO_2 and, because of that, the increase of cost-per-analysis ratio. But it has further disadvantages compared to the subsequently developed modulators applying N_2 and heat [31] [22]:

- re-mobilisation of analytes with a higher molecular weight are not efficient, because of the lack of additional heating,
- the peak width in the second dimension is larger,
- moving mechanical parts,
- CO_2, as a cooling agent, has a temperature of about $-70\,°C$ and this is not sufficient for a lot of very volatile compounds.

Nevertheless the LMCS device is still considered as a robust and often sufficiently efficient modulator [31].

Non-moving Cryogenic Modulator

In 1999, the era of non-moving modulators began. Combinations of a static cooling CO_2-jet and a thermal sweeper were developed. The next step was the dual-stage static modulator. At first, there were two CO_2-jets applied on the head of the second column. The disadvantage of the moving parts do not exist anymore, but there was still the problem of releasing the analyts of higher mass and lower volatility.

So further development was made by introducing two hot and two cold jets (quad-jet modulator) to focus and release the fractions. The modulation process for dual-stage two-jet respectively quad-jet is shown in figure 5.4.
In the quad-jet modulation process, the first step is to trap a fraction of the primary column effluent. Both cooling jets are activated. Then the fraction, collected and focused on the first stage, gets released by a hot jet, while the second stage is still cooled. By this way, the first fraction is transported to the second stage. To re-focus the coming fraction the first stage has to be cooled, while the second stage is heated to release the first fraction into the second column [22].

In 2003 CO_2 was replaced by liquid nitrogen which caused a further improvement for focusing highly volatile compounds like butane and propane. There is a large variety of arrangements with varying number of hot and cold jets, see Tranchida's review about

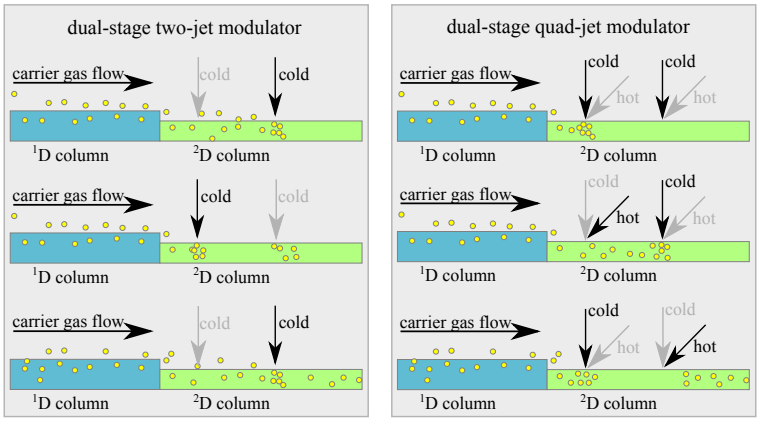

Figure 5.4.: Modulation processes in dual stage modulators, *left*: two-jet and *right*: quad-jet modulator. In both modulator types trapping of the effluent is obtained by cooling jets. In two-jet modulators effluent release occurs by the oven temperature while in quad-jet modulator two additional heating jets are applied to release the fraction.

modulators for comprehensive GC [31]. Non-moving cryogenic modulators were further developed resulting in a loop-type modulator. The loop-type modulator, shown in figure 5.5, is a dual-stage cryogenic modulator that also applies cold and hot jets. A segment of the column is looped through the pathway of a cold N_2-jet. By this way two stages are formed. The zone between those stages is called delay loop. Though, the delay loop can be formed by using the end of the first or the initial part of the second column this is not advisable because breakages can occur. The better way is to use an uncoated column or a segment of a capillary coated with stationary phase [22].
In a loop-type modulator there is a permanent cold gas jet to trap the sample. To release the sample the hot jet is turned on. The first fraction trapped on the first stage is released to the delay loop. Arrived on the second stage, the hot jet is inactive an the fraction is trapped. At the same time focussing of the next fraction occurs by the same cryogenic jet but on the first stage, that means the beginning of the loop. By the next hot jet the first fraction is released into the second column and the second fraction travels the delay loop. With this process a modulation period ends. The length of the loop, the modulation period and the velocity of the carrier gas must be aligned well to achieve efficient focusing. The advantage of this modulator is the reduced consumption of cryogenic agent compared to a quad-jet modulator. This technique can modulate compounds ranging from C2 to C55. There are also developments to reduce the consumption of cryogenic agent by applying a closed-cycle. But the cold jet has a only temperature of $-90\,°C$ and compounds over C7 can be modulated. [22]

5.3.2. Pneumatic or Valve-based Modulators

The other large group of modulators use a valve-based system. Although cryogenic modulators are very effective they are also very costly because of the consumption of cryogenic agents as discussed before. There was the interest to design a modulator which

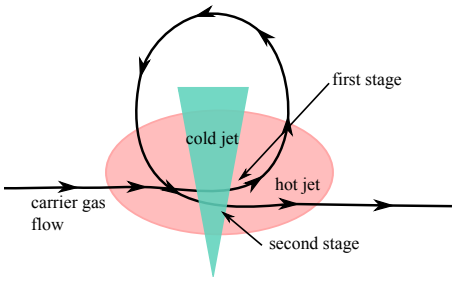

Figure 5.5.: A loop modulator is a dual stage. Two stages are received by forming a loop. Only one cooling and one heating jet has to be applied for trapping and releasing the effluent exiting the first dimensional column.

is cryogenic-free and low-cost [31].
Two kinds of pneumatic modulators have to be differentiated. There is the possibility of employing the valve "in-line" the column-set or "out-line".

The first modulator with an "in-line" valve system was developed in 1998 [22]. This interface used four ports of a six-port valve. It samples periodically the effluent of the primary column during a short period of time and transfers it into the second column. With a modulation period of 0.5 s and a brief injection period of 50 ms the value of the duty cycle is 0.1. The residual time of the period (450 ms) the primary column effluent is directed to waste [31]. That means that only 10 % of the sample eluted from the primary column is trapped and sent to the secondary column [22]. That is why this kind of modulators are less sensitive compared to cryogenic modulators. Although a huge part of the primary column effluent gets lost this method can be considered as comprehensive because the chromatography profile of the first dimension is still maintained. Reason for that are the short modulation period and the resulting high modulation frequency [31]. Besides the sensitivity problem pneumatic modulators have to deal with limiting operational temperature of the valve. A six-port, two position, diaphragm valve with a sampling loop of 20 µL. The six-port valve is located outside of the oven and the temperature is 125 °C. The accumulation state amounts to 80 % of the modulation period and the injection state amounts to the residual time (20 % of the modulation period). During the injection state the accumulated sample is launched to the secondary column by a high gas flow (15 mL min^{-1}) and the primary column effluent is directed into waste [31]. That means that about 80 % of the effluent is transferred to the secondary column [22]. With this method a second dimensional column with an internal diameter of 0.25 mm has to be employed because of the high gas flow applied to this column [31]. The problem of the high gas flow was solved by introducing a second secondary column and splitting the flow [31].

Main disadvantages of "in-line" valve techniques are [31]:

- the quite complex instrumental configuration
- the limited rotary valve life time
- the formation of artifact peaks (derived from the valve polymer)

- extended analysis time.

Through further development people try circumvent the last three issues by eliminating the valve from the column sequence [31]. But there is still the issue of the long total analysis time and the elaborate instrumentation.

There is also the approach to interrupt the flow of the first column before sampling by the modulator. It is called *stopped-flow modulation*. With this technique wraparound peaks can be avoided. However, analysis time is increased.

To overcome the problems caused by "in-line" valve systems "out-line" valve systems were developed. The breakthrough was done in 2004 with the construction of a thermally stable pneumatic modulator device.

5.3.3. Final Considerations

The commonly used modulator is the cryogenic two jet modulator.

In the review of Tranchida in 2011 [31] it is postulated that in the next ten years the popularity of pneumatic modulators will increase. But to compete against cryogenic modulators some efforts have to be taken and some characteristics would be beneficial, which are *i*) an accumulation step, *ii*) a high pressure injection step, *iii*) a duty cycle equal to one, and *iv*) no thermal restrictions. At the moment dual-stage nitrogen modulators can be considered as the most effective ones although the cost for the investment and the running expenses are quite high. [31]

5.4. Detectors

The comprehensive GC analysis has further requirements regarding the detection. In terms of the fast separation in the second dimension and the resulting narrow peak widths the detection has to be very fast and has to have a short response time. Just some detectors can cope with those requirements which are specified in the following section.

5.4.1. Special Requirements for GC×GC systems

For GC×GC, besides to column selection and modulator considerations, the detection is also an important part of this technique. [32]
In comparison to one-dimensional GC additional requirements have to be fulfilled for GC×GC [22]:

- a small internal volume
- a short detector response time
- a high data acquisition rate
- high sampling rates (at least 100 Hz)

The reason for that is the fast separation of the secondary column and consequently the narrow peak width (50 ms to 300 ms at the baseline) [22].

5.4.2. Non-mass selective Detectors

Flame ionisation detection with its high acquisition rate (50 Hz to 200 Hz) is popular for comprehensive two-dimensional gas chromatography. It is described in section 2.3. Alternatives are miniaturized electron-capture detectors, μECD, with a small internal volume and a data acquisition rate of about 50 Hz. But this technique requires an electronegative atom in the analyte molecule like halogens. This detector is suitable to analyse polychlorinated compounds for example. Some investigation were made using FPD and NPD but the data acquisition rate of both detectors were not constantly sufficient and peak tailing occurred. [9]
Even though FID and μECD can be used as detection systems for GC×GC they do not provide any structural information. Due to that mass selective detectors are often used for traditional one-dimensional gas chromatography and some of them also fulfil the requirements for GC×GC analysis. [9]

5.4.3. Mass selective detectors

A one-dimensional gas chromatographic analysis in combination with a non-specific detector, like FID or μECD, can be considered as a two-dimensional system [22]:

- retention time, t_R
- intensity

.

A GC × GC-MS analysis in general, can be seen as a four-dimensional analysis [22] with following dimensions:

- retention time of the primary column, ${}^1\mathrm{t}_R$
- retention time of the secondary column, ${}^2\mathrm{t}_R$
- intensity
- mass spectral information

As aforementioned there are several types of mass-selective detectors. However, in comprehensive two-dimensional GC analysis the aforementioned requirements cut down the options to the last two mass selective detectors which were specified in section 2.3. By using MS for GC×GC it has to be considered that there are different principles and from that different scanning time results. For example TOF can be used for comprehensive GC analysis because all ions generated in the ion source can be analysed at the same time unlike in quadrupoel MS analysis just a small range of m/z-values can be analysed in a given time frame. The other ions which are to light or to heavy get lost by crashing into the rods of the quadrupole. Hence, the qMS needs a longer scanning time than TOF-MS. But there are developments in the direction of fast-scanning qMS that are suitable for comprehensive GC analysis. [9]

TOF-MS Detection in Comprehensive GC Analysis

TOF-MS is characterized by a fast acquisition rate. Due to the principle of TOF-MS it can attain acquisition rates up to 500 spectra per second based on the summation of 10 transient peaks [22]. This allows 50 acquisitions per 100 ms-peak which is enough to describe even a narrow peak in GC×GC. Other mass selective detectors need more time because they have to scan a mass range while TOF-MS collects a full mass spectrum.

Quadrupole-MS Detection in Comprehensive GC Analysis

The first use of quadrupole hyphenated with comprehensive GC was reported in 1999. The major disadvantage is that the scan rate is limited when working in full-scan mode. With a reported scan rate of 2.43 scans/s it is too slow for the typical peak width in GC×GC.

Some effort was made to slow down the separation to achieve peak widths of about 1 s but the result were not satisfying. With *single ion-monitoring* (SIM) the scan speed can be increased and a acquisition rate of about 30 Hz can be achieved. Thus the amount of data points across the fastest peaks eluting from the secondary column increase to 5 to 6 points per peak. [9]

A further development are rapid-scanning quadrupole instruments. With this type of mass analyser an acquisition rate of 33 Hz can be achieved for a mass range of 200 Da. That corresponds to a minimum amount of 7 to 8 data points per peak, depending on the peak width. [32] [9]
In 2005, Adahchour *et al* carried out investigations with a Shimadzu rapid-scanning quadrupole having a scan speed of 10 000 amu/s and gained satisfactory results. [32]
Nowadays Shimadzu provides a rapid-scanning quadrupole, *QP2010 Ultra*, with a scan speed of 20 000 amu/s. [33]

5.5. Data Processing and Visualisation

After data aquisition the next important step is the data processing. For this purpose special software is provided. Figure 5.6 shows the way from the raw data to the two dimensional contour plot. First the raw signal from the detector has to be cut in pieces in the length of the modulation time. By aligning them in parallel a three dimensional plot is generated in which the x- and the y-axis represent the two retention times of the first respectively the second dimension and the z-axis represents the intensity of the detector signal. By using colors for the intensity a two dimensional plot, the contour plot, is generated.

5.6. Applications

Comprehensive GC has a lot of application fields, especially when samples are complex mixtures requiring a high peak capacity. Those areas are for example

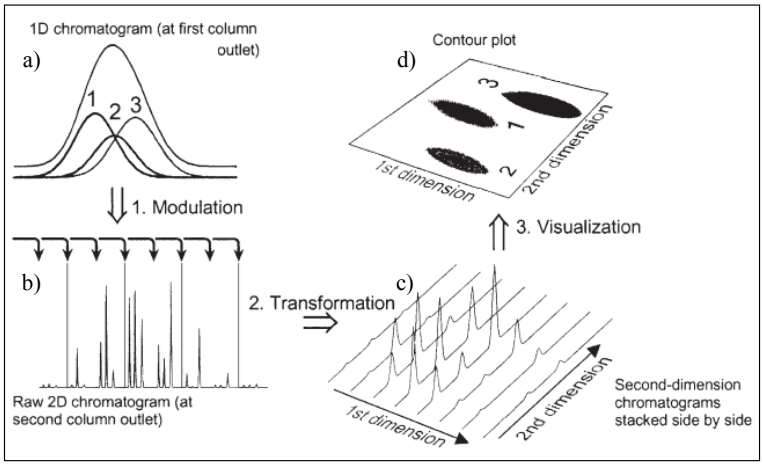

Figure 5.6.: Scheme of data aquisition and data processing in comprehensive GC analysis is shown. After modulation a raw chromatogram is received. By cutting the chromatogram in pieces with the length of a modulation period and aligning the parts in parallel a plot is generated where x-axis and the y-axis represent the retention time in either dimension (transoformation). The intensity of the detector signal is shown by the z-axis. By visualisation a two-dimensional contour plot is received where the intensity is displayed by color. [3]

- analysis in petrochemistry
- environmental analysis (pollutants and pesticides)
- analysis of volatiles components (plants, essential oils, fragrances)
- food analysis

It is beneficial for analysis of homologous series due to their appearance as clusters or bands in the contour plot, so-called *ordered structures*.

6. Summary and Final Considerations

6.1. Comparison of the two-dimensional GC-techniques

MDGC and GC×GC are, even though both two-dimensional, different techniques with different benefits and areas of application.
MDGC is suitable for the separation of few target analytes. Up to three cuts can be made, but the cuts have to be set carefully to avoid co-elution of compounds, deriving from different cuts, in the second dimension. One advantage of MDGC is, that it can also be used in combination with olfactometric detection. This is not possible when using comprehensive GC because of the narrow peak widths obtained by this method.
MDGC is more beneficial when co-elution of a target analyte is detected by another method like GC-O or GC-MS. A subsequent MDGC analysis with a comparable column in the first dimension provides more information about the target analyte and the co-eluting compounds. However, MDGC is not suitable for screening an unknown sample, especially when having an FID monitor detector analysing the first dimensional effluent, because no structural information is gained. Analysis with comprehensive GC, in contrast, gives good screening results.

With comprehensive GC a two dimensional plot is obtained and it is especially interesting for analysing homologous series because structured patterns can be achieved. But the data amount has to be considered as data processing is more complex. In GC×GC there is still potential in improving the system regarding the modulation system and the column dimensions. Also the possibilities of column combinations have not been exploited yet. This technique will improve further more in the next decade. But to establish such a system from scratch is very time- and cost-consuming.

6.2. Comparison one-dimensional and two-dimensional GC techniques

It is difficult to compare two-dimensional and one-dimensional GC techniques in general because there is a huge difference between comprehensive GC and MDGC as already mentioned above. That is why the two techniques are compared separately to 1D-GC in the following paragraphs.

6.2.1. MDGC vs. 1D-GC/MS

In some cases two-dimensional GC separation is not necessary when using one-dimensional GC in combination with a mass selective detector. By using the selected ion monitor-

ing (SIM) mode with a well-thought-out ion choice co-elution can be overcome. This might be the reason why the focus of research was concentrated on the field of GC/MS in the past 20 years than on MDGC. When co-elution of compounds occurs and these compounds have a similar fragmentation pattern, MDGC analysis is beneficial, because separation of these compounds via SIM cannot be done reliably. [24]

6.2.2. Comprehensive GC vs. 1D-GC/MS

By comparison of GC/MS and comprehensive GC it gets obvious that GC/MS is a technically mature method which is used for routine analysis in many laboratories. Therefore the instrumentation is highly developed and a good availability is given. Also the investment has to be considered. Purchasing a comprehensive GC is very cost-intensive and the running expenses are quite high especially when using a cryogenic modulator. Another drawback is the long time of analysis needed for GC×GC due to the low heating rate. Because of the complexity of the system it is also very time-consuming to establish a comprehensive GC system.
Advantages are given by a dramatically increase of sensitivity and improvements in LODs by GC×GC. It has been reported that the quantitative performance of a comprehensive GC is superior compared to one-dimensional GC. [34]
The above mentioned peak capacity increase is also an advantage of comprehensive GC compared to 1D-GC. For this reason, complex mixtures can be analysed.
Another benefit lies in the analysis of homologues via structured chromatograms are beneficial for some applications, e.g. in petrochemistry. [20]

After more than one decade there a few standard GC×GC methods compared to GC/MS. This is probably due to the enormous amount of options for method development.

Part III.

Practical Part

7. Samples

7.1. Elder Flowers

There are not many studies published about aroma-relevant compounds in elder flowers and the differences of odour between elder cultivars. Until now the aroma of elder flowers was not investigated to a high extent. There are some research on relevant aroma compounds in elder flowers which were carried out by Kaack *et al.*([35] [36]) and Jørgensen *et al.* [37]. However, the aroma of elder flowers and its conservation is an important issue for the food industries that produce elder flower syrups. Due to chemical reactions and enzymatic degradation the aroma active compounds can change during processing. In order to obtain consistency of the quality of the flowers have to be treated very gently. No long-time storage is possible as well as no high temperature and also no too low temperature have to be applied.
In Austria, especially in Styria, elder is cultivated in large amounts The blossoms are not the only reason but the elder berries have a high concentration of organic red dye. This dye is used especially in the food industry to color food, like yoghurt or other milk products, in order to make them more appetizing.
Elder flowers have a strong flowery and pleasant odour that is also present in elder flower syrups in combination with a honey-like taste. [37]
Elder flower odour consists of a large amount of compounds. In table 7.1 some important compounds are named and their contribution to the odour are given.

7.1.1. Standard Solution of aroma relevant Compounds in Elder Flowers

The standard solution contains 24 compounds that were found in elder flower in previous studies which were carried out by Nicole Pabi [38] whereby three of these compounds are cis/trans-mixtures. Because no ratio was declared these compounds were not used for quantitative analyses.
First a stock solution of each compound, relevant for the aroma of elder flower, was made with an concentration of 1 g/L for each compound. Subsequently a mix of all stock solutions was made which was diluted to different concentrations. This solution of 24 compounds was also used to optimize the parameters of the GC separation and of the MS detection and, in the following step, to measure calibration curves, described in the following chapter.
The compounds of the solution, the concentration of the stock solution and the solvent are shown in table 7.2. Their retention indices on a 5%-phenylmethylpolysiloxan column and a polyethylenglycol column are given in table 7.3.

Table 7.1.: Some compounds and their contribution to the aroma of elder flower [37] [35]

Odour description	Compound
floral green and floral odour	hotrienol
	rose oxides
	linalool
	linalool oxides
	nonanal
	(Z)-β-ocimene
	nerol oxide
fresh green odour	hexanol
	(Z)-3-hexen-1-ol
	(E)-3-hexen-1-ol
	(E)-2-hexen-1-ol
	hexanal
fruity odour	pentanal
	heptanal
	octanal
	limonene
	β-damascenone
	esters of lower carboxylic acids
waxy and fatty odour	relatively long chain alkanes

The standard solutions were analysed by pipetting 10 µL into a headspace vial which is crimp-capped.

7.1.2. Elder Flower Syrups of various Elder sorts/cultivars

The extracts were made with water, sugar, elder flowers and citric acid according to the following recipe:

- 50 g elder flowers
- 1 L water
- 500 g sugar
- 18.75 g citric acid

The extraction process lasts one to three days. The duration of the extraction is given with the code T1 to T3 for one day to respective three days. Twelve different elder sorts (S) were extracted by this way. The sorts and the corresponding codes are given in table 7.4.

The extraction was carried out by Nicole Pabi. The exact description of the preparation and the elder sorts themselves can be found in her Master Thesis. [38]
The extracts were stored in a freezer. The temperature is not specified but the samples were frozen. Analysed sample amounts were 100 µL and 500 µL.

Table 7.2.: Stock solutions, odour description of the compounds and their concentration

Compound	**Odour description** [39] [40]	**Concentration of the Stocksolution**	**Solvent**
(*E*)-2-hexenal	apple, green, leaf [40]	1.204 g/L	aceton
(*E*)-2-hexen-1-ol acetat	green, banana [40]	0.948 g/L	methanol
(*S*)-(-)-limonene	lemon, orange [40]	0.964 g/L	methanol
(*Z*)-3-hexen-1-ol	grass [40]	1.080 g/L	methanol
(*Z*)-3-hexen-1-ol acetat	sweet, green, sharp, fruity [39]	1.090 g/L	methanol
1-heptanol	herb [40]	1.038 g/L	methanol
2-ethyl furan	rubber, pungent, acid, sweet [39]	0.948 g/L	methanol
2,4-hexadienal	green vegetable [39]	1.128 g/L	aceton
6-methyl-5-hepten-2-one	mushroom, earthy, vinyl, rubber, woody, blackcurrant, boiled fruit [39]	1.148 g/L	methanol
α-terpinene	gasoline-like, ethereal, fruity, lemony [39]	0.986 g/L	methanol
β-myrcene	balsamic, must, spice [40]	0.988 g/L	methanol
citral (cis/trans)	citrus, lemon, peely, green [39]	1.076 g/L	methanol
citronellol	rose [40]	1.132 g/L	methanol
damascenone	apple, rose honey [40]	1.290 g/L	methanol
ethyl isovalerate	cashew, fruity, anise, sweet fruit, apple, blackcurrant [39]	0.908 g/L	methanol
geraniol	honey, sweet, fruity, apple, tobacco, canned peach [39]	1.032 g/L	methanol
heptanal	fat, citrus, rancid [40]	0.928 g/L	aceton
hexanal	grass, tallow, fat [40]	1.152 g/L	aceton
linalool	flower, lavender [40]	1.008 g/L	methanol
linalool oxide (cis/trans)	sweet, woody, floral, creamy, slight earthy [39]	1.238 g/L	methanol
methyl salicylate	wine-like, berry-like, warm, sweet, winter-green odor [39]	1.220 g/L	methanol
nerol	sweet [39]	1.515 g/L	methanol
p-cymene	solvent, gasoline, citrus [40]	0.968 g/L	methanol
rose oxide (cis/trans)	rose-like, floral [39]	1.350 g/L	methanol

Table 7.3.: Compounds of the standard solution their retention indices on a 5%-phenymethylsiloxane and a polyethylenglycol column. The compounds are arranged according to their retention index on the polyethlenglcol column. If no other notification is made the values are for DB-5 respectively DB-Wax columns.

