KAHO ST. LIEVEN

FORMATION AND DETERMINATION OF AROMATIC COMPOUNDS DURING BEER FERMENTATION

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B. ABBREVIATIONS

- ADP - Adenosine diphosphate
- ATP - Adenosine triphosphate
- CoA – Coenzyme A
- DMS – Dimethyl sulfide
- DMSO – Dimethyl sulfoxide
- FID – Flame Ionization Detector
- GC – Gas Chromatograph
- HS – Head Space
- MetSO – Methionine sulfoxide
- NAD^+ – reduced Nicotinamide adenine dinucleotide.
- NADH – Nicotinamide adenine dinucleotide
- P_i - phosphate
- SMM – S - methylmethionie
- TCD - Thermal Conductivity Detector

C. ABSTRACT

This study is an analysis verification method. The method consists in determining ten aromatic compounds present in beer. The compounds which have been analysed are acetaldehyde, ethyl acetate, isoamyl acetate, ethylhexanonate, ethyloctanoate, ethylbutyrat, n-Propanol, isobutanol, N-amyalcohol and dimethyl sulphur. This method analyses the compounds using GC-HS. The analysed method is internal standard addition in order to create calibration curves to find the original concentration of each compound. In this study we attempted to prove this method analysing samples of bottle beer. We obtained some unexpected results and we tried to explain the reason.

Key words: beer, aromatic compounds, gas chromatography

D. OBJECTIVES

The main objective of this project is the tuning and verification of an analysis method of aromatic compounds in any beer sample. This method is an adaptation of a method supplied by the company Bavaria which could be subsequently used by KAHO-Sint Lieven to analyse their own samples of beer. Therefore, the project consists in the optimization of a Gas Chromatograph.

The first part is a theoretical basis which consists in determining what kinds of compounds have to be analysed and when and why these compounds are being formed in the brewing process. Most of these compounds are undesirable in beer.

The second part, the experimental part is the optimization of the analytical method. We have to find the optimum operating conditions of the GC-HS to have an accurate and repeatable method to analyse the entire aromatic component chosen.

SUMMARY

E. INTRODUCTION

This study explains the formation and determination of aromatic compounds in beer fermentation. Aromatic compounds are the compounds that affect beer flavour. In some amounts these compounds could not be desired for the brewer because they affect negatively beer taste. Aromatic compounds are present in beer and the most common origin is yeast fermentation. Most of them are by-products of fermentation pathways. Determining which these compounds are and the reason of their existence is the key to improve fermentation parameters and finally improve beer flavour.

Developing an easy and precise method to determine the most important aromatic compounds is an important tool for following investigations in the fermentation area. We tried to develop a method to analyse ten of these compounds in one single analysis.

F. THEORETICAL PART

1. BREWING PROCESS

In this project the by-products in beer fermentation are studied. These by-products are aromatic products, and their levels in beer can make a huge difference in their flavour. Fermentation is produced after several steps in brewing.

Traditionally the raw materials in brewing are water, malt, hops and yeast. Most brewers use in addition adjuncts and/or various processing aids. Before beer is fermented, water, malt and hops are added to the brewing process. Just before fermentation, yeast is added, as is shown in