Compound	**5%-phenymethylsiloxane**	**polyethylenglycol**
2-ethylfuran	728 [HP5] [41]	945 (ZB-Wax) [42]
ethyl isovalerate	849 [43]	1070 [44]
hexanal	851 [43]	1088 [45]
β-myrcene	990 [45]	1158 [45]
α-terpinene	1018 [46]	1178 (HP20M) [47]
heptanal	901 [45]	1184 [45]
limonene	1030 [45]	1208 [45]
(*E*)-2-hexenal	848 [48]	1201 [48]
p-cymene	1026 [45]	1274 [45]
(*Z*)-3-hexen-1-ol acetate	1007 [46]	1308 [48]
(*E*)-2-hexen-1-ol acetate	1019 [48]	1323 [48]
6-methyl-5-hepten-2-one	985 [46]	1319 (HP20M) [47]
cis-rose oxide	1111 [46]	1338 (HP-Wax) [49]
trans-rose oxide	1127 [46]	1373 (C20M) [40]
(*Z*)-3-hexen-1-ol	865 [45]	1378 [48]
2,4-hexadienal	910 [43]	1337 (ZB-Wax) [42]
cis-linalool oxide	1074 [46]	1420 (C20M) [40]
1-heptanol	969 [46]	1436 (ZB-Wax) [42]
trans-linalool oxide	1088 [46]	1453 (HP-Wax) [49]
linalool	1100 [45]	1544 [45]
(*Z*)-citral	1240 [46]	1690 [50]
(*E*)-citral	1270 [46]	1744 [50]
citronellol	1235 [45]	1786 [44]
methyl salicylate	1190 [46]	1745 (C20M) [40]
nerol	1230 [45]	1792 [45]
damascenone	1393 [50]	1832 [44]
geraniol	1266 [45]	1852 [45]

Table 7.4.: Elder sorts and their code

Sort	Code
Sämling	S1
Klon17	S2
Hamburg	S3
Klon 14	S4
Tattin	S5
Rubin	S6
Haschberg	S7
Klon 311	S8
Klon B	S9
Klon 25	S10
Klon 13	S11
Wildholler	S12

7.2. Beer with Different Hop Sorts

In connection to another study some analysis with beer were made in this work. In special interest was the retention range where linalool elutes. Co-elution with other odour-active compounds were discovered by using a 5%-phenymethylsiloxane column in combination with olfactometric detection (GC-O). The aim was to investigate the difference in the linalool concentration between a beer with hop and a beer without hop. Analysed sample amounts were 200 µL and 1 mL.

8. One-Dimensional Gas Chromatography

In this chapter one-dimensional GC analyses with mass selective detection are described. The measurements were carried out on a MDGC/MS-system. The first dimensional GC with the FID can be used for analyses separately and the second dimension GC with the mass selective detection has an own inlet and can also be used separately.
Two columns are embedded in the second dimensional oven. One column connects the second dimensional inlet and the detector and the other column separates the effluent coming from the first dimension, i.e. from the interface. So for the one-dimensional measurement in the second oven and for two-dimensional analysis different column in used. The effluent exiting the second dimension is split up into two parts of which one part is directed to the mass analyser and the other part is directed to a sniffing port where the effluent can be analysed by the human nose (see chapter 2.3).
Using the first dimension without cut is necessary for screening a sample before doing heart-cuts. The dimensions of the separation column which is used in the one-dimensional GC is a polyethylenglycol column and is specified in table 8.1. Measurements with the second dimensional GC of separation were carried out to optimize the parameters of the sample preparation, the parameters of the GC separation in the second dimension, like the heating rate, and the parameters of the MS detection, like the ion source and the applied detection voltage.

Table 8.1.: Column Parameter for the one-dimensional GC separation

Gas Chromatograph	Shimadzu GC2010
Column	ZB-WAXplus
Column Length	20 m
Column Inner Diameter	0.18 mm
Column Film Thickness	0.18 µm
Mass Spectrometer	GCMS-QP2010 Plus

8.1. Optimization of SPME Parameters

The settings for the sample extraction are listed in table 8.3. The fiber that was used for all analyses was a SUPELCO 50/30 µm DVB/Carboxen/PDMS StableFlex/SS (2cm).

8.1.1. Variation of the Extraction Time

SPME extraction times of 15 min, 20 min, and 30 min were analysed and the signal-to-noise ratios were compared. For the analysis an EI ions source was applied with a detector voltage of 1.4 kV. The resulting S/N values are shown in table 8.2. 20 min extraction time were chosen for all following measurements. The final conditions for the extraction are specified in the following section.

Table 8.2.: Signal-to-noise ratio, SIM, EI, 1 ng sample, SPME: varying the extraction time

Compound	**Target Ion**	15 min	20 min	30 min
2-ethyl furan	96.00	11.4	9.8	12.1
ethyl isovalerate	88.00	107.1	102.2	101.1
hexanal	57.00	21.1	17.3	21.1
β-myrcene	55.00	7.0	3.8	6.5
α-terpinene	121.00	72.5	63.2	62.8
heptanal	70.00	181.1	211.1	175.7
limonene	93.00	90.2	128.5	95.8
(*E*)-2-hexanal	55.00	89.2	48.2	68.3
p-cymene	77.00	59.6	77.6	54.2
(*Z*)-3-hexen-1-ol acetate	67.00	67.7	56.7	67.1
(*E*)-2-hexen-1-ol acetate	67.00	24.2	20.5	24.0
6-methyl-5-hepten-2-one	108.00	275	574	251.6
(*Z*)-3-hexen-1-ol	67.00	165.7	134.3	132.5
2,4-hexadienal	81.00	202.6	185.0	205.8
1-heptanol	70.00	322.3	348.0	303.7
linalool	69.00	105.0	100.1	98.3
citronellol	69.00	54.6	43.0	53.4
methyl salicylate	92.00	426.6	502.9	524.3
nerol	96.00	119.3	99.6	122.3
damascenone	69.00	149.2	295.0	255.0
geraniol	69.00	84.6	109.0	77.7

8.1.2. Final SPME Conditions

The sample was agitated during extraction and the extraction temperature was 40 °C. The final SPME conditions for further measurements are summarized in table 8.3. The same conditions were chosen for MDGC analyses.

8.2. Optimization of GC Separation

The gas flow and further parameters for the one-dimensional GC analyses can be seen in table 8.4. The parameter which was varied to optimize the separation is the heating rate of the oven. It is discussed in the following subsections.

8.2.1. Temperature Rate

The measurements were performed at different heating rates: *i*) 4 °C/min, *ii*) 6 °C/min, *iii*) 8 °C/min, and *iv*) 10 °C/min. The concentration of the standard solution for this

Table 8.3.: SPME Conditions for 1D-GC Analysis

Fiber	SUPELCO 50/30 µm DVB/Carboxen/PDMS StableFlex/SS (2cm)
Pre-Incubation Time	1 min
Incubation Temperature	40 °C
Pre-Incubation Agitator Speed	40 min^{-1}
Agitator On Time	5 s
Agitator Off Time	2 s
Vial Needle Penetration	20 mm
Vial Fiber Exposure	22 mm
Extraction Time	20 min
Desorption Temperature	260 °C
Desorption Time	10 min

Table 8.4.: GC Parameters for GC Analysis

Flow Control Mode	Linear Velocity
Pressure	5.8 mL/min
Total Flow	0.69 mL/min
Column Flow	30 cm/s
Purge Flow	3 mL/min
Injection Temperature	260 °C
Injection Mode	split
Split Ratio	3

analysis was 50 ng. A repeat determination was performed for every heating rate.
In figure 8.1 the chromatograms for different heating rates are shown and it can be seen that a proper separation was obtained with a heating rate of 4 °C/min. By applying faster temperature programs 2-hexen-1-ol acetate and 6-methyl-5-hepten-2-one could not be resolved. For a heating rate of 4 °C/min the retention time for 2-hexen-1-ol acetate was 7.490 min while 6-methyl-5-hepten-2-one eluted at 7.540 min. But this separation problem could be solved by applying a SIM method and separating the compounds by specific ions.
Nevertheless, a heating rate of 10 °C/min was chosen for further analyses because of the advantage of a rapid separation.

8.3. Optimization of MS Parameters

Two different ion sources were used and compared. The combi ion source can be used for chemical ionisation and electron impact ionisation. The other source was a pure electron impact ion source. So three different ionisation modes were compared.
The sensitivity towards the compounds in the standard solution were determined by the slope of the calibration curve, S/N values were determined and the mass spectra of those

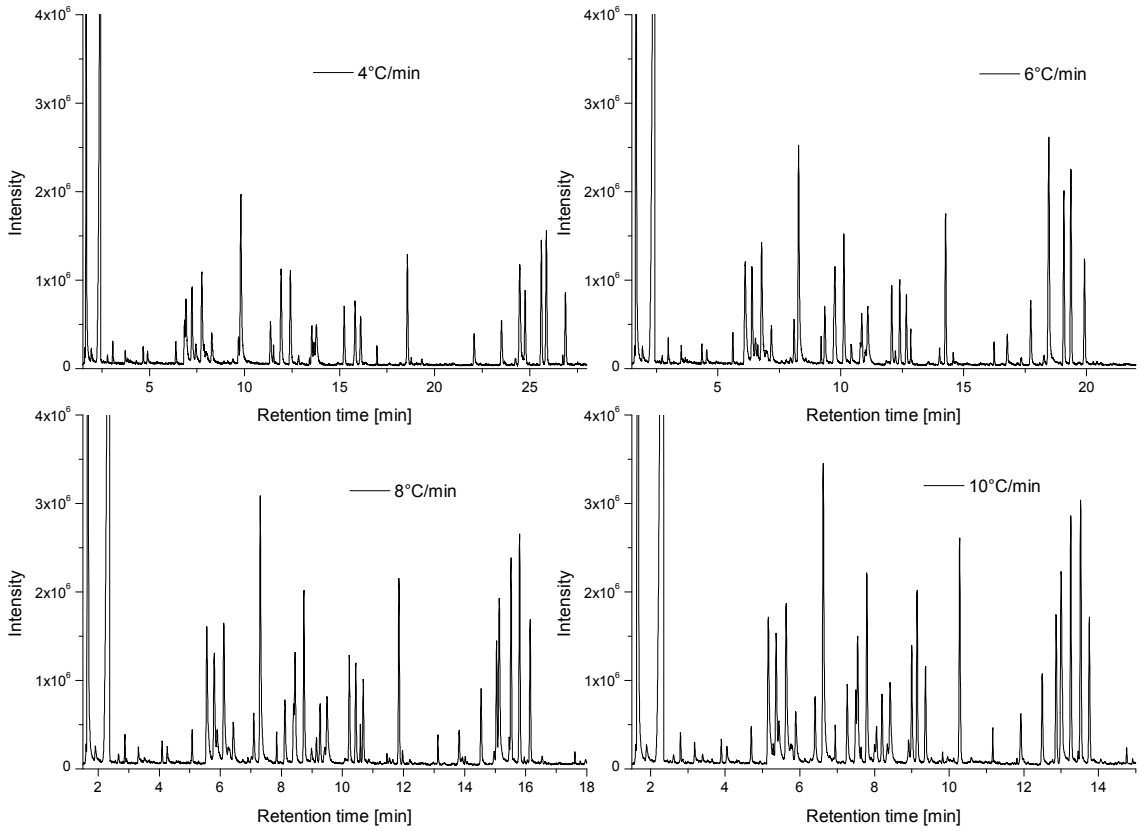

Figure 8.1.: Chromatograms of investigated temperature rates.

compounds were compared. The investigated parameters were the *detector voltage*, the *ion source temperature* and, for chemical ionisation, the *ionisation gas pressure*. All measurements threefold determinations. Further MS parameters are given in tabel 8.5.

Table 8.5.: MS parameters for 1D-GC analyses

Interface Temperature	220 °C
Ion Source Temperature	220 °C except for SCI
Electron Energy	70 keV
Scan mode	
Event Time	0.2 s
m/z**-Range**	46-300 amu
Scan Speed	1428 amu/s
SIM mode	
Event Time	0.2 s

8.3.1. Semi Electron Impact Ionization (SEI)

Semi-Electron Ionisation is a feature of the NCI ion source of Shimadzu. In SEI mode electron impact ionisation is performed.

Development of a SIM Method

The target ions and reference ions for the SIM method measured with SEI are given in table 8.6. The target ion is used for the quantification of a compound.
The reference ions for one compound are set to a percentage value relative to the m/z-peak with the highest intensity. One peak is identified when the measured percentage of the reference ions is within a range of ± 30 % of the defined value. Is the difference larger than 30 % then a peak is not identified.

Table 8.6.: Target ions and reference ions for 1D-GC-SEI measurements in SIM method.

Compound	Target Ion (m/z)	Reference Ions (m/z)
2-ethyl furan	96.00	53.00, 67.00
ethyl isovalerate	88.00	57.00, 60.00
hexanal	57.00	72.00, 100.00
β-myrcene	55.00	121.00, 70.00
α-terpinene	121.00	107.00, 93.00, 91.00
heptanal	70.00	55.00, 57.00
limonene	93.00	77.00, 107.00
(E)-2-hexanal	55.00	69.00, 77.00
p-cymene	77.00	51.00, 93.00
(Z)-3-hexen-1-ol acetate	67.00	82.00, 100.00
(E)-2-hexen-1-ol acetate	67.00	82.00, 100.00
6-methyl-5-hepten-2-one	108.00	126.00
cis-rose oxide	69.00	55.00, 154.00
trans-rose oxide	69.00	55.00, 84.00
(Z)-3-hexen-1-ol	67.00	82.00, 81.00
2,4-hexadienal	81.00	67.00, 82.00
cis-linalool oxide	55.00	81.00, 97.00
1-heptanol	70.00	55.00, 98.00
trans-linalool oxide	55.00	81.00, 97.00
linalool	69.00	53.00, 92.00
(Z)-citral	69.00	53.00, 109.00
(E)-citral	69.00	53.00, 109.00
citronellol	69.00	55.00, 53.00
methyl salicylate	92.00	121.00, 93.00
nerol	69.00	93.00, 53.00
damascenone	69.00	121.00, 77.00
geraniol	69.00	53.00, 55.00

8.3.2. Semi Chemical Ionization (SCI)

For this mode positive chemical ionisation was carried out by using methane as ionisation gas. First measurements in scan mode were done and subsequently a SIM method

was developed. The target ions and the reference ions are given in table 8.7. The following investigations, regarding the S/N ratio and the calibration curve were carried out by applying a SIM method. A scan method was used to compare the mass spectra when investigating different ion source temperatures and different ionisation gas pressure values.

Table 8.7.: Compounds of the standard solution their target ions, reference ions, and molecular mass for quantification in 1D-GC-SCI. The molecular mass of the compounds are shown due to the formation of pseudo-molecule peaks by SCI.

Compound	**Target Ion (m/z)**	**Reference Ions (m/z)**	**Molecular Mass (amu)**
2-ethyl furan	97.00	96.00, 81.00	96.13
ethyl isovalerate	131.00	103.00, 85.00	130.18
hexanal	83.00	85.00	100.16
β-myrcene	93.00	121.00, 81.00	136.23
α-terpinene	121.00	93.00, 81.00	136.23
heptanal	97.00	70.00	114.19
limonene	81.00	93.00, 121.00	136.23
(*E*)-2-hexanal	99.00	81.00, 70.00	98.14
p-cymene	119.00	93.00, 105.00	134.22
(*Z*)-3-hexen-1-ol acetate	83.00	69.00, 100.00	142.2
(*E*)-2-hexen-1-ol acetate	83.00	69.00, 100.00	142.2
6-methyl-5-hepten-2-one	109.00	126.00, 69,00	126.2
cis-rose oxide	139.00	81.00, 99.00	154.25
trans-rose oxide	139.00	81.00, 99.00	154.25
(*Z*)-3-hexen-1-ol	83.00	81.00, 82.00	100.16
2,4-hexadienal	97.00	81.00, 83.00	96.13
cis-linalool oxide	153.00	71.00, 94.00	170.25
1-heptanol	97.00	71.00, 111.00	116.2
trans-linalool oxide	153.00	71.00, 94.00	170.25
linalool	81.00	93.00, 137.00	154.25
(*Z*)-citral	95.00	135.00, 109.00	152.23
(*E*)-citral	95.00	81.00, 135.00	152.23
citronellol	83.00	95.00, 137.00	154.25
methyl salicylate	121.00	81.00, 109.00	152.15
nerol	81.00	95.00, 137.00	154.25
damascenone	191.00	95.00, 121.00	190.28
geraniol	81.00	95.00, 137.00	154.25

Ion Source Temperature

For this investigation the standard solution with an amount of 20 ng per compound was used. Three different temperatures were analysed: *i*) 150 °C, *ii*) 200 °C, and *iii*) 230 °C. By overlaying the three chromatograms it was visible that the highest intensities for the peaks resulted by using an ion source temperature of 150 °C. This temperature was used for the following studies with SCI ionisation not only because of its lower S/N values (see table 8.8), but also because of the higher intensities measured with 150 °C.
In figure 8.2 mass spectra of three compounds are shown recorded with different ion

source temperature. For all these plotted compounds it can be seen that the peak intensity of the pseudo-molecule peak, $[M+H]^+$, reduces with higher temperatures of the ion source (ethyl isovalerate m/z 131, damascenone m/z 191 and methyl salicylate m/z 153). A reason for this effect can be that by applying higher temperatures the kinetic energy of the molecules and ions is higher and the collisions in which the the proton is transferred from the ionisation gas ion to the analyte molecule are more energetic and fragmentation of the molecule is more likely.

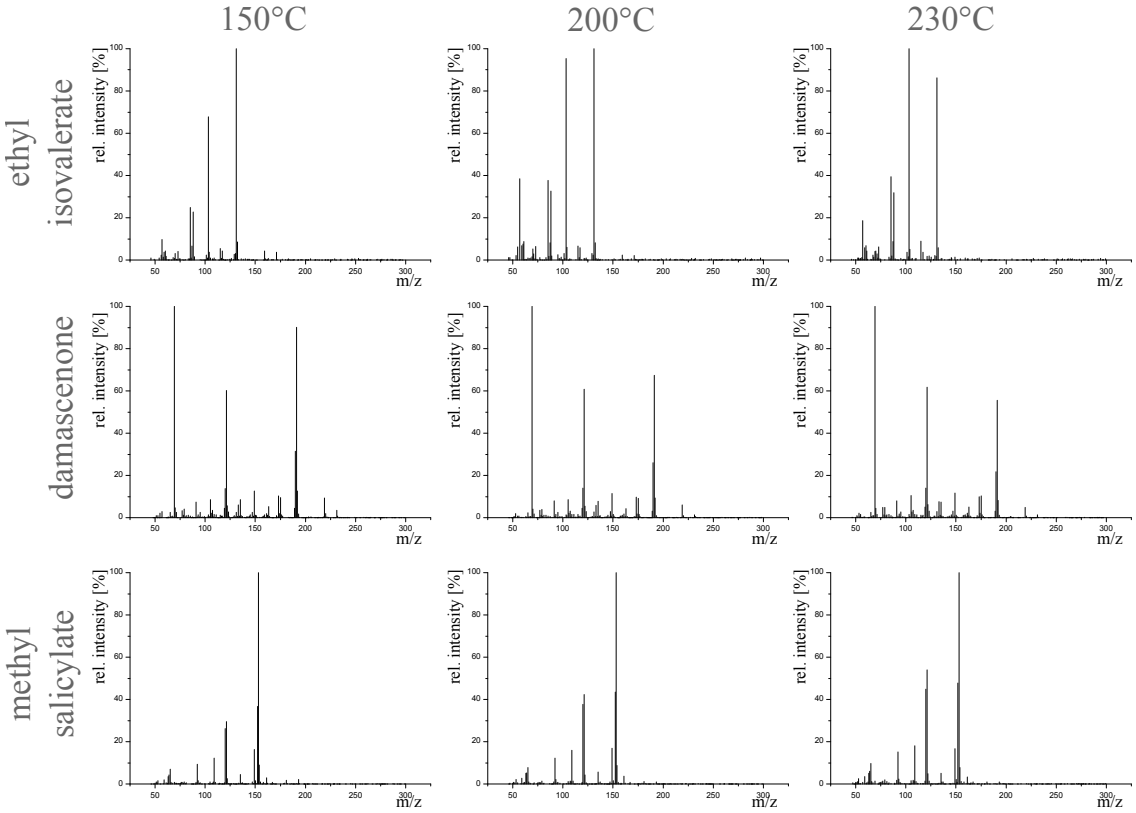

Figure 8.2.: Comparison of SCI mass spectra measured with different ion source temperature for ethyl isovalerate, damascenone and methyl salicylate. The mass spectra recorded at different temperatures vary in the intensity of the pseudo-molecule peak (ethyl isovalerate m/z 131, damascenone m/z 191 and methyl salicylate m/z 153).

Ionisation Gas Pressure

Three different gas pressure values have been investigated at an ion source temperature of 150 °C. The investigated gas pressure values in the ion source were: *i*) 200 kPa, *ii*) 300 kPa, and *iii*) 350 kPa.
In figure 8.3 the mass spectra of three target analytes are plotted and it can be seen that the lower the gas pressure was the more fragmentation was observed. A higher pseudo-molecule peak, $[M + H]^+$, was found for higher pressure values.

Table 8.8.: S/N values measured with different ion source temperatures with SCI in SIM mode. Sample amount were 20 ng.

Compound	150 °C	200 °C	230 °C
2-ethyl furan	64.4	82.2	53.1
ethyl isovalerate	852.6	410.5	313.4
hexanal	357.4	317.7	264.2
β-myrcene	351.4	327.7	278.1
α-terpinene	263.6	290.1	185.6
heptanal	388.9	n.d.	292.9
limonene	110.5	102.2	103.9
(*E*)-2-hexanal	663.6	504.6	412.9
p-cymene	1736.0	1672.6	1233.9
(*Z*)-3-hexen-1-ol acetate	83.0	67.0	72.5
(*E*)-2-hexen-1-ol acetate	67.9	60.2	65.7
6-methyl-5-hepten-2-one	780.9	396.1	n.d.
(*Z*)-3-hexen-1-ol	1057.9	377.7	1181.5
2,4-hexadienal	978.7	827.5	774.5
1-heptanol	266.5	207.8	156.9
linalool	630.7	531.9	465.5
citronellol	183.3	148.4	115.7
methyl salicylate	257.3	216.5	214.2
nerol	596.6	498.1	460.8
damascenone	1290.2	756.5	972.9
geraniol	245.0	228.6	185.8

By applying a relatively low pressure of 200 kPa the occurring ionisation is more like a electron impact ionisation. Due to low concentration of ionisation gas the electrons, which should react with the ionisation gas first, collide with the analyte molecules directly. The analyte molecules get ionised and fragmented like in EI. That is why the spectra which were achieved with 200 kPa are quite similar to those which were recorded by SEI and EI. Peaks of ions that consist of the analyte molecule and the reacting ion, for example $[M + C_2H_5]^+$ can be found to a higher extent by applying higher pressure. For further measurements in SCI mode an ionisation gas pressure of 350 kPa was chosen to guarantee a proper positive chemical ionisation.

8.3.3. Electron Impact Ionization (EI)

After comparing the two possibilities with the combi-source, SEI and SCI, the ion source was changed and the EI source was installed. After scan measurements a SIM method was performed and afterwards the signal-to-noise ratio measured with different detector voltages were compared. Afterwards quantification of elder flower extracts were carried out.

SIM Methods

The target ions and the reference ions using for identification and quantification with GC-EI-MS are given in table 8.9.

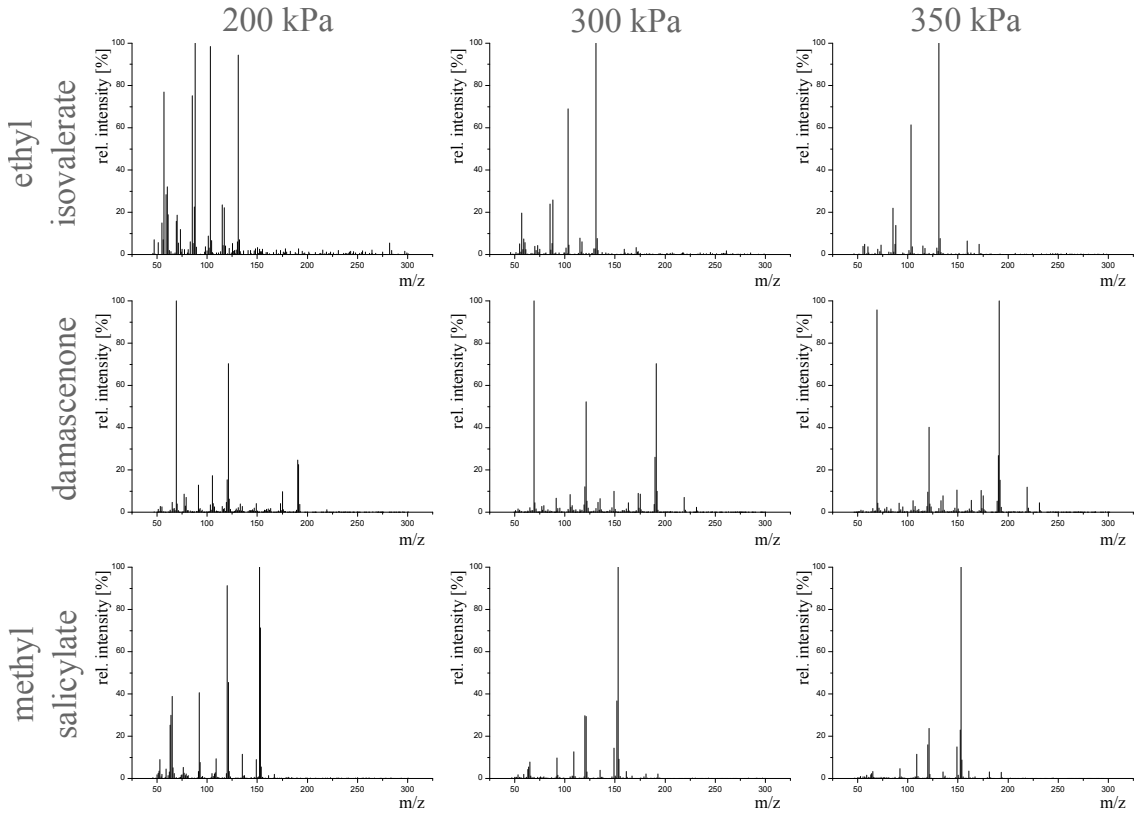

Figure 8.3.: Comparison of mass spectra measured with values for the different ionisation gas pressure, for ethyl isovalerate, damascenone and methyl salicylate. The mass spectra recorded with a relatively low gas pressure exhibit fragmentation to a higher extend than spectra recorded with a higher pressure. Is the gas pressure to low in PCI the conditions are similar to EI conditions and a higher fragmentation rate occurs. Peaks of ions that consist of the analyte molecule and the reacting ion can be found to a higher extent in the measurement with 350 kPa.

Detector Voltage

Different detector voltages were applied to increase the detector sensitivity. The applied voltages in the measurement of the standard solution with the sample amount of 1 ng in SIM mode are: *i*) 1.3 kV *ii*) 1.4 kV, and *iii*) 1.5 kV. It can be observed that the higher the detector voltage is the higher intensities of the signal can be achieved. However by considering the S/N-ratio it can be seen that there is a maximum. 1.4 kV were chosen for further analyses.
S/N values for SIM analyses are shown in table 8.10.

Table 8.9.: Compounds with their belonging target ion and reference ions ^{1}D-GC-EI

Compound	Target Ion (m/z)	Reference Ions (m/z)
2-ethyl furan	96.00	53.00, 67.00
ethyl isovalerate	88.00	57.00, 60.00
hexanal	57.00	72.00, 100.00
β-myrcene	55.00	121.00, 70.00
α-terpinene	121.00	107.00, 93.00, 91.00
heptanal	70.00	55.00, 57.00
limonene	93.00	77.00, 107.00
(*E*)-2-hexanal	55.00	69.00, 77.00
p-cymene	77.00	51.00, 93.00
(*Z*)-3-hexen-1-ol acetate	67.00	82.00, 100.00
(*E*)-2-hexen-1-ol acetate	67.00	82.00, 100.00
6-methyl-5-hepten-2-one	108.00	126.00
cis-rose oxide	69.00	55.00, 154.00
trans-rose oxide	69.00	55.00, 84.00
(*Z*)-3-hexen-1-ol	67.00	82.00, 81.00
2,4-hexadienal	81.00	67.00, 82.00
cis-linalool oxide	55.00	81.00, 97.00
1-heptanol	70.00	55.00, 98.00
trans-linalool oxide	55.00	81.00, 97.00
linalool	69.00	53.00, 92.00
(*Z*)-citral	69.00	53.00, 109.00
(*E*)-citral	69.00	53.00, 109.00
citronellol	69.00	55.00, 53.00
methyl salicylate	92.00	121.00, 93.00
nerol	69.00	93.00, 53.00
damascenone	69.00	121.00, 77.00
geraniol	69.00	53.00, 55.00

8.4. Quantification of aroma-relevant Compounds in Elder Flower Extracts

The quantification was done by external calibration of a standard solution specified in chapter 7.

8.4.1. Calibration Curves

Calibration curves were made with the standard solution described in section 7.1.1 and were recorded for all three ionization modes (SEI, SCI and EI). All measurements are at least threefold determinations. The slopes of the linear regression curves are shown in table 8.12. When using the EI mode the slopes are steeper which shows that this ionisation method is more sensitive than SEI and SCI. The concentration ranges of the calibration were adapted to the sensitivity of the ionisation mode, see table 8.11.

For all calibration curves a coefficient of determination, R^2, of at least 0.98 was achieved. All curves with related standard deviations are shown in appendix A. The values for the S/N ratios and the calculated LOQs are shown in table 8.13. For de-

Table 8.10.: Signal-to-noise ratio of 1 ng standard solution measured with different detector volatges in SIM mode, split 3:1.

Compound	**Target Ion** (m/z)	1.3 kV	1.4 kV	1.5 kV
2-ethyl furan	96.00	8.4	9.3	8.2
ethyl isovalerate	88.00	88.5	90.1	109.1
hexanal	57.00	8.34	15.5	22.7
β-myrcene	55.00	1.9	3.7	5.3
α-terpinene	121.00	129.7	144.2	205.1
heptanal	70.00	35.9	37.7	51.1
limonene	93.00	109.4	110.2	129.9
(E)-2-hexanal	55.00	40.1	58.3	42.1
p-cymene	77.00	78.73	75.5	76.3
(Z)-3-hexen-1-ol acetate	67.00	49.7	44.3	55.8
(E)-2-hexen-1-ol acetate	67.00	17.8	15.9	19.8
6-methyl-5-hepten-2-one	108.00	421.1	373.4	399.8
(Z)-3-hexen-1-ol	67.00	26.9	76.3	87.7
2,4-hexadienal	81.00	203.5	179.3	204.7
1-heptanol	70.00	341.4	313.5	182.8
linalool	69.00	129.7	114.0	80.5
citronellol	69.00	58.8	60.2	38.2
methyl salicylate	92.00	499.8	371.1	277.7
nerol	96.00	154.7	192.0	100.5
damascenone	69.00	195.8	258.6	104.5
geraniol	69.00	106.5	125.8	63.3

terming the LOQ a concentration is calculated with a S/N of 10.

8.4.2. Quantification of Odour-active Compounds in Elder Flower Extracts

Elder flower extracts specified in chapter 7 were analysed and selected compounds were quantified with the calibrations mentioned above. The extracts were also measured with different ionisation modes and the results were compared. Quantification of selected compounds were carried out with SEI, SCI and SEI by applying the above mentioned SIM methods and calculate the concentration via the calibration curves (10.2). In table 8.14 it is shown with which ionisation method and with what sample amount the samples were measured. The obtained results are shown in tables 8.15 to 8.21. When it was not possible to detect a peak *n.d.* is shown in the table. There can be two reasons for that: *i*) the peak was not identified because the ratio of the reference ions do not match (difference is >30 %) or *ii*) the S/N ratio is <2.
< LOQ is shown in the table when the measured value is lower than the limit of quantification. Limit of quantification is defined as the concentration that makes a S/N of 10.
The investigated extracts with their corresponding sample amount and ionisation method are shown in table 8.14. All measurements are threefold determinations.

Table 8.11.: Concentration of standard solutions used for calibration curves

Amount of Analyte	SEI	SCI	EI
0.1 ng	–	–	×
0.25 ng	–	–	×
0.5 ng	–	–	×
1 ng	×	×	×
5 ng	×	×	×
10 ng	×	×	×
20 ng	×	×	×
50 ng	×	×	–
100 ng	×	×	–

Table 8.12.: Slopes of calibration curves for different ionisation methods. Complete calibration curves are shown in appendix A

Compound	SEI	SCI	EI
	intensity/ng	intensity/ng	intensity/ng
2-ethyl furan	1.37×10^3	7.13×10^3	2.16×10^4
ethyl isovalerate	3.93×10^3	2.27×10^4	9.54×10^4
hexanal	3.44×10^3	5.45×10^4	8.57×10^4
β-myrcene	2.18×10^3	5.67×10^4	2.56×10^4
α-terpinene	2.05×10^4	3.63×10^4	3.39×10^5
heptanal	1.08×10^4	9.59×10^4	2.47×10^5
limonene	1.99×10^4	6.20×10^4	4.91×10^5
(E)-2-hexanal	7.46×10^3	6.63×10^4	1.66×10^5
p-cymene	1.14×10^4	1.02×10^5	1.15×10^5
(Z)-3-hexen-1-ol acetate	3.18×10^4	1.10×10^5	7.23×10^5
(E)-2-hexen-1-ol acetate	1.33×10^4	1.21×10^5	2.91×10^5
6-methyl-5-hepten-2-one	1.22×10^4	1.14×10^5	2.50×10^5
(Z)-3-hexen-1-ol	1.56×10^4	5.79×10^4	3.28×10^5
2,4-hexadienal	3.36×10^4	2.97×10^5	5.30×10^5
1-heptanol	2.63×10^4	3.12×10^4	5.80×10^5
linalool	1.54×10^4	7.81×10^4	2.56×10^5
citronellol	2.23×10^4	3.11×10^4	3.79×10^5
methyl salicylate	3.39×10^4	5.98×10^4	5.56×10^5
nerol	6.71×10^4	8.97×10^4	1.06×10^6
damascenone	5.55×10^4	7.07×10^4	8.72×10^5
geraniol	4.93×10^4	6.10×10^4	8.70×10^5

Comparison of the results obtained with different ionisation methods

A comparison of the results obtained with SEI, SCI and EI can be seen in table 8.15 for the sample S3Tx and in table 8.16 for the sample S7Tx. For both samples the sample amount was 500 µL. The results of different ionisation methods do not differ significantly. Variation can occur because of storage and frosting-defrosting cycles. These effects are discussed in section 8.4.3.

Table 8.13.: S/N values for 1 ng sample and LOQs for each method and compound. LOQ is defined as the amount of analyte that effects a peak with a S/N of 10.

Compound	**SEI**		**SCI**		**EI**	
		ng		ng		ng
2-ethyl furan	n.d.	–	5.12	1.95	9.28	1.08
ethyl isovalerate	7.91	1.26	18.70	0.53	90.06	0.11
hexanal	5.91	1.69	16.64	0.60	15.46	0.65
β-myrcene	n.d.	–	12.52	0.80	3.66	2.73
α-terpinene	12.36	0.81	6.95	1.44	144.22	0.07
heptanal	4.84	2.07	15.22	0.66	37.72	0.27
limonene	12.24	0.82	11.46	0.87	110.22	0.09
(*E*)-2-hexanal	8.80	1.14	25.96	0.39	58.32	0.17
p-cymene	11.73	0.85	62.91	0.16	75.48	0.13
(*Z*)-3-hexen-1-ol acetate	22.68	0.44	19.34	0.52	44.28	0.23
(*E*)-2-hexen-1-ol acetate	8.85	1.13	16.39	0.61	15.88	0.63
6-methyl-5-hepten-2-one	52.24	0.19	41.30	0.24	373.41	0.03
(*Z*)-3-hexen-1-ol	34.61	0.29	153.27	0.07	76.30	0.13
2,4-hexadienal	27.24	0.37	37.96	0.26	179.28	0.06
1-heptanol	43.82	0.23	8.59	1.16	313.45	0.03
linalool	21.61	0.46	24.37	0.41	113.98	0.09
citronellol	24.07	0.42	4.39	2.28	60.15	0.17
methyl salicylate	122.38	0.08	6.53	1.53	371.07	0.03
nerol	37.11	0.27	17.70	0.56	191.95	0.05
damascenone	45.07	0.22	39.93	0.25	258.63	0.04
geraniol	20.53	0.49	6.80	1.47	125.79	0.08

Table 8.14.: Measured elder flower extracts, corresponding sample amount and ionisation method.

Sample	**Sample Amount**	**SEI**	**SCI**	**EI**
S3T3	100 µL			×
	500 µL		×	×
S3Tx	100 µL	×		×
	500 µL	×	×	×
S5T3	100 µL			×
	500 µL		×	×
S6T3	100 µL			×
	500 µL		×	×
S7T3	100 µL			×
	500 µL		×	×
S7Tx	100 µL	×		×
	500 µL	×	×	×
S9Tx	100 µL	×		
	500 µL			
S10T3	100 µL			
	500 µL		×	
S10Tx	100 µL	×		×
	500 µL	×	×	
S11Tx	100 µL	×		
	500 µL			
S11Tx	100 µL	×		
	500 µL			

Table 8.15.: Comparision of the results for the sample S3Tx gained with different ionisation modes, sample amount 500 µL, measured with 1D-GC-qMS

Compound	**SEI** µg/L	**SCI** µg/L	**EI** µg/L
2-ethyl furan	n.d.	n.d.	<LOQ
ethyl isovalerate	2.0 ± 0.2	1.3 ± 0.1	1.44 ± 0.07
hexanal	23 ± 1	19.4 ± 0.7	17.2 ± 0.3
β-myrcene	n.d.	2.3 ± 0.1	n.d.
α-terpinene	n.d.	n.d.	1.26 ± 0.02
heptanal	11.8 ± 0.3	10.16 ± 0.08	11.7 ± 0.7
limonene	<LOQ	2.63 ± 0.04	0.58 ± 0.03
(*E*)-2-hexanal	6.54 ± 0.06	n.d.	6.3 ± 0.2
p-cymene	n.d.	n.d.	0.47 ± 0.02
(*Z*)-3-hexen-1-ol acetate	1.37 ± 0.05	n.d.	1.68 ± 0.03
(*E*)-2-hexen-1-ol acetate	n.d.	n.d.	n.d.
6-methyl-5-hepten-2-one	1.2 ± 0.2	0.86 ± 0.06	1.50 ± 0.06
(*Z*)-3-hexen-1-ol	48 ± 1	37 ± 2	48 ± 2
2,4-hexadienal	n.d.	n.d.	n.d.
1-heptanol	5.3 ± 0.2	n.d.	5.0 ± 0.1
linalool	156 ± 5	171 ± 4	154 ± 1
citronellol	32 ± 1	33 ± 2	36 ± 2
methyl salicylate	16.2 ± 0.9	12.1 ± 0.2	19.7 ± 0.4
nerol	10.6 ± 0.3	7.9 ± 0.2	6.0 ± 0.2
damascenone	5.80 ± 0.06	2.0 ± 0.1	1.81 ± 0.05
geraniol	14.3 ± 0.4	7.7 ± 0.4	9.8 ± 0.5

Comparison of the results obtained by different sample amounts

The quantification was carried out with 100 µL sample and 500 µL sample and the results are presented in table 8.17.

By comparing the results obtained with different sample amounts (table 8.17) it can be seen that the concentration of the compounds is higher for 100 µL than for 500 µL. This tendency is the same for all compounds in all samples.
By changing the sample amount the ratio between the surface and the bulk is also different, it is higher for less sample amount, and therefore more compounds go into the gas phase. Another possible reason is that the headspace is saturated with molecules and this reduces the vapour pressure of the compounds for higher sample amounts. An alternative explanation is that the SPME fiber is saturated and no more molecules can adsorb on the fiber anymore. The difference between the concentrations of both sample amounts is not given by a constant factor. That is be explained by the difference in volatility of the compounds and the difference in adsorption affinity to the fiber. It is probable that all these effects are reasons for the different results.
In fact, with this method, the concentration of the compounds in the headspace is determined and not the concentration in the sample itself. The equilibria of the compounds between the sample and the headspace are neglected, because a standard solution was used for external calibration and not a model solution. By using 10 µL of the standard solution nearly all molecules are in the gas phase. If a model solution was used

Table 8.16.: Comparision of the results for the sample S7Tx gained with different ionisation modes, sample amount 500 µL, measured with 1D-GC-qMS

Compound	**SEI** µg/L	**SCI** µg/L	**EI** µg/L
2-ethyl furan	n.d.	n.d.	<LOQ
ethyl isovalerate	4.7 ± 0.3	4.85 ± 0.08	4.10 ± 0.11
hexanal	23.9 ± 0.4	22.5 ± 0.6	21 ± 2
β-myrcene	n.d.	n.d.	n.d.
α-terpinene	n.d.	n.d.	1.26 ± 0.01
heptanal	6.2 ± 0.8	n.d.	8 ± 1
limonene	<LOQ	2.8 ± 0.1	1.6 ± 0.9
(*E*)-2-hexanal	9.7 ± 0.1	9.2 ± 0.3	10.5 ± 0.5
p-cymene	n.d.	n.d.	0.85 ± 0.06
(*Z*)-3-hexen-1-ol acetate	9.7 ± 0.4	4.2 ± 0.2	5.00 ± 0.03
(*E*)-2-hexen-1-ol acetate	n.d.	n.d.	n.d.
6-methyl-5-hepten-2-one	1.20 ± 0.02	0.72 ± 0.03	1.45 ± 0.07
(*Z*)-3-hexen-1-ol	53 ± 2	38 ± 1	51 ± 3
2,4-hexadienal	n.d.	n.d.	n.d.
1-heptanol	2.76 ± 0.06	n.d.	2.26 ± 0.08
linalool	174 ± 6	171 ± 189	164 ± 3
citronellol	36 ± 1	36.3 ± 0.7	40 ± 1
methyl salicylate	30 ± 1	20.7 ± 0.8	34 ± 1
nerol	9.25 ± 0.05	6.09 ± 0.04	4.05 ± 0.06
damascenone	n.d.	n.d.	1.49 ± 0.06
geraniol	13.6 ± 0.1	6.4 ± 0.1	8.3 ± 0.3

for the calibration, having the same amount of sugar, citric acid and water, and if this was applied in the same sample amount the equilibrium between the sample and the headspace is also considered. A quantification of the compounds in the sample and not in the headspace can be done. In this case there will be no significant difference between 100 µL and 500 µL sample amount. However, in this case two calibration curves have to be taken, one for each sample amount. A problem can occur by using a sample amount in which the concentration of the investigated compounds is lower than the limit of quantification. This problem however is not relevant for analyses with the EI ion source because of the high sensitivity.

Comparison of the results from different elder sorts

The concentration of the analysed compounds varies for different elder cultivars. It confirms the other investigations where the differentiation of elder sorts according to the odour of the flowers was carried out. [38]
The results of the quantification can be seen in tables 8.18 (SEI with a sample amount of 500 µL), 8.19 (SCI with a sample amount of 500 µL), 8.20 (EI with a sample amount of 100 µL), and 8.21 (EI with a sample amount of 500 µL).

Table 8.17.: Comparison of the results of elder flower syrups, sample amount: 100 µL and 500 µL , measured with 1D-GC-EI-qMS

Compound	**S3T3**		**S5T3**	
	100 µL µg/L	500 µL µg/L	100 µL µg/L	500 µL µg/L
2-ethyl furan	n.d.	<LOQ	1.1 ± 0.1	1.42 ± 0.06
ethyl isovalerate	n.d.	2.24 ± 0.04	4.26 ± 0.04	2.86 ± 0.06
hexanal	46.8 ± 0.7	37.0 ± 0.6	48 ± 1	36.0 ± 0.2
β-myrcene	n.d.	<LOQ	n.d.	n.d.
α-terpinene	5.80 ± 0.03	1.35 ± 0.01	5.53 ± 0.02	1.17 ± 0.01
heptanal	12 ± 2	7.58 ± 0.09	9.4 ± 0.6	3.5 ± 0.1
limonene	4 ± 1	2.3 ± 0.1	2.50 ± 0.03	0.8 ± 0.4
(*E*)-2-hexanal	32.9 ± 0.9	18.3 ± 0.4	44 ± 1	25.3 ± 0.2
p-cymene	<LOQ	0.49 ± 0.04	n.d.	n.d.
(*Z*)-3-hexen-1-ol acetate	n.d.	1.68 ± 0.01	6.56 ± 0.05	3.63 ± 0.02
(*E*)-2-hexen-1-ol acetate	n.d.	<LOQ	n.d.	0.80 ± 0.01
6-methyl-5-hepten-2-one	4.6 ± 0.8	1.88 ± 0.09	3.4 ± 0.2	1.12 ± 0.01
(*Z*)-3-hexen-1-ol	243 ± 3	71 ± 2	301 ± 8	89.4 ± 0.3
2,4-hexadienal	n.d.	n.d.	n.d.	n.d.
1-heptanol	10.48 ± 0.02	3.59 ± 0.07	7.1 ± 0.1	2.06 ± 0.01
linalool	675 ± 3	343 ± 6	39.1 ± 0.6	87 ± 2
citronellol	163 ± 6	63.4 ± 0.8	261 ± 5	97 ± 2
methyl salicylate	79 ± 5	33.6 ± 0.8	71 ± 3	28.0 ± 0.2
nerol	29 ± 1	8.8 ± 0.1	49 ± 1	15.5 ± 0.3
damascenone	5.1 ± 0.3	1.48 ± 0.05	6.6 ± 0.2	1.6 ± 0.1
geraniol	36.2 ± 0.8	10.4 ± 0.2	25 ± 1	89 ± 3

8.4.3. Possible Improvements of the Quantification

The main focus of this work was the comparison of different ionisation methods and the application of multidimensional gas chromatography. Therefore, some improvements regarding the quantification are considered.

Internal Standard

Using an internal standard is beneficial to examine errors in the analytical process. Fluctuations are better recognized with an internal standard and can be calculated with the recovery rate.

Modell Solution

Using a model solution for the external calibration the concentration of the sample can be analysed. This was already discussed in the section 8.4.2 where the effect of different sample amounts on the quantification was discussed.

Adaptation of the Concentration Range of the Calibration

It is advantageous to use a calibration range were the expected concentration in the sample is nearly in the middle of this range. Though the concentration ranges of each compound vary and also the concentration ranges of one compound in different elder extracts differ from each other.

Table 8.18.: Results of elder flower syrups, sample amount: 500 µL, measured with 1D-GC-SEI-qMS

Compound	**S3Tx** µg/L	**S7Tx** µg/L	**S10Tx** µg/L
2-ethyl furan	n.d.	n.d.	n.d.
ethyl isovalerate	<LOQ	4.7 ± 0.3	n.d.
hexanal	23 ± 1	23.9 ± 0.4	32.9 ± 0.8
β-myrcene	n.d.	n.d.	n.d.
α-terpinene	n.d.	n.d.	n.d.
heptanal	11.8 ± 0.3	6.2 ± 0.8	13 ± 1
limonene	<LOQ	<LOQ	2.44 ± 0.09
(*E*)-2-hexanal	6.54 ± 0.06	9.7 ± 0.4	12.4 ± 0.2
p-cymene	n.d.	n.d.	n.d.
(*Z*)-3-hexen-1-ol acetate	1.37 ± 0.05	9.7 ± 0.4	n.d.
(*E*)-2-hexen-1-ol acetate	n.d.	n.d.	n.d.
6-methyl-5-hepten-2-one	1.2 ± 0.2	1.20 ± 0.02	1.95 ± 0.03
(*Z*)-3-hexen-1-ol	48 ± 1	53 ± 2	79 ± 1
2,4-hexadienal	n.d.	n.d.	n.d.
1-heptanol	5.3 ± 0.2	2.76 ± 0.06	n.d.
linalool	156 ± 5	174 ± 6	424 ± 4
citronellol	32 ± 1	36 ± 1	28.1 ± 0.6
methyl salicylate	16.2 ± 0.9	30 ± 1	28.4 ± 0.5
nerol	10.6 ± 0.3	9.25 ± 0.05	9.6 ± 0.1
damascenone	5.80 ± 0.06	n.d.	5.69 ± 0.03
geraniol	14.3 ± 0.4	13.6 ± 0.1	14.3 ± 0.2

Storage and Frosting–Defrosting-Cycles

The syrups were stored in a freezer. The temperature was not specified, but the samples are frozen (chapter 7). For the analysis the sample has to be defrosted gently in a water bath and pipetted into the vial. Afterwards the sample is refrozen again. Maybe the process has a slight effect on the sample. The samples measured at a different point of time were also measured with a different ionisation method and hence these differences cannot be quantified.

cis/trans-Mixtures

Especially the linalool oxides and the rose oxides are very important compounds for the odour of elder flowers. It is beneficial to analyse them as pure substances and not as mixtures. Then, it is possible to quantify these compounds.

8.5. Comparison of the Different Ionization Methods

The combi ion source provides two different ionisation modes, chemical and electron impact ionisation. That is an advantage if chemical ionisation is needed.

8.5.1. Sensitivity and Signal-to-Noise-Ratio

The S/N ratios measured with different ionisation methods and their LOQ were compared in table 8.13. It can be easily seen that there is a big difference in the S/N values

Table 8.19.: Results of elder flower syrups, sample amount: 500 µL, measured with 1D-GC-SCI-qMS

Compound	**S3T3** µg/L	**S5T3** µg/L	**S6T3** µg/L	**S7T3** µg/L	**S10T3** µg/L	**S10Tx** µg/L
2-ethyl furan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ethyl isovalerate	2.7 ± 0.2	2.3 ± 0.5	2.2 ± 0.1	n.d.	6.4 ± 0.4	<LOQ
hexanal	45.2 ± 0.6	42 ± 6	95 ± 4	98 ± 2	62 ± 5	31 ± 2
β-myrcene	<LOQ	<LOQ	4 ± 2	<LOQ	<LOQ	1.74 ± 0.08
α-terpinene	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ
heptanal	n.d.	n.d.	<LOQ	n.d.	n.d.	1.4 ± 0.3
limonene	4 ± 1	2.50 ± 0.03	2.87 ± 0.06	2.36 ± 0.08	2.8 ± 0.1	<LOQ
(*E*)-2-hexanal	n.d.	24 ± 4	38.6 ± 0.9	55 ± 1	41 ± 1	11.1 ± 0.1
p-cymene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(*Z*)-3-hexen-1-ol acetate	n.d.	n.d.	n.d.	n.d.	2.1 ± 0.2	n.d.
(*E*)-2-hexen-1-ol acetate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6-methyl-5-hepten-2-one	0.56 ± 0.04	<LOQ	1.36 ± 0.01	<LOQ	0.59 ± 0.07	1.14 ± 0.04
(*Z*)-3-hexen-1-ol	53.4 ± 0.7	75 ± 6	74 ± 2	47.6 ± 0.3	47 ± 2	60 ± 4
2,4-hexadienal	2.37 ± 0.01	2.67 ± 0.05	2.82 ± 0.03	2.66 ± 0.04	2.80 ± 0.04	3.0 ± 0.01
1-heptanol	n.d.	n.d.	n.d.	n.d.	n.d.	
linalool	292 ± 4	37 ± 4	466 ± 5	100 ± 1	261 ± 14	465 ± 12
citronellol	55 ± 1	106 ± 5	39 ± 2	51 ± 1	59 ± 2	25 ± 1
methyl salicylate	20 ± 1	18 ± 2	30.7 ± 0.17	11.0 ± 0.3	28 ± 1	19 ± 1
nerol	8.8 ± 0.3	18 ± 1	10.8 ± 0.4	7.71 ± 0.03	9.90 ± 0.04	6.5 ± 0.2
damascenone	2.70 ± 0.01	4.4 ± 0.2	3.96 ± 0.02	3.38 ± 0.05	3.88 ± 0.01	1.88 ± 0.06
geraniol	9.5 ± 0.2	20.5 ± 0.7	9.3 ± 0.2	11.3 ± 0.3	10.3 ± 0.2	7.0 ± 0.3

between SEI respectively SCI and EI. With EI a much higher S/N ratio can be achieved. By comparing the limits of quantification in table 8.13 it can be seen that the LOQ values for EI are much lower than for SEI and SCI. It is beneficial for quantification of low amounts of analyte.
The sensitivity difference between the ionisation methods can also be seen by analysing the slopes of the regression curves that is shoen in figure 8.4. Steeper slopes are observed by applying EI, i.e. a higher change of detector signal per change in concentration can be observed resulting in a higher sensitivity. In figure 8.4 the slopes for SEI and SCI are shown relatively to EI slope that is 100 % for all compounds.It shows that the slope of different compounds react differently to the change of the ion source. For compounds eluting at a high retention time, like citronellol, geraniol, and nerol, the EI source is more than ten times more sensitive than the combi source. In some cases like for hexanal and p-cymene with 63 % and 87 % the sensitivity with SCI is almost as high as with EI. The differences are also dependent on the choice of the target ion.

8.5.2. Mass Spectra in Scan Mode

The different ionisation modes are also indicated in the mass spectra. Compounds ionized by SEI and EI show the same spectra because the ionization process is the same, while spectra analysed with SCI are different. There is less fragmentation and some peaks of ions, consisting of the molecule and the reacting ions, can be found. In figure

Table 8.20.: Results of elder flower syrups, sample amount: 100 µL, measured with 1D-GC-EI-qMS

Compound	**S6T3** µg/L	**S7T3** µg/L	**S3T3** µg/L	**S7Tx** µg/L	**S10Tx** µg/L
2-ethyl furan	12.1 ± 0.4	<LOQ	n.d.	<LOQ	<LOQ
ethyl isovalerate	6 ± 1	n.d.	n.d.	n.d.	n.d.
hexanal	103 ± 4	137 ± 2	25.2 ± 0.7	27.3 ± 0.5	34.0 ± 0.9
β-myrcene	n.d.	n.d.	n.d.	n.d.	n.d.
α-terpinene	6.17 ± 0.02	5.50 ± 0.03	5.50 ± 0.02	5.51 ± 0.03	5.98 ± 0.02
heptanal	19.4 ± 0.5	11.0 ± 0.2	17 ± 2	13 ± 1	16 ± 1
limonene	2.87 ± 0.06	2.36 ± 0.08	2.68 ± 0.09	2.63 ± 0.04	2.8 ± 0.1
(E)-2-hexanal	71.1 ± 0.8	114 ± 1	12.90 ± 0.05	17.8 ± 0.1	20.6 ± 0.5
p-cymene	1.71 ± 0.05	n.d.	<LOQ	1.5 ± 0.2	1.6 ± 0.3
(Z)-3-hexen-1-ol acetate	4.63 ± 0.06	7.36 ± 0.09	4.60 ± 0.02	7.62 ± 0.09	4.77 ± 0.03
(E)-2-hexen-1-ol acetate	n.d.	n.d.	n.d.	n.d.	n.d.
6-methyl-5-hepten-2-one	5.3 ± 0.2	3.08 ± 0.04	3.7 ± 0.3	3.32 ± 0.08	4.1 ± 0.1
(Z)-3-hexen-1-ol	312 ± 3	231 ± 4	165 ± 5	162 ± 3	152 ± 11
2,4-hexadienal	n.d.	n.d.	n.d.	n.d.	n.d.
1-heptanol	11.5 ± 0.2	9.02 ± 0.04	13.7 ± 0.1	7.24 ± 0.08	12.1 ± 0.3
linalool	879 ± 5	256 ± 4	388 ± 9	414 ± 3	809 ± 10
citronellol	119 ± 2	179 ± 3	94 ± 3	95 ± 2	72 ± 3
methyl salicylate	113 ± 1	43 ± 1	49.2 ± 0.5	72.7 ± 0.9	77 ± 3
nerol	20.6 ± 0.4	29.6 ± 0.6	20.6 ± 0.5	13.2 ± 0.3	15.1 ± 0.7
damascenone	5.03 ± 0.09	4.6 ± 0.1	5.1 ± 0.1	4.44 ± 0.04	4.65 ± 0.08
geraniol	30.9 ± 0.5	53.4 ± 0.8	34 ± 1	26.8 ± 0.4	29 ± 1

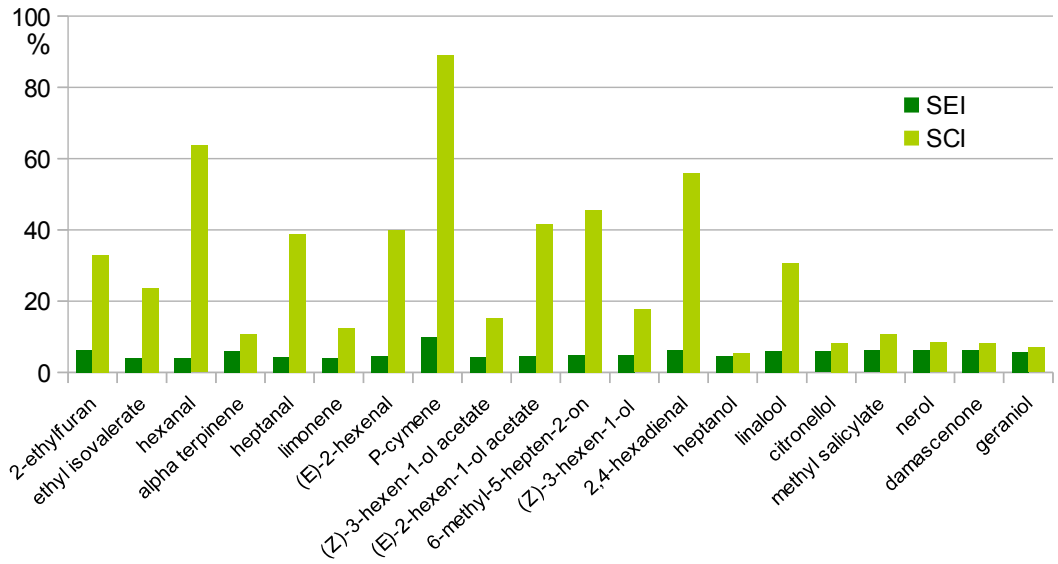

Figure 8.4.: Comparison of the slopes of the calibration curves recorded with EI, SEI and SCI. EI is the method where the steepest slopes, i.e. the highest values, are obtained. The slopes for SEI and SCI are pictured relatively to the EI slope. This shows that the slope of different compounds react differently to the change of the ion source.

Table 8.21.: Results for elder flower syrups, sample amount: 500 µL, measured with 1D-GC-EI-qMS

Compound	**S3T3** µg/L	**S5T3** µg/L	**S6T3** µg/L	**S7T3** µg/L	**S3Tx** µg/L	**S7Tx** µg/L
2-ethyl furan	<LOQ	<LOQ	2.7 ± 0.3	3.00 ± 0.03	<LOQ	<LOQ
ethyl isovalerate	2.24 ± 0.04	2.86 ± 0.06	3.28 ± 0.06	1.47 ± 0.06	1.44 ± 0.07	4.1 ± 0.01
hexanal	37.0 ± 0.6	36.0 ± 0.2	81 ± 2	117 ± 5	17.2 ± 0.3	21 ± 2
β-myrcene	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
α-terpinene	1.35 ± 0.01	1.17 ± 0.01	1.52 ± 0.03	1.21 ± 0.02	1.26 ± 0.02	1.26 ± 0.01
heptanal	7.58 ± 0.09	3.5 ± 0.1	10.6 ± 0.2	7.6 ± 0.5	11.7 ± 0.7	8 ± 1
limonene	2.3 ± 0.1	0.8 ± 0.4	0.77 ± 0.03	1.2 ± 0.5	0.58 ± 0.03	1.6 ± 0.9
(*E*)-2-hexanal	18.3 ± 0.4	25.3 ± 0.2	41.1 ± 0.8	70 ± 2	6.3 ± 0.2	10.5 ± 0.5
p-cymene	0.49 ± 0.04	n.d.	0.62 ± 0.07	n.d.	0.47 ± 0.02	0.85 ± 0.06
(*Z*)-3-hexen-1-ol acetate	1.68 ± 0.01	3.63 ± 0.02	1.76 ± 0.02	4.4 ± 0.2	1.68 ± 0.03	5.00 ± 0.03
(*E*)-2-hexen-1-ol acetate	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.
6-methyl-5-hepten-2-one	1.88 ± 0.09	1.12 ± 0.01	2.24 ± 0.06	1.57 ± 0.08	1.50 ± 0.06	1.45 ± 0.07
(*Z*)-3-hexen-1-ol	71 ± 2	89.4 ± 0.3	91.7 ± 0.8	69 ± 4	48 ± 2	51 ± 3
2,4-hexadienal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-heptanol	3.59 ± 0.07	2.06 ± 0.01	3.87 ± 0.05	2.8 ± 0.2	5.0 ± 0.1	2.26 ± 0.08
linalool	343 ± 6	39.1 ± 0.6	478 ± 6	125 ± 7	154 ± 1	164 ± 3
citronellol	63.4 ± 0.8	97 ± 2	46.4 ± 0.9	72 ± 3	36 ± 2	40 ± 1
methyl salicylate	33.6 ± 0.8	28.0 ± 0.2	50.5 ± 0.5	17 ± 1	19.7 ± 0.4	34 ± 1
nerol	8.8 ± 0.1	15.5 ± 0.3	6.2 ± 0.1	8.9 ± 0.8	6.0 ± 0.2	4.05 ± 0.06
damascenone	1.48 ± 0.05	1.6 ± 0.1	1.23 ± 0.05	1.63 ± 0.06	1.81 ± 0.05	1.49 ± 0.06
geraniol	10.4 ± 0.2	25 ± 1	8.3 ± 0.2	15.9 ± 0.8	9.8 ± 0.5	8.3 ± 0.3

8.5 it can be easily seen that the spectra for SEI and EI are similar while the spectra for SCI is completely different. There is less fragmentation in SCI-spectra and the pseudo-molekule peak shows a high intensity. That results from the relatively soft chemical ionisation process.

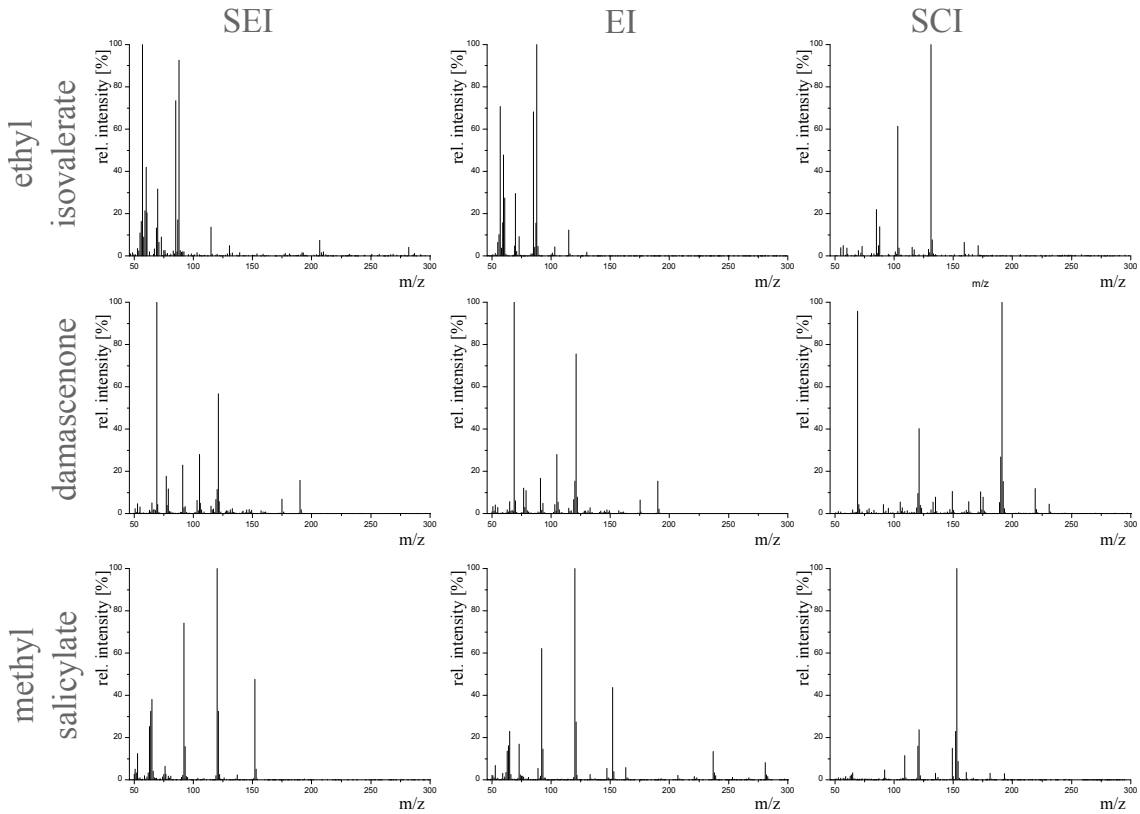

Figure 8.5.: A comparison of mass spectra, SEI, EI and SCI for ethyl isovalerate, damascenone and methyl salicylate. SEI and EI mass spectra are similar while SCI mass spectra is differing due to the different ionisation mechanism. For SCI more peudo-molecule peaks are obtained.

9. Multidimensional GC Separation Techniques

9.1. MDGC Analysis

In MDGC analyses the first step is to record the chromatogram of the first dimensional separation to define retention time ranges were co-elution of interesting compounds occurs. It is also advantageous to collect more information by GC-MS or GC-O previously on a similar column to specify interesting retention time ranges and compare the data with those obtained by the first dimensional separation with FID detection. However more often MDGC analyses is a solution for a problem that occurs in 1D-GC. For example the analysis of beer resulted from the information that there are overlapping compounds in a specific retention time part.
The aim was to define standard settings for the two-dimensional GC separation, which can be applied, when co-elution is of an target analyte is detected in one-dimensional GC separation. In this investigation measurements with the standard solution, some elder flower extracts and beer samples, all of them specified in chapter 7, were carried out.

9.1.1. Parameters

The instrument parameters chosen for the separation by MDGC are given in table 9.1. In the first dimensional oven a 5%-phenylmethylsiloxane column is embedded (table 9.1), because a lot of analyses of odour-active compounds use this type of column. So the separation problem can be easily transferred to the first dimension of MDGC analysis. The ^{2}D-column is a polyethylenglycole column to achieve a separation by another selectivity. A FID is applied as monitor detector and its conditions are shown in table 9.2.
The second dimensional detector is a quadrupole MS with EI ionisation. A detector voltage of 1.4 kV was applied (see table 9.3).

9.1.2. MDGC Analysis of the Standard Solution in Scan Mode

The standard solution was analysed by MDGC. Before making a heart-cut a one-dimensional scan was carried out to find out which parts should be transferred onto the second column. The FID signal of the standard solution without cut can be seen in figure 9.1. The sample amount was 10 ng per compound. Later different heart-cuts and

Table 9.1.: Parameters for MDGC separation

	First Dimension	**Second Dimension**
Instrumental Setting and Separation Columns		
Gas Chromatograph	Shimadzu GC2010	Shimadzu GC2010
Column	Optima5-Accent	ZB-WAXplus
Column Length	30 m	30 m
Column Inner Diameter	0.25 mm	0.25 mm
Column Film Thickness	0.25 µm	0.25 µm
Detection	FID	GCMS-QP2010 Plus
GC parameters		
Start Temperature	35 °C	35 °C
Hold time	1 min	1 min
Temperature Ramp	5 °C/min	5 °C/min
End Temperature	280 °C	240 °C
Hold Time	3 min	depending on the heart-cut
Inlet temperature	260.0 °C	–
Injection Mode	splitless	–
Flow Control Mode	Pressure	Linear Velocity
Pressure	342.2 kPa	32.5 kPa
Total Flow	34.5 mL/min	4.1 mL/min
Column Flow	1.97 mL/min	0.79 mL/min
Linear Velocity	16.8 cm/s	32.0 cm/s
Purge Flow	1.97 mL/min	1.0 mL/min
Split Ratio	15.0	–
Carrier Gas	Helium	Helium
Heart-Cut Parameters		
Switching Pressure	290.0 kPa	
Interface Temperature	240.0 °C	

Table 9.2.: FID Conditions

Temperature	280 °C
Air Flow	400 mL/min
Hydrogen Gas Flow	47.0 mL/min
Make Up Gas	Helium
Make Up Gas Flow	1.0 mL/min
Sampling Rate	40 ms

Table 9.3.: MS Conditions

Interface Temperature	220 °C
Ion Source Temperature	220 °C
Electron Energy	70 keV
Scan mode	
Event Time	0.2 s
m/z-Range	46-250 amu
Scan Speed	1111 amu/s
SIM mode	
Event Time	0.2 s

different numbers of heart-cuts were made. The results are discussed in the following paragraphs.

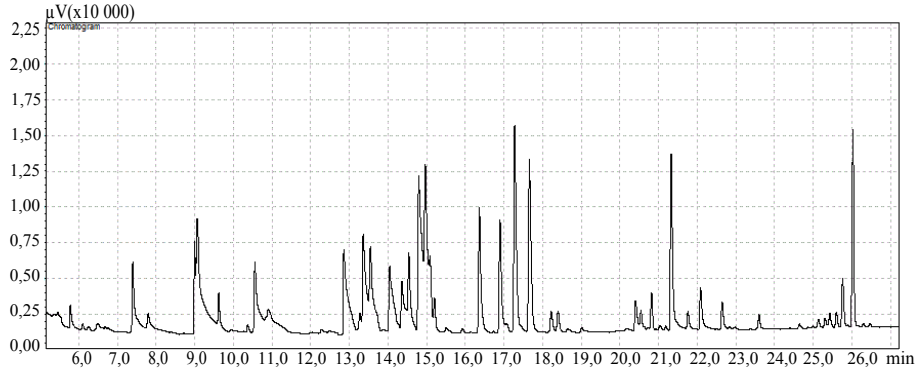

Figure 9.1.: FID signal of the standard solution, without heart-cut. Sample amount was 10 ng per compound.

One-dimensional Scan of Different Concentrations

By scanning standard solutions of different concentrations interesting retentions time ranges could be chosen by overlaying those FID-chromatograms. Peaks varying in height and area are peaks of interest. Those parts were transferred to the second column and analysed mass-selectively.

All cuts and the compounds which are detected in these cuts are shown in figure 9.2. For further discussion the analyses are categorized in one cut in one run and more cuts in one run measurements.

One Heart-Cut in One Run

Interesting retention time parts were cut and carried over to the second dimensional column. The cuts are specified in table 9.4. It was found that it is beneficial for the separation to set the hold time of the second dimension to the retention time were the

Table 9.4.: Heart-Cuts for the Analysis of the Standard Solution in MDGC

First Dimension Cut	Second Dimension Hold Time
7.2 min – 7.7 min	8.5 min
8.9 min – 9.9 min	8.5 min
10.3 min – 11.5 min	8.5 min
12.8 min – 13.85 min	13.5 min
13.9 min – 14.3 min	14 min
14.75 min – 15.35 min	15 min
14.9 min – 15.1 min	15 min
16.8 min – 17.2 min	17 min
16.8 min – 17.6 min	17.5 min
17.2 min – 17.6 min	17.5 min
20.3 min – 20.76 min	20.5 min

heart-cut is set. That means the temperature of 35 °C is hold until the fraction of the first dimensional effluent is transferred to the second dimension.

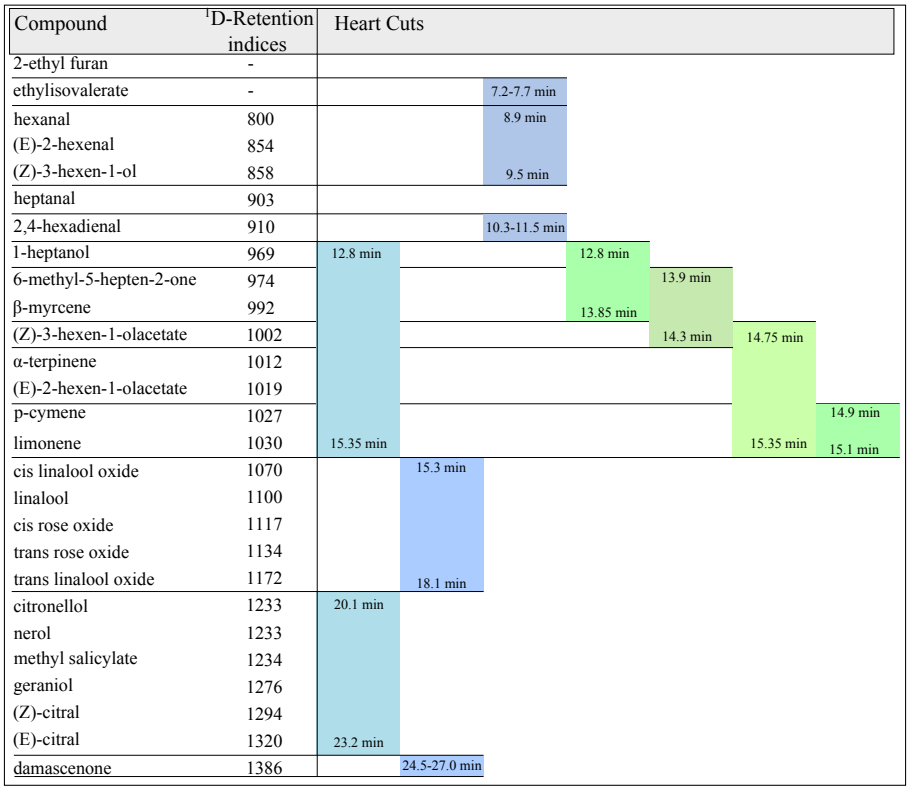

Figure 9.2.: The heart cuts that were made an the compounds which were found are shown, same colour means one run

Table 9.5.: Two and three Heart-Cuts for the Analysis of the Standard Solution in MDGC

First Dimension Cuts	Second Dimension Hold Time
12.8 min to 15.35 min 20.1 min to 23.2 min	17.5 min and 24 min
15.3 min to 18.1 min 24.5 min to 27.0 min	17.5 min
12.8 min to 13.85 min 14.75 min to 15.35 min	15 min
7.2 min to 7.7 min 8.9 min to 9.5 min 10.3 min to 11.5 min	8.5 min

Two and Three Heart-Cuts in One Run

Measurements with more than one cut in one GC run were also made. Different hold times of the second dimension were investigated. It could be seen that it is advantageous for the separation when the heating rate starts at the moment of the first cut especially when the time between the different cuts is more than few minutes. As shown in figure 9.3 two heart-cuts were made and different hold times of the second dimension were investigated. One hold time was between the cuts (17.5 min) and the other investigated hold time was during the second cut (24 min). It was found that the peaks in the chromatogram with a longer hold time are broader and have a lower intensity.
It can be seen that the retention time of the first column is not effected by the previous cuts so that all retention time frames can be chosen after a first run without cut. There is no shift in retention time due to pressure differences between the detector and the second dimensional column. This is the advantage of the multi Deans switch which is described in section 4.3.2.

Investigation of Linearity in Scan Mode

One method with two heart-cuts was measured with different concentrations to investigate the linearity of the response. The heart-cuts were made from 12.5 min to 15.5 min and from 20.1 min to 23.2 min during one run.

Following concentrations of the standard solution were used:

- 0.5 ng
- 1 ng
- 5 ng
- 10 ng

In table 9.6 the compounds transferred to the second column and detected by MS and their elution order in the first and second dimension are listed. Their regression curves

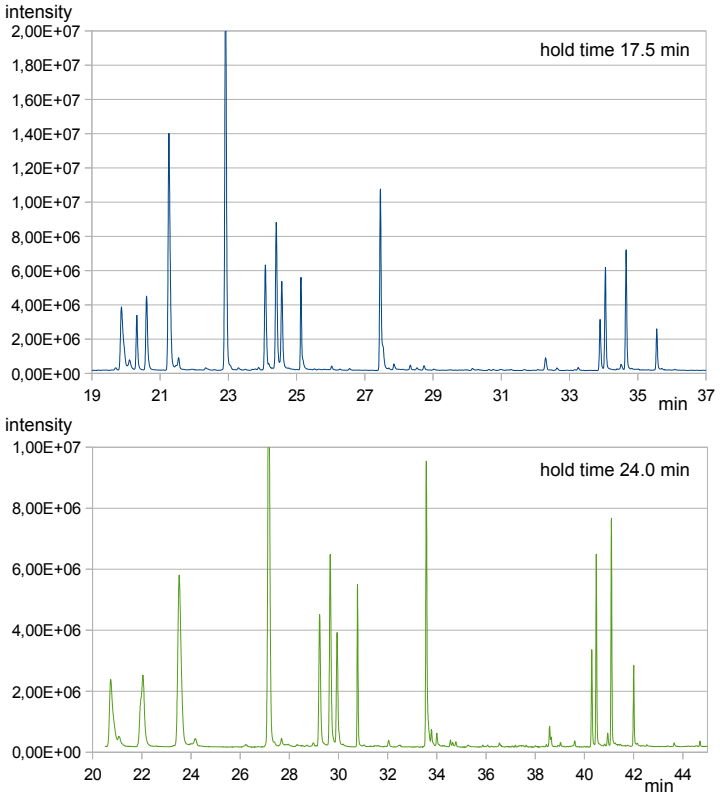

Figure 9.3.: Two chromatograms 2 cuts (12.8 min to 15.35 min and 20.1 min to 23.2 min) and different hold times in the second dimension. Broader peaks and lower intensities are found for a longer hold time.

are shown in table 9.7. Some were not satisfactory because of a relatively large negative intercept on the y-axis. The order of the elution of the compounds were given in this table because of the possibility that the compounds located at the edge of the cut are not transferred properly to the second dimensional column. But a direct relationship could not be found. A closer look is required before carrying out quantitative analyses with this system.
All regression curves are shown in appendix B.

9.1.3. MDGC Analysis of the Standard Solution in SIM Mode

Development of a SIM-Method

The same two-cut-one-run analysis used for the calibration curve in scan mode was chosen to be turned into a SIM-method. The order of the elution of the compounds is the same like in the scan method and is specified in table 9.6.

Reference ions and target ions for the analysed compounds are shown in table 9.8. As already definded for 1D-GC the target ion of a compound is the ion which is integrated

Table 9.6.: Compounds in the 2-cuts-1-run scan (heart-cuts from 12.5 min to 15.5 min and from 20.1 min to 23.2 min) with different concentrations, * compounds at the edge of a cut

Compound	**^{2}D elution order**	**^2t_R**	**^{1}D elution order**
Compounds deriving from the first cut			
β-myrcene	1	20.927 min	3
α-terpinene	2	21.583 min	5
limonene	3	22.287 min	8*
p-cymene	4	24.240 min	7
(Z)-3-hexen-1-olacetat	5	25.683 min	4
6-methyl-5-hepten-2-one	6	26.087 min	2
(E)-2-hexen-1-olacetat	7	26.243 min	6
1-heptanol	8	29.683 min	1*
Compounds deriving from the second cut			
cis-citral	9	35.213 min	13
trans-citral	10	36.427 min	14*
methyl salicylate	11	37.150 min	11
citronellol	12	37.430 min	9*
nerol	13	38.143 min	12
geraniol	14	39.223 min	10

Table 9.7.: Regression curves of the compounds in the second dimension in scan mode.

Compound	**Slope** intensity/ng	**Intercept** intensity
Compounds deriving from the first cut		
β-myrcene	7.54×10^5	-6.17×10^5
α-terpinene	3.25×10^5	-2.11×10^5
limonene*	1.11×10^6	3.16×10^5
p-cymene	3.23×10^6	-2.96×10^5
(Z)-3-hexen-1-olacetat	9.52×10^5	-3.63×10^5
6-methyl-5-hepten-2-one	3.96×10^5	-7.34×10^3
(E)-2-hexen-1-olacetat	3.95×10^5	-1.37×10^5
1-heptanol*	8.32×10^5	-2.82×10^5
Compounds deriving from the second cut		
methyl salicylate	2.11×10^5	-9.74×10^4
citronellol*	2.66×10^5	-0.191×10^4
nerol	5.08×10^5	-6.63×10^5
geraniol	3.68×10^5	-3.50×10^5

Table 9.8.: Target ions and reference ions for the MDGC SIM method

Compound	Target ion	Reference ions
Compounds deriving from the first cut		
β-myrcene	93.00	69.00, 91.00
α-terpinene	93.00	121.00, 136.00
limonene	68.00	67.00, 93.00
p-cymene	119.00	134.00, 91.00
(Z)-3-hexen-1-olacetat	67.00	82.00, 54.00
6-methyl-5-hepten-2-one	108.00	55.00, 69.00
(E)-2-hexen-1-olacetat	67.00	82.00, 100.00
1-heptanol	70.00	56.00, 55.00
Compounds deriving from the second cut		
cis-citral	69.00	55.00, 84.00
trans-citral	69.00	84.00, 94.00
methyl salicylate	120.00	92.00, 152.00
citronellol	69.00	55.00, 67.00
nerol	69.00	93.00, 68.00
geraniol	69.00	68.00, 93.00

for the determination of the peak area and the reference ions are chosen to check the ratio between the ions. The tolerable range <30 %-points more or less the defined ratio.

Investigation of Linearity in SIM Mode

The SIM method was applied to answer the question if the problem of the negative intercept also occurs for SIM. Therefore, different concentrations were analysed. The applied concentrations of the standard solution were

- 1 ng,
- 5 ng, and
- 10 ng.

The resulting calibration curves (see table 9.9) have the same properties like the calibration curves which were measured in scan mode. Most of them have a negative intercept which cannot be explained properly. The first suggestion that the compounds are not transferred properly, because the substances are located at the edges of a cut, can not be confirmed.
The regression curve for p-cymene and for 6-methyl-5-hepten-2-one have a relatively small y-axis intercept while the other compounds show a notable negative intercept.
All regression curves are illustrated in appendix B.

9.1.4. MDGC Analysis of Elder Flower Extracts

Three sorts of elder flower extracts were analysed via MDGC. The heart-cuts that were made are shown in tables 9.10 for the elder sort S3Tx and 9.11 for S10Tx and S6T3.

Table 9.9.: Calibration curves of the compounds after a second dimensional separation, measured in SIM mode. The curve for p-cymene and for 6-methyl-5-hepten-2-one show a relatively small y-axis intercept. The other compounds have a notable negative intercept. Compounds marked with * elute at the edges of the cuts.

Compound	**Slope** intensity/ng	**Intercept** intensity
Compounds deriving from the first cut		
β-myrcene	9.72×10^5	-9.12×10^5
α-terpinene	4.25×10^5	-3.44×10^5
limonene*	1.40×10^6	-5.52×10^5
p-cymene	3.79×10^6	1.04×10^5
(Z)-3-hexen-1-olacetat	1.18×10^6	-5.86×10^5
6-methyl-5-hepten-2-one	4.87×10^5	-1.18×10^4
(E)-2-hexen-1-olacetat	2.00×10^5	-9.81×10^4
1-heptanol*	1.02×10^6	-5.16×10^5
Compounds deriving from the second cut		
methyl salicylate	2.44×10^5	-1.02×10^4
citronellol*	2.94×10^5	-2.60×10^5
nerol	5.52×10^5	-9.09×10^5
geraniol	4.00×10^5	-4.69×10^5

Table 9.10.: One-cut-one-run analysis elder flower syrup, S3Tx

First Dimension Cuts	**Second Dimension Hold Time**
8.9 min to 9.5 min	10 min
10.3 min to 11.5 min	11 min
13.9 min to 14.3 min	14 min
24.5 min to 27.0 min	24 min

The sample amount for all measurements of the extracts are 100 µL. Compounds found in the two heart-cuts are given in tables 9.12 and 9.13.

9.1.5. MDGC Analysis of Beer Samples

Beer samples specified in chapter 7 were analysed in two-dimensions by MDGC. Two different heart-cuts were carried out. One part was in the retention time range of linalool, because in previous study with GC-O it was found out that this range is odour active to a great extent. The other part that was cut out was the retention part of hexanoic acid. Results of both cuts are described in the following sections.

Linalool Problem

On a 5%-phenylmethylsiloxane column linalool is co-eluting with a lot of other compounds which are odour-active and therefore it is relevant. In figure 9.6 the second dimensional signal is shown which is obtained by MS. In this case co-elution also occurs

Table 9.11.: One-cut-one-run analysis elder flower extract, sorts S10Tx and S6T3

First Dimension Cuts	Second Dimension Hold Time
8.9 min to 9.9 min	10 min
14.75 min to 15.35 min	15 min
16.8 min to 17.6 min	17 min
20.3 min to 20.7 min	20.5 min

Table 9.12.: Compounds of different elder flower extracts in a cut, sample S3Tx: 8.9 min to 9.5 min, samples S10Tx and S6T3: 8.9 min to 9.9 min

Compounds	Retention Index 5%-phenylmethylsiloxane	S3Tx	S10Tx	S6T3
(*E*)-2-hexenal	854 [46]	×	×	×
1-hexanol	851 [40]	×	×	×
(*E*)-3-hexenal	802 [48]	×	×	×
(*Z*)-3-hexenal	801 [48]	×	×	×
furfural	830 [46]	×	×	×
2-furan methanol	866 [40]		×	×

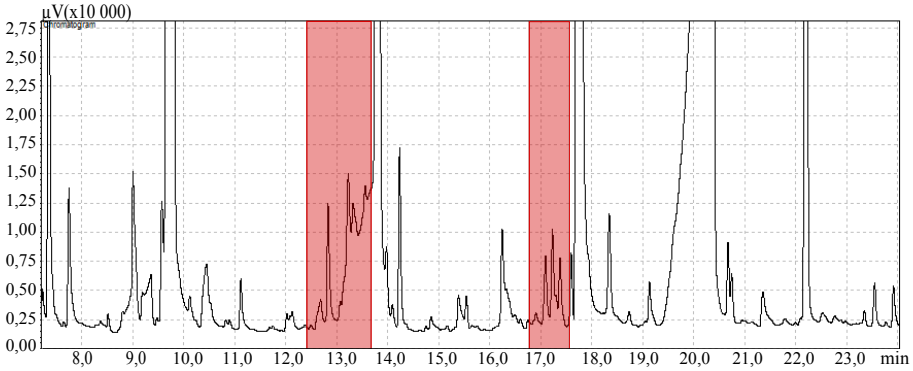

Figure 9.4.: The signal of the monitor detector for beer *A*. The red rectangles represent the heart-cuts which were made. One cut from 12.4 min to 13.7 min and the other from 16.7 min to 17.6 min.

Table 9.13.: Compounds in cut 14.75 min to 15.35 min, samples S10Tx and S6T3

Compounds	Retention Index 5%-phenylmethylsiloxane	S10Tx	S6T3
D-limonene	1031 [46]	×	×
β-phellandrene	1031 [46]	×	×
p-cymene	1026 [46]	×	×
2-ethyl-1-hexanol	1032 [40]	×	×
benzyl alcohol	1039 [40]	×	×

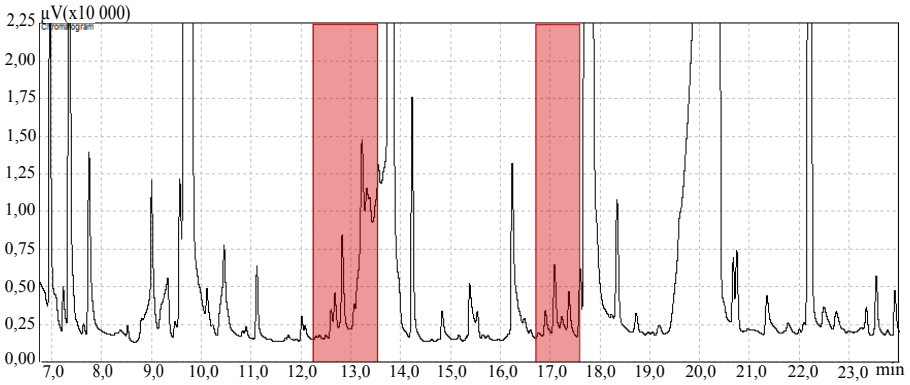

Figure 9.5.: The signal of the monitor detector for beer *B* which does not contain linalool. Not much of a difference can be seen to the FID signal of beer *A* (figure 9.4) which contains linalool.

in the second dimension because of the large window which was cut and transferred. Although the intensity of the signal for beer *B* be was low, an ion extraction by the software has to be executed before the lack of linalool in beer *B* could definitively be approved. The peaks for the ions 71, 93, 126, are shown in figure 9.6. The ions 71 and 93 were extracted to represent linalool. These ions are the fragments of linalool with the highest intensities as it can be seen in figure 9.7 where the mass spectrum of linalool is displayed.

Hexanoic Acid Problem

The shape of the peaks evoked by carboxylic acids on a 5%-phenylmethylsiloxane column have a triangular shape. In samples with high amount of carboxylic acids they can co-elute with other compounds. Therefore a second dimensional separation is advantageous. In the beer samples a high concentration of hexanoic acid was found and it was obvious that there are compounds eluting at the same retention time (figure 9.4). That is why a cut was made to transfer this retention part to the second dimension. On a polyethylenglycol column the acid elute in an almost bell-shaped curve and so it can be separated from the other compounds as it can be seen in figure 9.8.

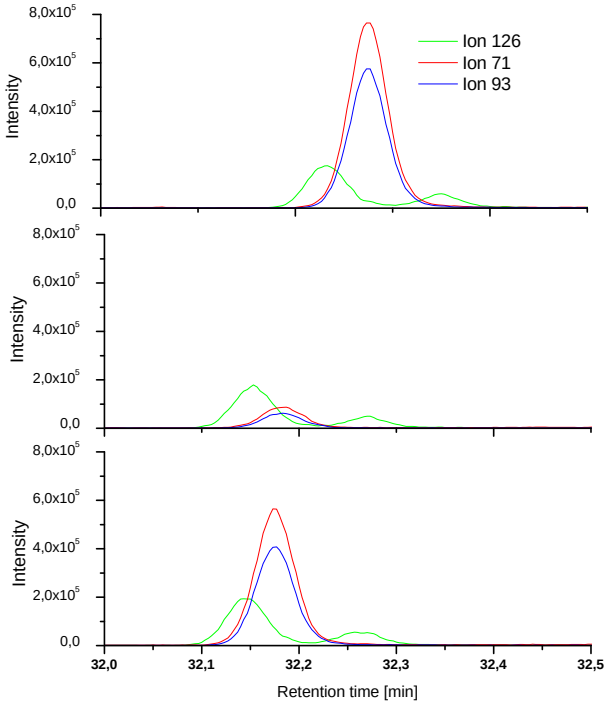

Figure 9.6.: Extracted ions for linalool with another compound after the second dimensional separation. Ions 71 and 93 derive from linalool while ion 126 derive from another compound. Ion 71 has a higher intensity than ion 93 which can also be seen in the mass spectrum of linalool in figure 9.7

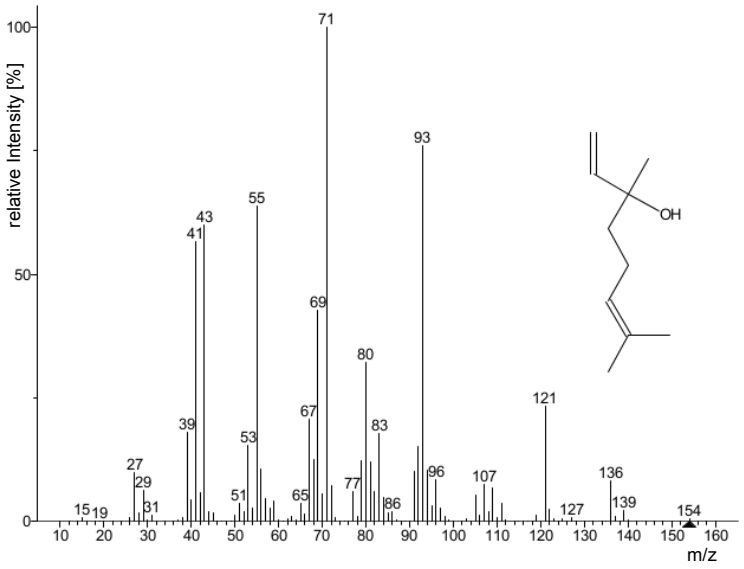

Figure 9.7.: In the EI mass spectrum of linalool (data base: NIST) it can be seen that the ions 71 and 93 are the fragments with the highest intensities for linalool. That is why these ions were chosen for the ion extraction to identify linalool in the heart-cut of the beer sample.

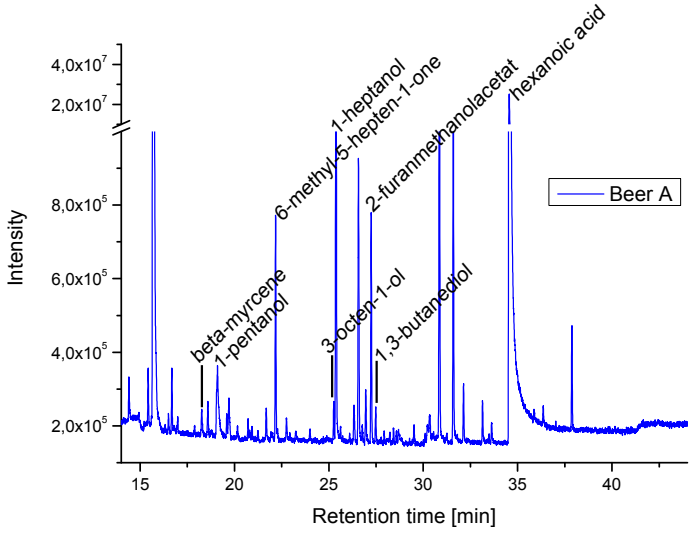

Figure 9.8.: Second dimensional separation of the retention time range of hexanoic acid to show how much compounds can hide behind a carboxylic acid that elutes like a triangle on a 5%-phenylmethylsiloxane column.

10. Results

10.1. 1D-GC Results

In the investigations carried out by one-dimensional GC/MS differences of the ion sources were specified. By analysing S/N-values and slopes of regression curves it was found that the pure electron impact ion source is more sensitive than the combi source. The factor varies depending on the compound from two to 20.
Parameter for positive chemical ionisation with the combi ion source were defined. With a temperature of 150 °C a relatively low ion source temperature has to be chosen for a proper chemical ionisation as well as a high ionisation gas pressure of 350 kPa.
Finally, differences of the odour of elder flowers of different sorts were analysed quantitatively regarding some odour-relevant compounds. These analyses were carried out by an external calibration. It was discovered, that the amount of odour-active compounds differs for in dependence of the type of elder sorts. The results obtained with different ionisation methods are comparable and show no significant differences. Slight fluctuation can be explained by degradation reactions during storage and frosting–defrosting processes.

10.2. MDGC Results

A method is established where measurement can be carried out by MDGC in order to solve separation problems occurring due to co-elution of two or more compounds on a 5%-phenylmethylsiloxane column. Important for the second dimensional separation is the hold time of the ^{2}D-oven temperature. It has to be defined according to the heart-cut. Linearity of the response was investigated in scan and in SIM mode. A negative intercept of some regression curves was obtained. For further measurements, especially for quantifications, this effect has to be investigated more closer.
In cooperation with another study it was discovered by means of MDGC that beer without hop has a negligible amount of linalool. That means that linalool in beer, which has a floral and lemony odour, derives from the hop.

11. List of Figures

12. List of Tables

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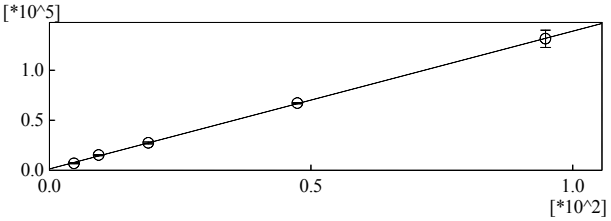
A. Appendix: Calibration Curves, 1D

A.1. SEI

ID#:1 Mass:96.00 Name:2-Ethylfuran
f(x)=1372.465907*x+1383.651701
rr1=0.999897 rr2=0.999794



#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
2	4.740	6932.00	577.84	8.34
3	9.480	15262.67	556.64	3.65
4	18.960	27192.67	937.63	3.45
5	47.400	67035.33	706.01	1.05
6	94.800	131198.67	8712.03	6.64

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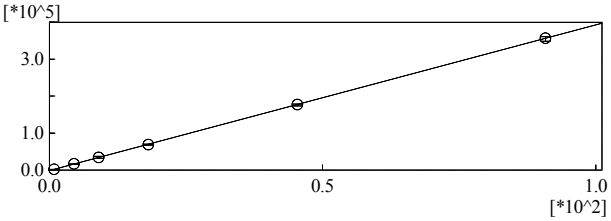
f(x)=3934.363464*x-1600.021175
rr1=0.999992 rr2=0.999984



#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.908	2283.00	100.41	4.40
2	4.540	16574.00	401.98	2.43
3	9.080	34503.00	2812.92	8.15
4	18.160	68955.33	1363.20	1.98
5	45.400	176558.33	2605.55	1.48
6	90.800	355993.00	6747.28	1.90

ID#:3 Mass:57.00 Name:Hexanal
f(x)=3463.788064*x-6707.833696
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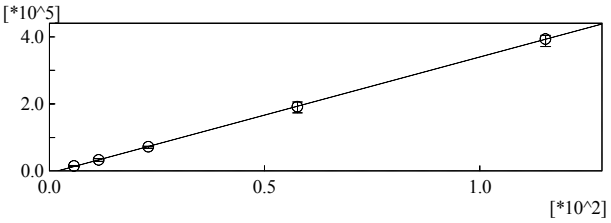

#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
2	5.760	15231.33	1368.81	8.99
3	11.520	32971.67	3794.43	11.51
4	23.040	71682.00	2686.42	3.75
5	57.600	191697.00	16704.17	8.71
6	115.200	393081.33	19036.86	4.84

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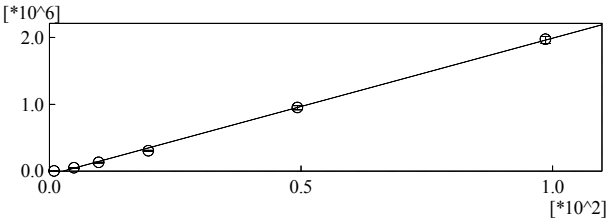
rr1=0.999378 rr2=0.998755



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1	0.986	3784.00	--	--
2	4.930	50060.00	4019.57	8.03
3	9.860	131250.33	6998.24	5.33
4	19.720	305530.67	6578.29	2.15
5	49.300	952210.67	29638.59	3.11
6	98.600	1973053.33	55211.57	2.80

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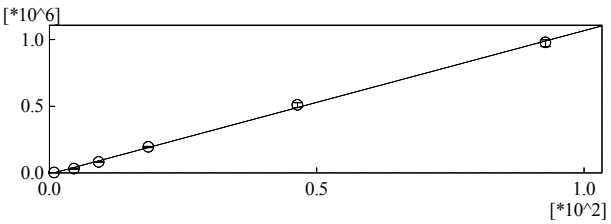
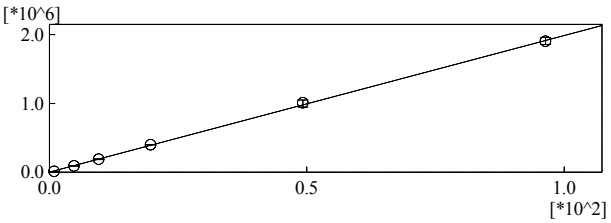
rr1=0.999495 rr2=0.998991



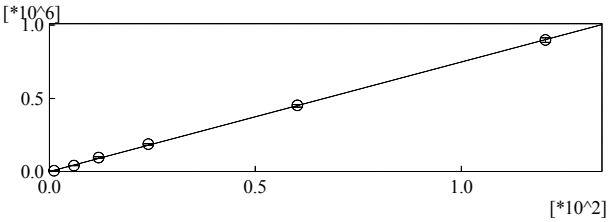
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.928	2910.50	516.90	17.76
2	4.640	32129.33	5394.19	16.79
3	9.280	83067.00	2209.92	2.66
4	18.560	194698.67	5626.53	2.89
5	46.400	511628.00	17809.47	3.48
6	92.800	978745.67	26942.65	2.75

ID#:6 Mass:93.00 Name:Limonene
f(x)=19865.014638*x+2335.158002
rr1=0.999757 rr2=0.999513



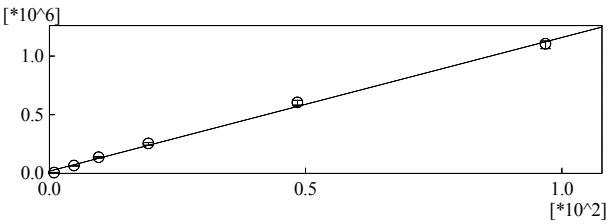
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.964	10942.00	3294.15	30.11
2	4.820	92258.33	1192.44	1.29
3	9.640	190138.33	7039.23	3.70
4	19.720	400285.33	4177.27	1.04
5	49.300	1011065.33	55440.58	5.48
6	96.400	1901790.33	50862.66	2.67

ID#:7 Mass:55.00 Name:(E)-2-Hexenal
f(x)=7464.035182*x+954.348191
rr1=0.999919 rr2=0.999838



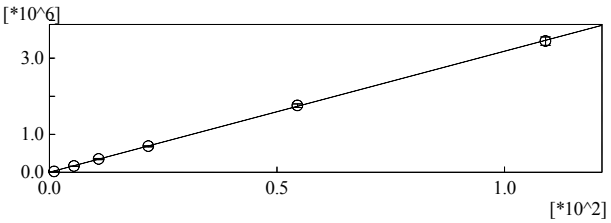
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.204	5518.00	292.74	5.31
2	6.020	41164.33	1834.13	4.46
3	12.040	95592.67	4790.70	5.01
4	24.080	185833.00	4834.21	2.60
5	60.200	451201.67	9551.22	2.12
6	120.400	897942.33	14520.22	1.62

ID#:8 Mass:77.00 Name:p-Cymene
f(x)=11369.093044*x+21138.967471
rr1=0.998648 rr2=0.997299



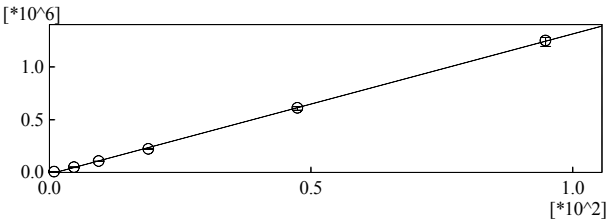
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.968	7803.33	2230.51	28.58
2	4.840	65805.33	434.65	0.66
3	9.680	138294.00	6397.23	4.63
4	19.360	255226.00	11311.54	4.43
5	48.400	604128.00	21502.35	3.56
6	96.800	1102559.67	35612.48	3.23

ID#:9 Mass:67.00 Name:3-Hexen-1-ol acetat (Z)
f(x)=31838.010508*x-4739.784826
rr1=0.999934 rr2=0.999868



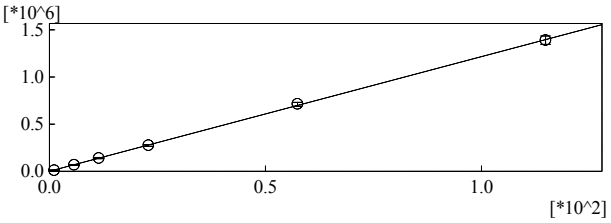
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.090	22952.50	730.44	3.18
2	5.450	160501.67	1941.71	1.21
3	10.900	346248.00	15288.37	4.42
4	21.800	685433.67	12856.84	1.88
5	54.500	1759153.67	46724.67	2.66
6	109.000	3452110.00	109700.09	3.18

ID#:10 Mass:67.00 Name:2-Hexen-1-ol, acetate (E)
f(x)=13301.562300*x-16488.986173
rr1=0.999841 rr2=0.999682



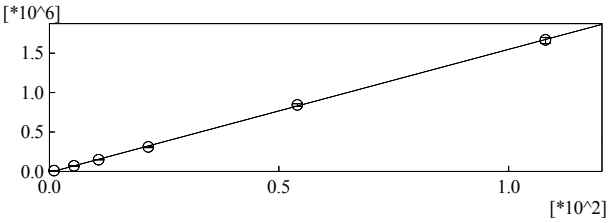
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.948	7972.33	1054.47	13.23
2	4.740	49429.67	3010.79	6.09
3	9.480	107511.67	2774.04	2.58
4	18.960	222176.33	4001.95	1.80
5	47.400	610516.00	16661.82	2.73
6	94.800	1248898.00	47226.28	3.78

ID#:11 Mass:108.00 Name:6-methyl-5-Hepten-2-on
f(x)=12165.721490*x+1587.019786
rr1=0.999888 rr2=0.999775



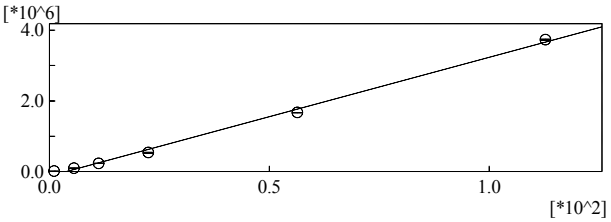
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.148	12838.33	1488.37	11.59
2	5.740	69904.33	2410.03	3.45
3	11.480	140339.67	7482.01	5.33
4	22.960	277275.00	8707.13	3.14
5	57.400	715608.33	22357.42	3.12
6	114.800	1391278.67	42980.41	3.09

ID#:12 Mass:67.00 Name:3-Hexen-1-ol (Z)
f(x)=15611.315705*x-13644.629437
rr1=0.999889 rr2=0.999777



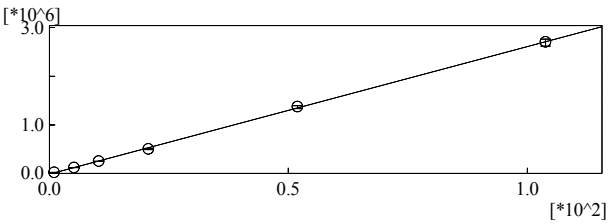
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.080	11747.33	166.32	1.42
2	5.400	71180.67	2447.51	3.44
3	10.800	150168.00	2570.74	1.71
4	21.600	309780.33	5593.59	1.81
5	54.000	842146.00	16441.15	1.95
6	108.000	1669111.00	39641.48	2.38

ID#:13 Mass:81.00 Name:2,4-Hexadienal
f(x)=33634.723836*x-130291.765869
rr1=0.998360 rr2=0.996723



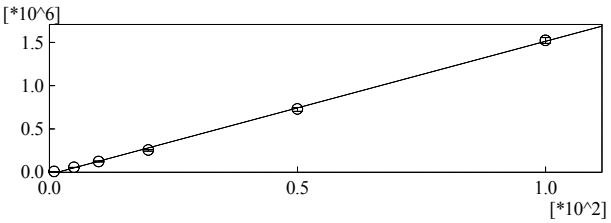
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.128	10673.33	883.93	8.28
2	5.640	95237.33	4441.09	4.66
3	11.280	233497.67	5333.38	2.28
4	22.560	536954.33	13736.38	2.56
5	56.400	1669225.00	2958.88	0.18
6	112.800	3729496.00	19651.13	0.53

ID#:14 Mass:70.00 Name:Heptanol
f(x)=26290.208558*x-15979.130523
rr1=0.999872 rr2=0.999745



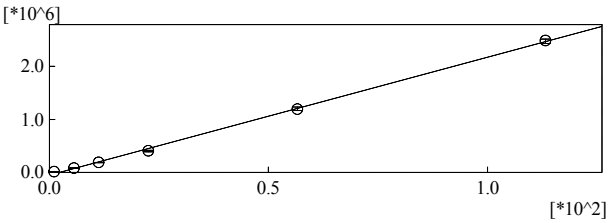
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.038	21056.33	1118.08	5.31
2	5.190	122128.67	838.40	0.69
3	10.380	252331.33	2961.05	1.17
4	20.760	505430.33	13439.50	2.66
5	51.900	1373276.00	33995.34	2.48
6	103.800	2705700.67	76348.75	2.82

ID#:15 Mass:69.00 Name:Linalool
f(x)=15373.535904*x-26048.279706
rr1=0.999613 rr2=0.999227



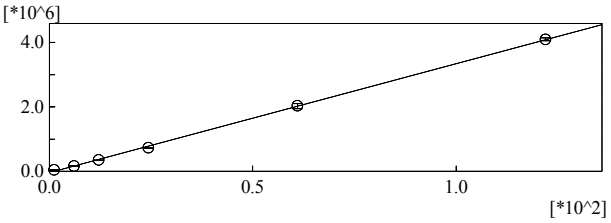
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.000	9838.67	620.63	6.31
2	5.000	58227.33	378.88	0.65
3	10.000	125624.67	4820.90	3.84
4	20.000	257133.00	9359.13	3.64
5	50.000	730512.33	22428.35	3.07
6	100.000	1521852.00	32974.56	2.17

ID#:16 Mass:69.00 Name:Citronellol
f(x)=22281.835088*x-52677.135813
rr1=0.999536 rr2=0.999072



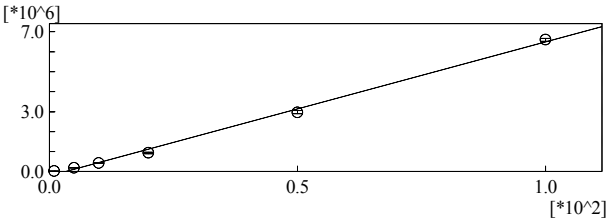
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.132	14601.33	2198.29	15.06
2	5.660	84105.33	2012.52	2.39
3	11.320	188066.67	4472.23	2.38
4	22.640	409993.33	15255.71	3.72
5	56.600	1192477.33	36010.80	3.02
6	113.200	2486178.00	47899.27	1.93

ID#:17 Mass:92.00 Name:Methyl salicylate
f(x)=33866.414859*x-44852.250800
rr1=0.999766 rr2=0.999532



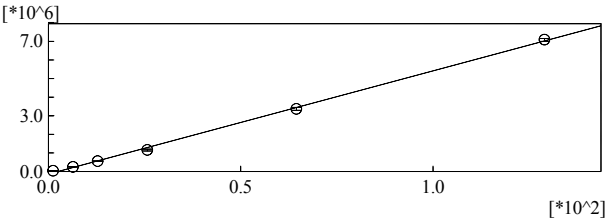
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.220	44065.00	11120.44	25.24
2	6.100	168688.33	6649.88	3.94
3	12.200	349903.00	6092.26	1.74
4	24.400	727096.33	6320.90	0.87
5	61.000	2033709.33	83270.14	4.09
6	122.000	4092391.33	47794.03	1.17

ID#:18 Mass:69.00 Name:Nerol
f(x)=67140.309366*x-226861.534803
rr1=0.998271 rr2=0.996545



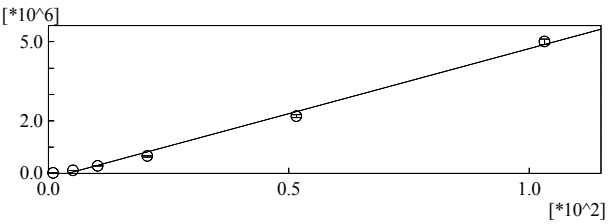
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.000	26119.33	3283.83	12.57
2	5.000	179745.67	2278.64	1.27
3	10.000	418407.33	16303.10	3.90
4	20.000	932697.67	34944.15	3.75
5	50.000	2967705.67	84903.85	2.86
6	100.000	6602252.67	80410.07	1.22

ID#:19 Mass:69.00 Name:Damascenone
f(x)=55492.359339*x-141997.715028
rr1=0.999477 rr2=0.998955



#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.290	39962.00	4224.25	10.57
2	6.450	256753.67	3970.34	1.55
3	12.900	561121.67	10824.24	1.93
4	25.800	1169685.33	58519.62	5.00
5	64.500	3356098.33	82643.71	2.46
6	129.000	7079229.33	76176.41	1.08

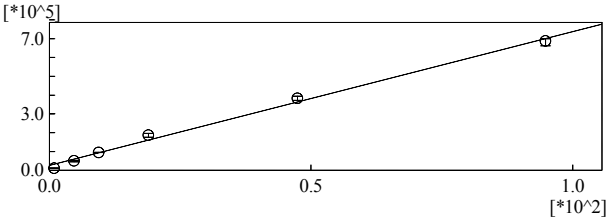
ID#:20 Mass:69.00 Name:Geraniol
f(x)=49328.967826*x-203031.625165
rr1=0.997308 rr2=0.994623



#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	1.032	15367.00	2074.65	13.50
2	5.160	118375.67	1203.39	1.02
3	10.320	287397.00	6820.76	2.37
4	20.640	659851.33	25293.35	3.83
5	51.600	2165648.33	60329.07	2.79
6	103.200	5003964.67	104109.37	2.08

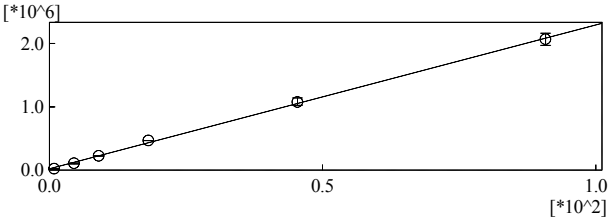
A.2. SCI

ID#:1 Mass:97.00 Name:2-Ethylfuran
f(x)=7123.547967*x+25936.502137
rr1=0.997412 rr2=0.994831



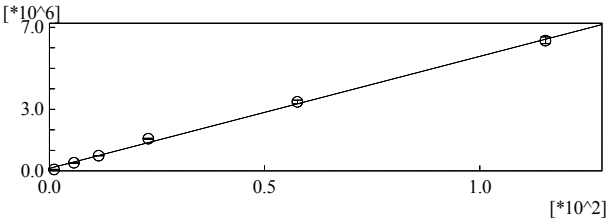
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.948	11486.67	668.33	5.82
4	4.740	49465.67	4238.68	8.57
5	9.480	95018.33	816.88	0.86
6	18.960	185919.00	10613.12	5.71
7	47.400	382360.67	11695.14	3.06
8	94.800	687449.67	21269.40	3.09

ID#:2 Mass:131.00 Name:Ethylisovalerate
f(x)=22668.295861*x+22056.514080
rr1=0.999541 rr2=0.999083



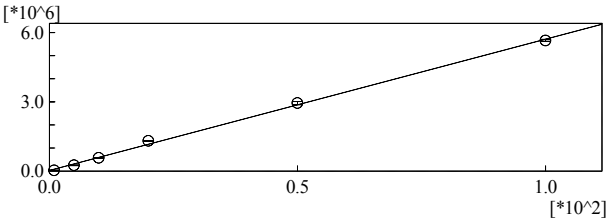
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.908	21502.67	2604.57	12.11
4	4.540	108005.67	7553.06	6.99
5	9.080	224834.67	1777.97	0.79
6	18.160	468904.67	1087.77	0.23
7	45.400	1074464.00	59263.57	5.52
8	90.800	2063030.67	94560.36	4.58

ID#:3 Mass:83.00 Name:Hexanal
f(x)=54598.475117*x+135754.561933
rr1=0.998898 rr2=0.997797



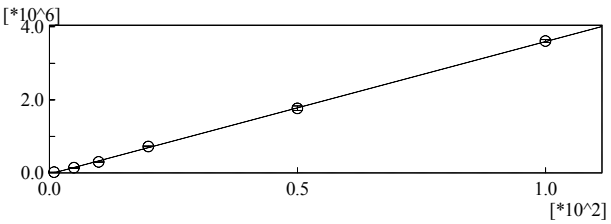
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.152	73815.67	14495.96	19.64
4	5.760	398844.67	18085.48	4.53
5	11.520	749169.67	13923.63	1.86
6	23.040	1572002.00	23846.95	1.52
7	57.600	3368186.33	88932.09	2.64
8	115.200	6351433.33	167258.42	2.63

ID#:4 Mass:93.00 Name:beta Myrcene
f(x)=56674.870523*x+38448.513774
rr1=0.999270 rr2=0.998540



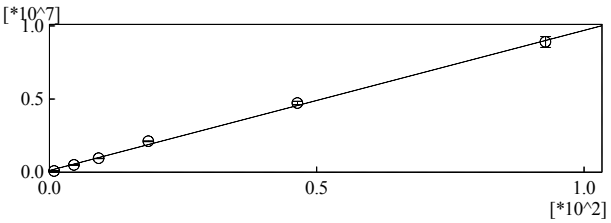
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.000	40563.67	5854.68	14.43
4	5.000	260888.00	16713.69	6.41
5	10.000	570468.67	14972.02	2.62
6	20.000	1307789.00	14661.46	1.12
7	50.000	2941159.00	71016.99	2.41
8	100.000	5651348.67	44603.04	0.79

ID#:5 Mass:121.00 Name:alpha Terpinene
f(x)=36288.812948*x-31218.868044
rr1=0.999861 rr2=0.999723



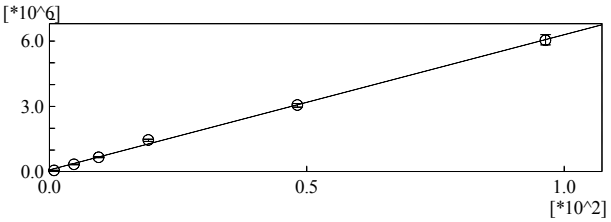
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.000	21034.67	2752.68	13.09
4	5.000	145082.67	15002.99	10.34
5	10.000	302335.00	1160.16	0.38
6	20.000	726791.00	10007.51	1.38
7	50.000	1762610.33	63208.46	3.59
8	100.000	3604552.33	40312.02	1.12

ID#:6 Mass:97.00 Name:Heptanal
f(x)=95920.778561*x+116235.984549
rr1=0.999075 rr2=0.998151



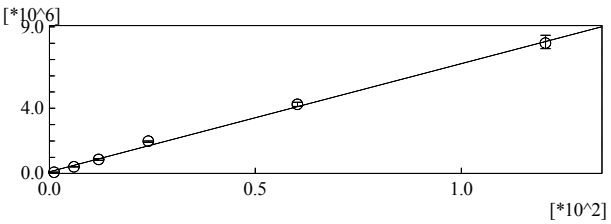
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.928	76382.67	12385.64	16.22
4	4.640	489325.00	22758.78	4.65
5	9.280	949233.33	13970.51	1.47
6	18.560	2106952.67	31774.68	1.51
7	46.400	4725069.00	112856.84	2.39
8	92.800	8907147.33	370291.76	4.16

ID#:7 Mass:81.00 Name:Limonene
f(x)=61958.722620*x+78673.552875
rr1=0.999195 rr2=0.998391



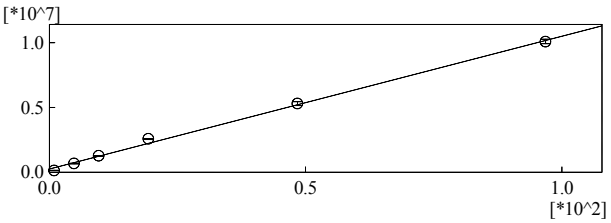
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.964	58305.67	6451.71	11.07
4	4.820	330108.67	13252.50	4.01
5	9.640	659773.67	22125.05	3.35
6	19.280	1452251.00	58541.85	4.03
7	48.200	3056072.00	84911.31	2.78
8	96.400	6024977.33	238680.74	3.96

ID#:8 Mass:99.00 Name:(E)-2-Hexanal
f(x)=66312.797353*x+114468.405581
rr1=0.998650 rr2=0.997301



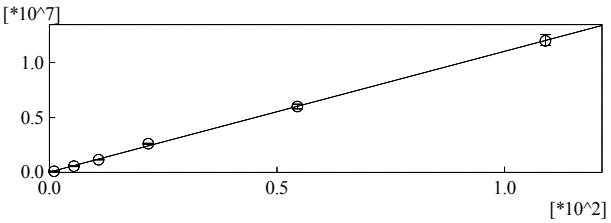
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.204	75497.67	5262.24	6.97
4	6.020	408457.00	8636.60	2.11
5	12.040	850092.33	48636.22	5.72
6	24.080	1973993.67	43305.67	2.19
7	60.200	4234319.67	135262.45	3.19
8	120.400	7994803.33	443379.65	5.55

ID#:9 Mass:119.00 Name:p-Cymene
f(x)=102169.908457*x+270852.179875
rr1=0.998730 rr2=0.997462



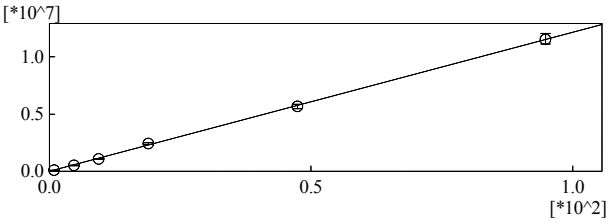
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.968	140478.00	14600.26	10.39
4	4.840	679535.67	29978.72	4.41
5	9.680	1273232.33	25178.62	1.98
6	19.360	2575017.00	34782.29	1.35
7	48.400	5286971.33	154734.78	2.93
8	96.800	10065367.00	173794.13	1.73

ID#:10 Mass:83.00 Name:3-Hexen-1-ol acetate (Z)
f(x)=109624.140277*x+29249.823811
rr1=0.999809 rr2=0.999618



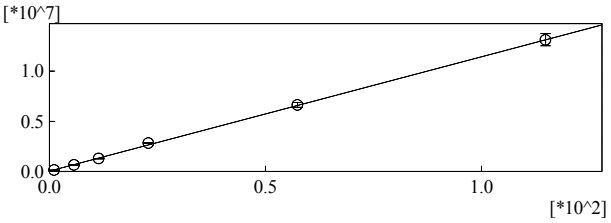
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.090	115947.67	3382.61	2.92
4	5.450	580893.33	37168.18	6.40
5	10.900	1163365.67	23029.93	1.98
6	21.800	2597713.00	65854.29	2.54
7	54.500	5979295.67	216137.02	3.61
8	109.000	11963481.67	503956.88	4.21

ID#:11 Mass:83.00 Name:2-Hexen-1-ol acetat (E)
f(x)=121369.349230*x-23389.217847
rr1=0.999823 rr2=0.999645



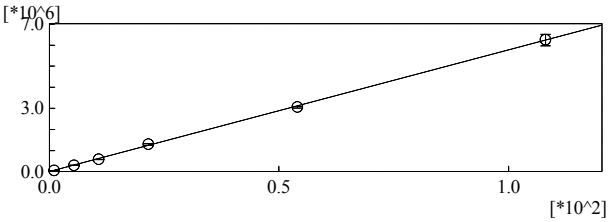
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.948	100224.00	1257.61	1.25
4	4.740	513596.67	34540.52	6.73
5	9.480	1064602.00	28361.19	2.66
6	18.960	2429336.33	68000.65	2.80
7	47.400	5655520.00	190672.47	3.37
8	94.800	11497200.67	478108.61	4.16

ID#:12 Mass:109.00 Name:6-Methyl-5-Hepten-2-on
f(x)=113859.680427*x+51811.636470
rr1=0.999836 rr2=0.999671



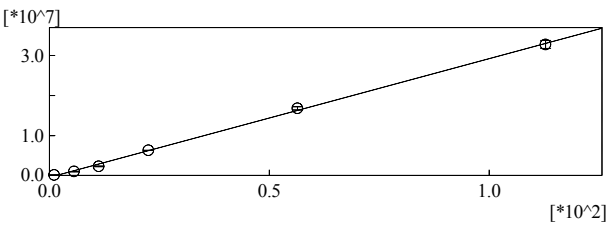
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.148	150228.00	8343.90	5.55
4	5.740	651322.33	31480.70	4.83
5	11.480	1282956.00	25268.82	1.97
6	22.960	2834103.00	54133.66	1.91
7	57.400	6621803.33	229121.42	3.46
8	114.800	13082687.33	610047.48	4.66

ID#:13 Mass:83.00 Name:3-Hexen-1-ol
f(x)=57887.749215*x-14424.072788
rr1=0.999865 rr2=0.999730



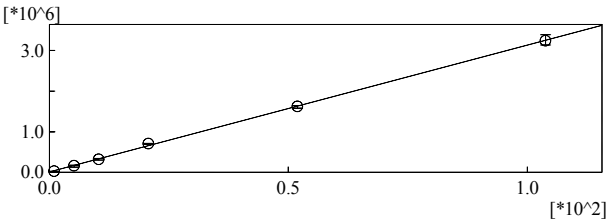
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.080	49225.00	2899.71	5.89
4	5.400	298236.33	10424.56	3.50
5	10.800	577719.67	14684.10	2.54
6	21.600	1300823.67	40111.92	3.08
7	54.000	3064833.33	48364.77	1.58
8	108.000	6251108.67	275499.06	4.41

ID#:14 Mass:97.00 Name:2,4-Hexadienal
f(x)=296698.427322*x-502239.787790
rr1=0.999469 rr2=0.998938



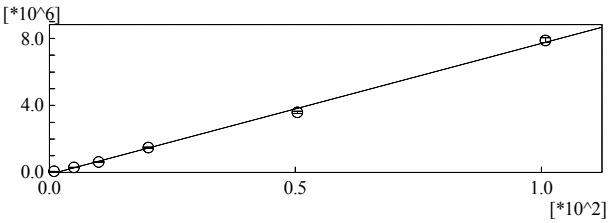
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.128	151179.67	7978.06	5.28
4	5.640	981205.00	35642.48	3.63
5	11.280	2282335.00	73466.85	3.22
6	22.560	6290767.33	27813.04	0.44
7	56.400	16814059.67	397104.88	2.36
8	112.800	32716719.33	1007735.95	3.08

ID#:15 Mass:97.00 Name:Heptanol
f(x)=31179.203319*x+9318.958695
rr1=0.999829 rr2=0.999658



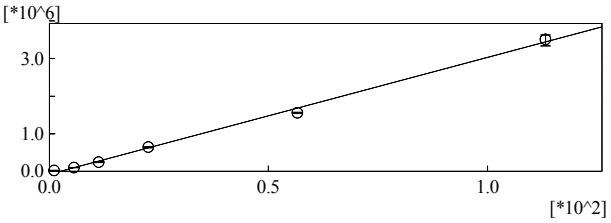
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.038	25337.33	2059.63	8.13
4	5.190	161992.33	25413.87	15.69
5	10.380	323176.33	20780.07	6.43
6	20.760	702434.67	11606.41	1.65
7	51.900	1621535.00	32914.40	2.03
8	103.800	3241144.67	131667.10	4.06

ID#:16 Mass:81.00 Name:Linalool
f(x)=78123.228129*x-119567.633358
rr1=0.999127 rr2=0.998256



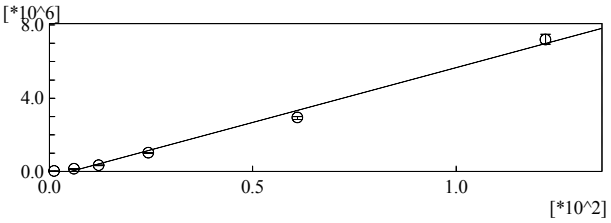
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.008	59518.00	1480.24	2.49
4	5.040	304784.00	12619.73	4.14
5	10.080	622277.67	19571.65	3.15
6	20.160	1489289.33	34985.53	2.35
7	50.400	3588933.00	71662.28	2.00
8	100.800	7864960.33	150719.01	1.92

ID#:17 Mass:83.00 Name:Citronellol
f(x)=31122.753097*x-80677.511111
rr1=0.998540 rr2=0.997082



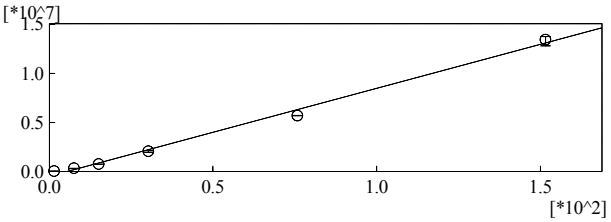
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.132	25103.33	2401.27	9.57
4	5.660	101248.00	5980.60	5.91
5	11.320	243858.67	12788.29	5.24
6	22.640	641067.33	18709.45	2.92
7	56.600	1552808.00	18900.18	1.22
8	113.200	3504807.33	154674.31	4.41

ID#:18 Mass:121.00 Name:Methyl salicylate
f(x)=59787.866722*x-317330.251937
rr1=0.996157 rr2=0.992329



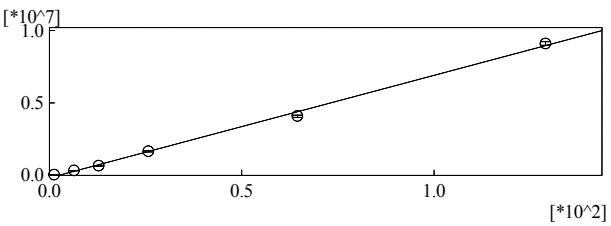
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.220	24551.50	5943.23	24.21
4	6.100	142970.33	8394.92	5.87
5	12.200	337552.00	20207.57	5.99
6	24.400	1020817.67	21525.28	2.11
7	61.000	2942832.67	94379.19	3.21
8	122.000	7194357.00	277050.95	3.85

ID#:19 Mass:81.00 Name:Nerol
f(x)=89634.975509*x-486737.937974
rr1=0.997439 rr2=0.994884



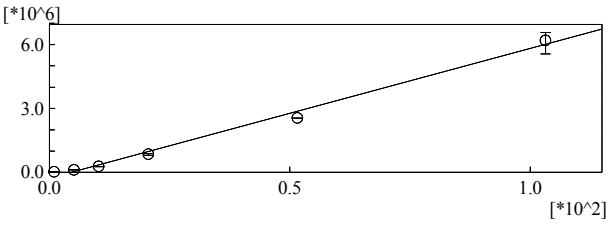
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.515	50497.67	2842.80	5.63
4	7.575	329879.67	5533.93	1.68
5	15.150	750408.33	22302.80	2.97
6	30.300	2080833.00	84092.60	4.04
7	75.750	5701633.33	17265.88	0.30
8	151.500	13424560.00	523092.43	3.90

ID#:20 Mass:191.00 Name:Damascenone
f(x)=70630.704658*x-173177.974214
rr1=0.998857 rr2=0.997715



#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.290	59116.33	1709.37	2.89
4	6.450	333836.67	16739.36	5.01
5	12.900	670305.67	15939.64	2.38
6	25.800	1675574.67	43914.33	2.62
7	64.500	4084621.67	79027.95	1.93
8	129.000	9084608.33	121182.46	1.33

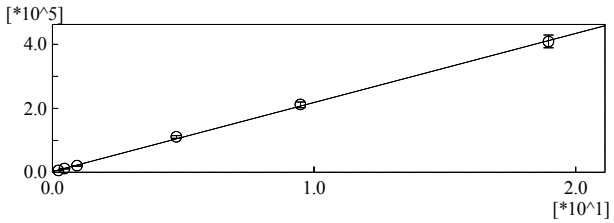
ID#:21 Mass:81.00 Name:Geraniol
f(x)=60951.449678*x-283753.442431
rr1=0.996224 rr2=0.992463



#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.032	20767.00	2941.40	14.16
4	5.160	115370.00	7853.33	6.81
5	10.320	272633.00	8503.31	3.12
6	20.640	853651.00	40388.42	4.73
7	51.600	2545922.00	7336.23	0.29
8	103.200	6188888.67	544994.32	8.81

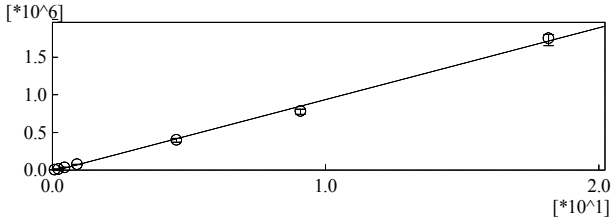
A.3. EI

ID#:1 Mass:96.00 Name:2-Ethylfuran
f(x)=21574.130597*x+2770.313582
rr1=0.999659 rr2=0.999319



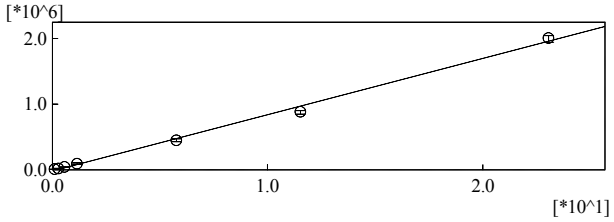
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
2	0.237	5210.00	--	--
3	0.474	11598.33	1236.99	10.67
4	0.948	20341.25	487.83	2.40
5	4.740	111042.00	4359.48	3.93
6	9.480	211758.40	6602.07	3.12
7	18.960	408293.00	19764.51	4.84

ID#:2 Mass:88.00 Name:Ethyl isovalerate
f(x)=95417.985844*x-17698.478030
rr1=0.998736 rr2=0.997473



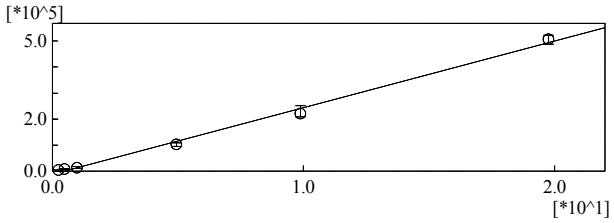
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.091	6002.50	798.32	13.30
2	0.227	14584.67	788.10	5.40
3	0.454	36435.33	2345.28	6.44
4	0.908	77100.25	2943.15	3.82
5	4.540	400985.00	26765.04	6.67
6	9.080	782973.60	27895.23	3.56
7	18.160	1750696.00	84358.13	4.82

ID#:3 Mass:57.00 Name:Hexanal
f(x)=85681.693311*x-17471.054111
rr1=0.998224 rr2=0.996452



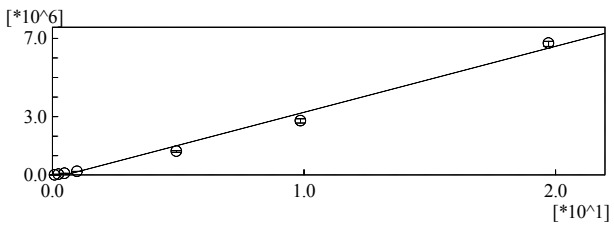
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.115	11959.33	1936.48	16.19
2	0.288	22843.67	2097.31	9.18
3	0.576	45744.33	1714.16	3.75
4	1.152	96250.25	10164.53	10.56
5	5.760	450115.00	22094.32	4.91
6	11.520	883120.20	24750.59	2.80
7	23.040	2004960.67	53249.46	2.66

ID#:4 Mass:55.00 Name:beta myrcene
f(x)=25616.367285*x-12492.139821
rr1=0.997750 rr2=0.995505



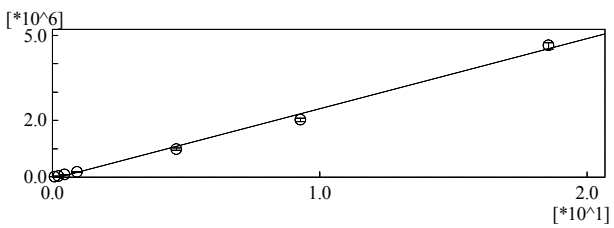
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
2	0.247	4697.00	--	--
3	0.494	7881.50	1635.54	20.75
4	0.988	13390.75	3182.30	23.76
5	4.940	102271.33	6624.89	6.48
6	9.880	220674.60	18010.64	8.16
7	19.760	506236.67	17202.46	3.40

ID#:5 Mass:121.00 Name:alpha terpinene
f(x)=338505.941294*x-173141.764150
rr1=0.995365 rr2=0.990752



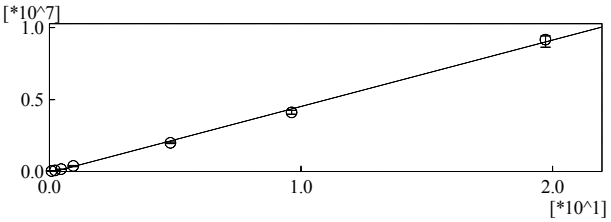
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.099	16184.00	63.98	0.40
2	0.247	37928.67	3002.14	7.92
3	0.493	91827.33	1005.48	1.09
4	0.986	188826.50	2380.89	1.26
5	4.930	1215618.33	41934.37	3.45
6	9.860	2777382.80	82772.90	2.98
7	19.720	6759548.33	145794.39	2.16

ID#:6 Mass:70.00 Name:Heptanal
f(x)=247106.369440*x-64333.395480
rr1=0.997840 rr2=0.995684



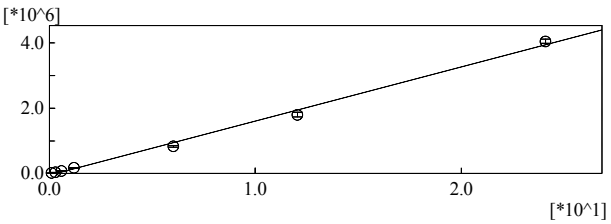
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.093	22119.67	3768.39	17.04
2	0.232	42955.00	1250.25	2.91
3	0.464	91345.33	7481.94	8.19
4	0.928	186662.75	6612.54	3.54
5	4.640	989931.33	39937.15	4.03
6	9.280	2021012.00	50553.20	2.50
7	18.560	4645887.00	115520.09	2.49

ID#:7 Mass:93.00 Name:Limonene
f(x)=461430.022898*x-94710.930320
rr1=0.999122 rr2=0.998245



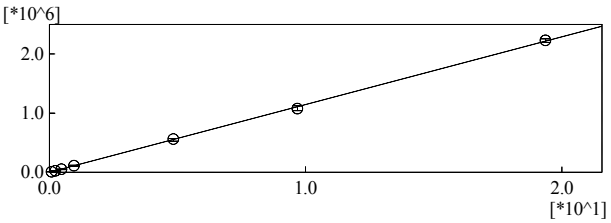
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.096	42322.33	5443.17	12.86
2	0.241	81189.67	6668.24	8.21
3	0.482	166072.00	6053.52	3.65
4	0.964	387019.75	41515.13	10.73
5	4.820	1998802.33	52500.70	2.63
6	9.640	4099374.80	108350.60	2.64
7	19.720	9156835.00	449039.58	4.90

ID#:8 Mass:55.00 Name:(E)-2-Hexenal
f(x)=166211.450614*x-58153.736681
rr1=0.998099 rr2=0.996201



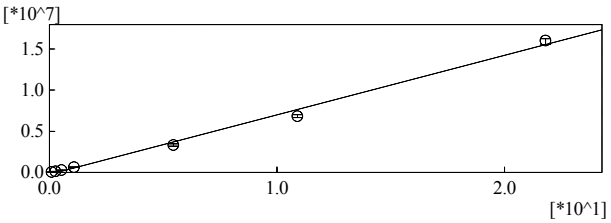
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.120	17022.33	380.26	2.23
2	0.301	32096.00	--	--
3	0.602	75774.00	6316.89	8.34
4	1.204	171757.00	7691.72	4.48
5	6.020	834941.00	27060.94	3.24
6	12.040	1792419.40	55273.52	3.08
7	24.080	4043284.00	66927.59	1.66

ID#:9 Mass:77.00 Name:p-Cymene
f(x)=114716.100755*x-4102.724986
rr1=0.999869 rr2=0.999738



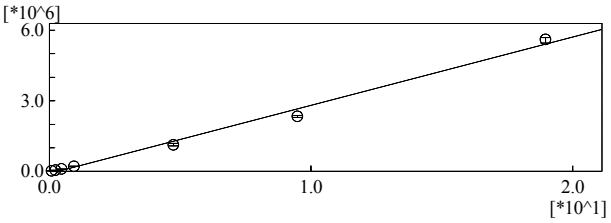
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.097	9590.00	2147.74	22.40
2	0.242	24916.33	5637.70	22.63
3	0.484	51314.00	5203.95	10.14
4	0.968	112603.00	10830.14	9.62
5	4.840	558214.00	24386.74	4.37
6	9.680	1077529.80	30042.33	2.79
7	19.360	2229129.00	30037.26	1.35

ID#:10 Mass:67.00 Name:3-Hexen-1-ol acetat (Z)
f(x)=723173.638718*x-250838.093007
rr1=0.997212 rr2=0.994432



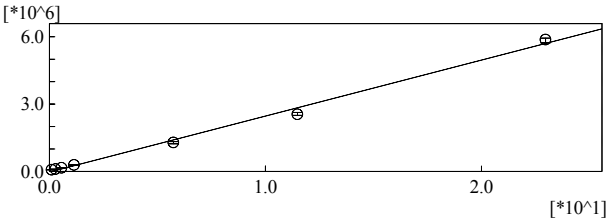
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.109	60384.00	3101.46	5.14
2	0.273	138296.50	4165.57	3.01
3	0.545	297268.33	6899.99	2.32
4	1.090	644149.75	22474.32	3.49
5	5.450	3340735.67	125196.89	3.75
6	10.900	6811492.80	154806.21	2.27
7	21.800	15999159.33	390397.36	2.44

ID#:11 Mass:67.00 Name:2-Hexen-1-ol, acetate (E)
f(x)=290867.623006*x-102910.139584
rr1=0.996484 rr2=0.992981



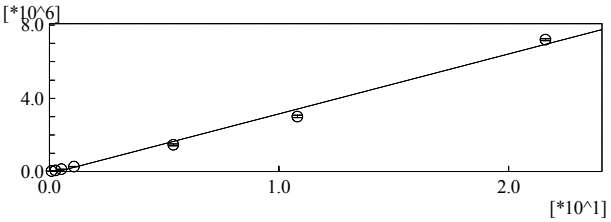
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.095	19428.00	2688.13	13.84
2	0.237	44896.67	1688.02	3.76
3	0.474	97902.33	2858.63	2.92
4	0.948	212309.00	6376.81	3.00
5	4.740	1123824.67	54129.07	4.82
6	9.480	2340389.60	38192.78	1.63
7	18.960	5601989.67	132380.06	2.36

ID#:12 Mass:108.00 Name:6-methyl-5-Hepten-2-on
f(x)=250055.614087*x-32201.295772
rr1=0.997499 rr2=0.995003



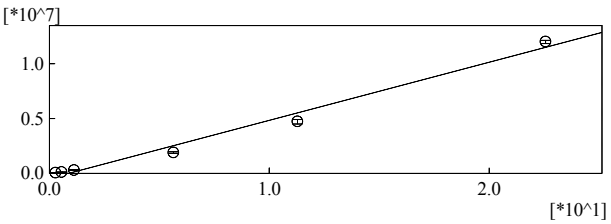
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.115	77990.33	4657.41	5.97
2	0.287	102890.67	2297.34	2.23
3	0.574	164115.33	3182.81	1.94
4	1.148	291717.75	9516.16	3.26
5	5.740	1286157.67	54544.15	4.24
6	11.480	2553697.80	54446.97	2.13
7	22.960	5876323.67	126249.39	2.15

ID#:13 Mass:67.00 Name:3-Hexen-1-ol (Z)
f(x)=327692.207264*x-124894.901282
rr1=0.996491 rr2=0.992993



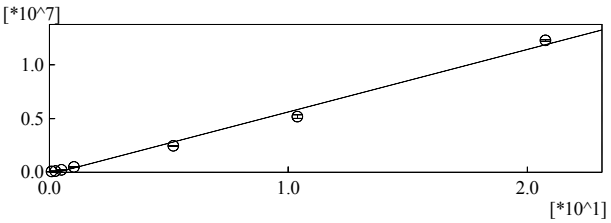
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.108	32482.33	1418.03	4.37
2	0.270	59578.33	671.47	1.13
3	0.540	131903.33	1690.68	1.28
4	1.080	276747.00	6708.96	2.42
5	5.400	1466754.33	47720.53	3.25
6	10.800	3002276.40	62528.16	2.08
7	21.600	7197488.67	48335.80	0.67

ID#:14 Mass:81.00 Name:2,4-Hexadienal
f(x)=530312.007007*x-465920.440943
rr1=0.993001 rr2=0.986050



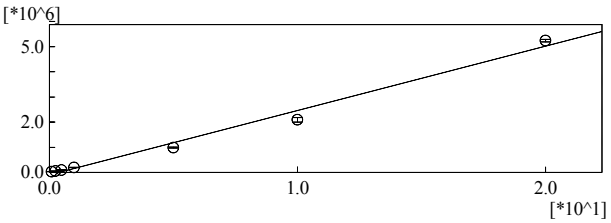
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
2	0.282	66739.00	2277.73	3.41
3	0.564	136580.67	4764.09	3.49
4	1.128	317954.75	9638.59	3.03
5	5.640	1907135.33	83713.00	4.39
6	11.280	4741771.40	149343.00	3.15
7	22.560	12017849.67	151268.08	1.26

ID#:15 Mass:70.00 Name:Heptanol
f(x)=580351.161271*x-204871.951398
rr1=0.996597 rr2=0.993206



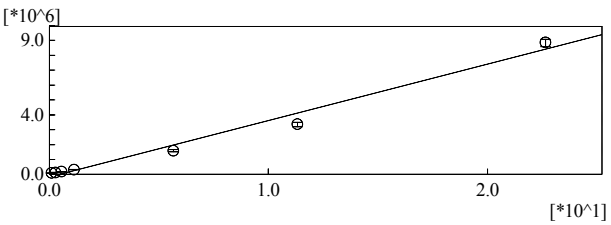
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.104	60910.33	5528.84	9.08
2	0.259	115477.67	6079.62	5.26
3	0.519	231993.33	3687.46	1.59
4	1.038	493980.25	18209.47	3.69
5	5.190	2451749.33	43788.01	1.79
6	10.380	5160200.00	116727.60	2.26
7	20.760	12250191.67	81500.79	0.67

ID#:16 Mass:69.00 Name:Linalool
f(x)=255783.509968*x-101351.798956
rr1=0.994443 rr2=0.988918



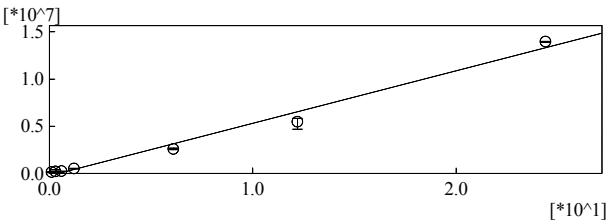
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.100	35224.67	2668.67	7.58
2	0.250	59565.00	1175.81	1.97
3	0.500	100844.33	2003.16	1.99
4	1.000	200726.75	5991.26	2.98
5	5.000	986742.00	26653.15	2.70
6	10.000	2097461.00	66404.91	3.17
7	20.000	5235596.00	47657.02	0.91

ID#:17 Mass:69.00 Name:Citronellol
f(x)=379254.428473*x-185794.648394
rr1=0.991771 rr2=0.983610



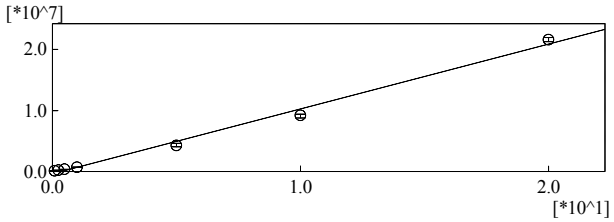
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.113	92113.00	8520.94	9.25
2	0.283	125218.00	7985.91	6.38
3	0.566	180127.33	15291.72	8.49
4	1.132	321720.25	10834.95	3.37
5	5.660	1575876.67	82083.22	5.21
6	11.320	3371308.20	117295.88	3.48
7	22.640	8853368.67	249911.00	2.82

ID#:18 Mass:92.00 Name:Methyl salicylate
f(x)=556451.577485*x-256866.810875
rr1=0.993364 rr2=0.986772



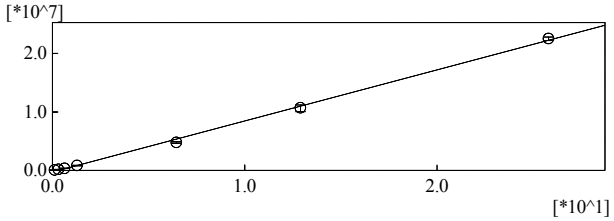
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.122	162741.33	69598.26	42.77
2	0.305	226764.33	129477.33	57.10
3	0.610	249526.67	15444.98	6.19
4	1.220	526464.75	38613.04	7.33
5	6.100	2607114.00	79173.17	3.04
6	12.200	5483455.80	470886.28	8.59
7	24.400	13962258.67	69704.87	0.50

ID#:19 Mass:69.00 Name:Nerol
f(x)=1063565.481342*x-381001.941292
rr1=0.996843 rr2=0.993695



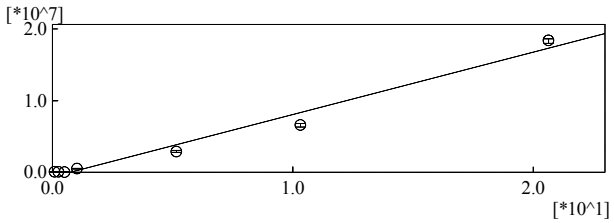
#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.100	122718.50	41285.84	33.64
2	0.250	238569.33	16953.56	7.11
3	0.500	394435.67	32340.82	8.20
4	1.000	724995.50	31986.62	4.41
5	5.000	4289611.33	309414.45	7.21
6	10.000	9180454.40	270673.53	2.95
7	20.000	21574589.67	341755.72	1.58

ID#:20 Mass:69.00 Name:Damascenone
f(x)=872322.572481*x-294797.293513
rr1=0.999295 rr2=0.998590



#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.129	77464.67	1792.79	2.31
2	0.322	179058.00	4824.18	2.69
3	0.645	380699.00	3363.45	0.88
4	1.290	838423.75	38027.27	4.54
5	6.450	4768978.33	95453.35	2.00
6	12.900	10669992.60	436141.28	4.09
7	25.800	22488963.33	294003.55	1.31

ID#:21 Mass:69.00 Name:Geraniol
f(x)=869924.274469*x-653433.503775
rr1=0.989362 rr2=0.978837

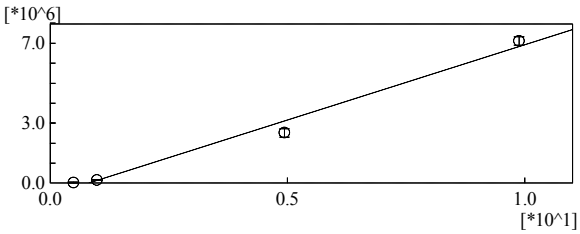


#	Conc. (ppm)	Mean Area	AreaSD	Area%RSD
1	0.103	46107.67	14162.71	30.72
2	0.258	48974.00	4913.30	10.03
3	0.516	42778.00	9823.08	22.96
4	1.032	508885.75	25895.00	5.09
5	5.160	2900867.00	145178.51	5.00
6	10.320	6583576.60	225069.64	3.42
7	20.640	18377299.67	343494.16	1.87

B. Appendix: Calibration Curves, MDGC

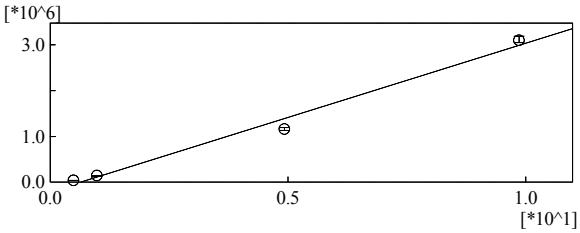
B.1. Scan

ID#:1 Mass:93.00 Name:.beta.-Myrcene
f(x)=753690.888307*x-617460.657433
rr1=0.992566 rr2=0.985188



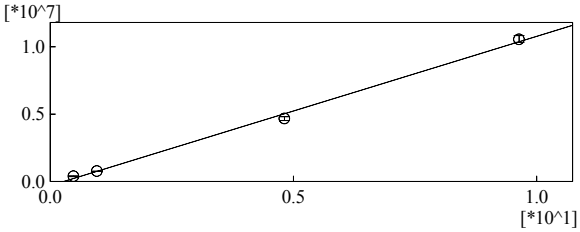
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.494	30071.00	26131.84	86.90
3	0.988	157894.33	11549.89	7.31
4	4.940	2527601.33	213527.47	8.45
5	9.880	7101259.67	212936.76	3.00

ID#:2 Mass:93.00 Name:alpha Terpinene
f(x)=324626.841668*x-210825.146820
rr1=0.993754 rr2=0.987547



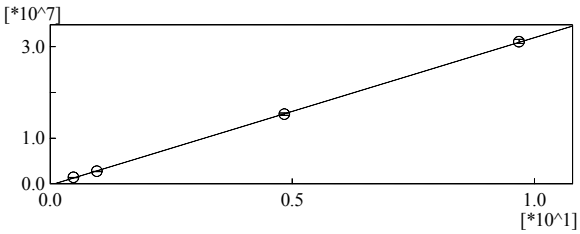
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.493	41505.00	--	--
3	0.986	140845.33	7740.97	5.50
4	4.930	1157376.67	31403.64	2.71
5	9.860	3098326.33	76369.08	2.46

ID#:3 Mass:68.00 Name:Limonene
f(x)=1109874.620159*x-315973.731063
rr1=0.998671 rr2=0.997343



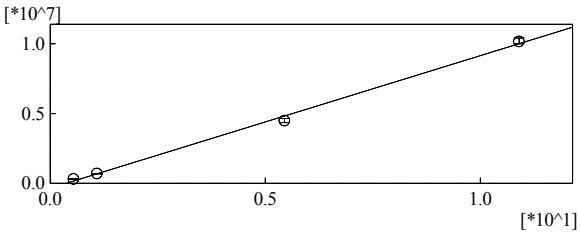
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.482	388717.67	17339.17	4.46
3	0.964	768373.33	11968.30	1.56
4	4.820	4685161.33	144807.97	3.09
5	9.640	10547519.00	259194.73	2.46

ID#:4 Mass:119.00 Name:P Cymene
f(x)=3232902.839704*x-296314.152557
rr1=0.999966 rr2=0.999932



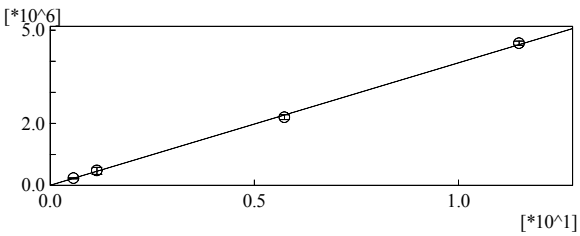
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.484	1407347.00	31754.92	2.26
3	0.968	2734011.33	56566.47	2.07
4	4.840	15265381.33	259803.82	1.70
5	9.680	31043929.33	520122.61	1.68

ID#:5 Mass:67.00 Name:3-Hexen-1-ol, acetate, (Z)-
f(x)=952379.227205*x-363144.559335
rr1=0.998648 rr2=0.997297



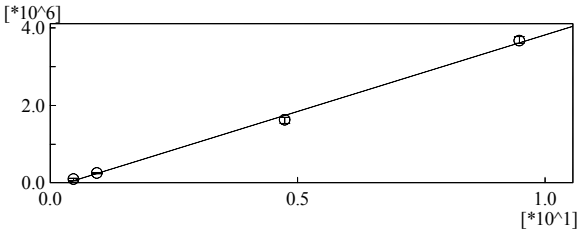
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.545	320078.00	16457.77	5.14
3	1.090	691512.67	9292.02	1.34
4	5.450	4485573.00	157235.82	3.51
5	10.900	10178798.00	217269.36	2.13

ID#:6 Mass:108.00 Name:5-Hepten-2-one, 6-methyl-
f(x)=395579.982848*x-7336.482818
rr1=0.999656 rr2=0.999311



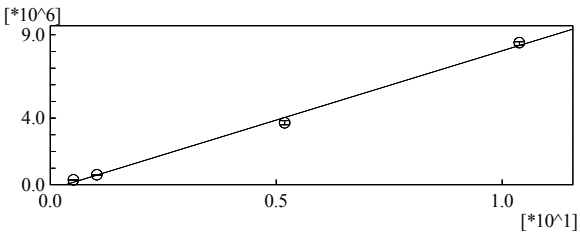
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.574	227312.50	17845.25	7.85
3	1.148	481242.67	110849.24	23.03
4	5.740	2186863.00	79372.61	3.63
5	11.480	4568311.67	70964.76	1.55

ID#:7 Mass:67.00 Name:2-Hexen-1-ol, acetate, (E)-
f(x)=395416.095339*x-136620.824996
rr1=0.998863 rr2=0.997727



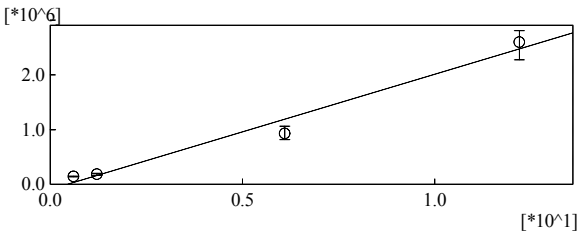
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.474	96213.00	36413.60	37.85
3	0.948	254579.67	18157.62	7.13
4	4.740	1621956.00	80709.58	4.98
5	9.480	3665866.33	94323.57	2.57

ID#:8 Mass:70.00 Name:1-Heptanol
f(x)=832292.849025*x-282331.534296
rr1=0.998372 rr2=0.996748



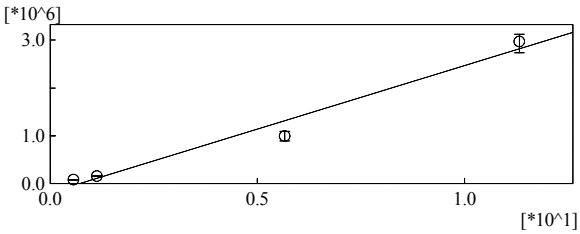
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.518	294614.00	2633.27	0.89
3	1.038	601840.33	27490.45	4.57
4	5.190	3723733.00	126371.62	3.39
5	10.380	8504334.00	116655.67	1.37

ID#:9 Mass:120.00 Name:Methyl salicylate
f(x)=210731.890840*x-97413.186171
rr1=0.987922 rr2=0.975991



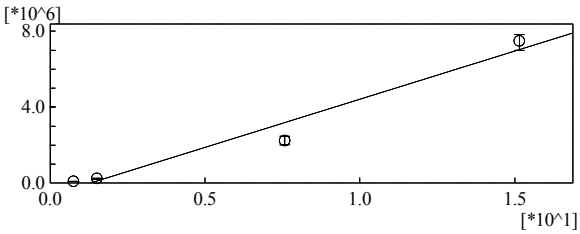
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.610	143273.50	5142.79	3.59
3	1.220	184841.33	19913.53	10.77
4	6.100	929694.00	124842.41	13.43
5	12.200	2594571.33	280893.79	10.83

ID#:10 Mass:69.00 Name:Citronellol
f(x)=265565.745394*x-190752.589689
rr1=0.986849 rr2=0.973871



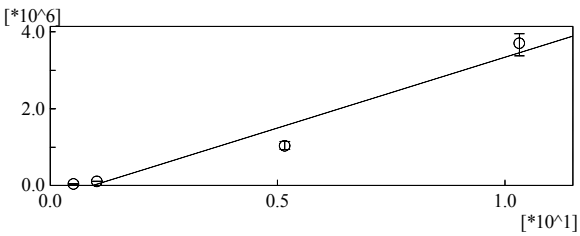
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.566	82269.50	4990.05	6.07
3	1.132	157501.67	3905.29	2.48
4	5.660	993456.00	103165.47	10.38
5	11.320	2963999.33	202630.61	6.84

ID#:11 Mass:69.00 Name:nerol
f(x)=507698.758622*x-662661.694624
rr1=0.982031 rr2=0.964385



#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.758	100065.50	1182.99	1.18
3	1.515	238342.67	26737.51	11.22
4	7.575	2227874.00	233228.61	10.47
5	15.150	7474524.33	427925.77	5.73

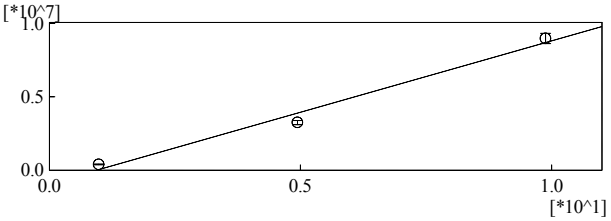
ID#:12 Mass:69.00 Name:geraniol
f(x)=368321.732528*x-349710.274046
rr1=0.978596 rr2=0.957650



#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
2	0.516	40252.50	2332.75	5.80
3	1.032	106898.00	4635.88	4.34
4	5.160	1033302.67	105447.29	10.20
5	10.320	3692488.00	294831.29	7.98

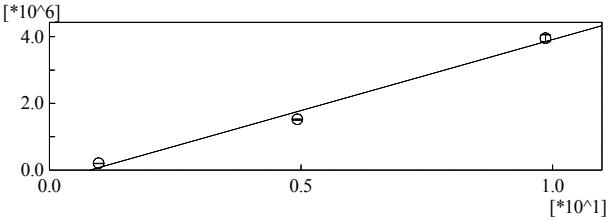
B.2. SIM

ID#:1 Mass:93.00 Name:.beta.-Myrcene
f(x)=972158.576295*x-912024.639104
rr1=0.991950 rr2=0.983966



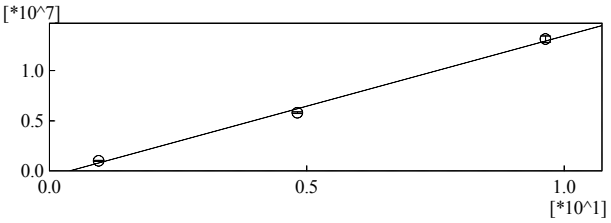
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.988	402416.50	26309.32	6.54
4	4.940	3253331.50	195094.30	6.00
5	9.880	8976061.00	479909.13	5.35

ID#:2 Mass:93.00 Name:alpha Terpinene
f(x)=424939.443118*x-343721.645466
rr1=0.994316 rr2=0.988664



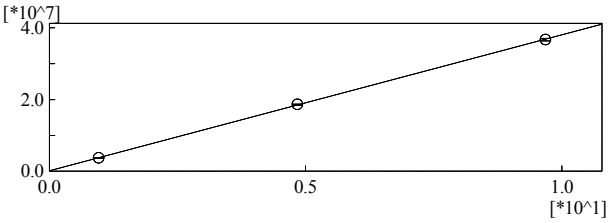
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.986	204783.50	5442.60	2.66
4	4.930	1518103.00	17660.70	1.16
5	9.860	3949793.00	144319.08	3.65

ID#:3 Mass:68.00 Name:limonene
f(x)=1400860.194302*x-552203.946543
rr1=0.998413 rr2=0.996829



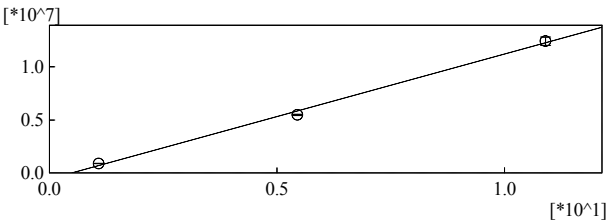
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.964	1018085.50	52670.26	5.17
4	4.820	5804194.00	97136.67	1.67
5	9.640	13127977.00	446153.27	3.40

ID#:4 Mass:119.00 Name:p-cymene
f(x)=3789407.677998*x+103555.556131
rr1=0.999955 rr2=0.999911



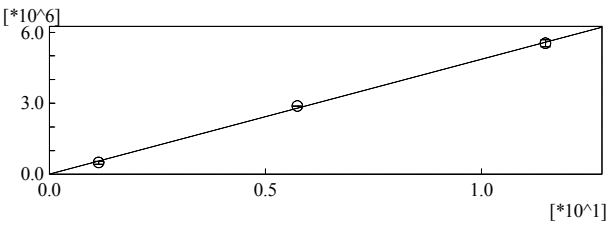
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.968	3671614.00	121076.48	3.30
4	4.840	18624448.00	195103.49	1.05
5	9.680	36704952.50	511477.91	1.39

ID#:5 Mass:67.00 Name:3-hexen-1-ol acetate, (Z)-
f(x)=1176314.393949*x-585556.798878
rr1=0.998569 rr2=0.997140



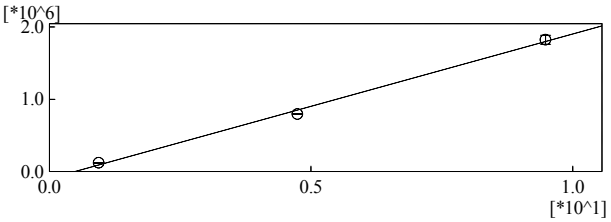
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.090	894852.00	24477.21	2.74
4	5.450	5468549.50	80206.42	1.47
5	10.900	12394850.50	533092.75	4.30

ID#:6 Mass:108.00 Name:5-hepten-2-one, 6-methyl-
f(x)=486673.891250*x-11798.233743
rr1=0.999435 rr2=0.998869



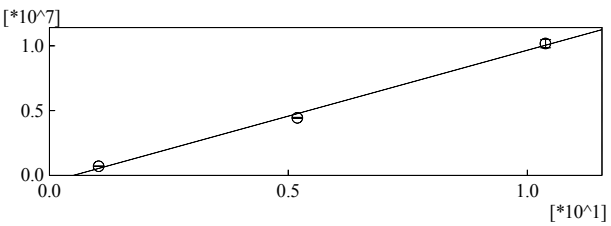
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.148	492640.50	83582.14	16.97
4	5.740	2879383.00	7759.79	0.27
5	11.480	5531807.50	207682.21	3.75

ID#:7 Mass:100.00 Name:2-hexen-1-ol acetate, (E)-
f(x)=200212.888038*x-98082.150349
rr1=0.998534 rr2=0.997070



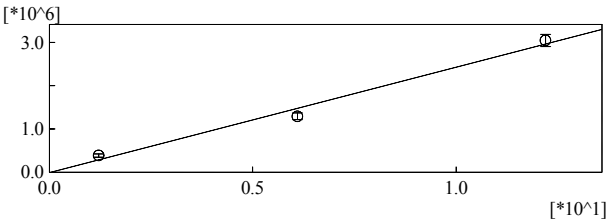
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	0.948	121421.00	5345.73	4.40
4	4.740	797464.50	10665.29	1.34
5	9.480	1823697.00	88420.87	4.85

ID#:8 Mass:70.00 Name:1-heptanol
f(x)=1016768.997389*x-515991.223525
rr1=0.998402 rr2=0.996807



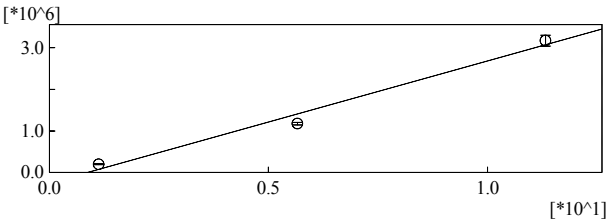
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.038	711860.50	15101.68	2.12
4	5.190	4450638.00	59590.72	1.34
5	10.380	10176027.50	470807.96	4.63

ID#:9 Mass:120.00 Name:methyl salicylate
f(x)=243801.909637*x-10188.904446
rr1=0.993065 rr2=0.986178



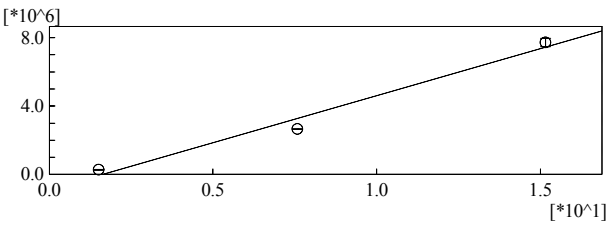
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.220	388901.50	52435.50	13.48
4	6.100	1294029.00	112212.19	8.67
5	12.200	3045516.00	194154.55	6.38

ID#:10 Mass:69.00 Name:citronellol
f(x)=294065.197929*x-260138.573770
rr1=0.991369 rr2=0.982813



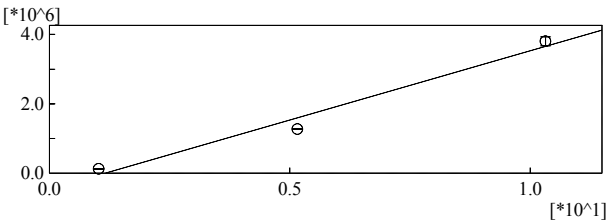
#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.132	199819.00	19200.78	9.61
4	5.660	1175534.00	39401.40	3.35
5	11.320	3170340.00	186182.63	5.87

ID#:11 Mass:69.00 Name:nerol
f(x)=551598.551394*x-908388.520754
rr1=0.990274 rr2=0.980643



#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.515	266208.50	28374.07	10.66
4	7.575	2659905.00	4358.61	0.16
5	15.150	7719469.50	336251.19	4.36

ID#:12 Mass:69.00 Name:geraniol
f(x)=399806.136836*x-468901.408898
rr1=0.988981 rr2=0.978083



#	Conc. (ng)	Mean Area	AreaSD	Area%RSD
3	1.032	121995.00	12339.01	10.11
4	5.160	1273164.50	21194.11	1.66
5	10.320	3799735.00	182113.94	4.79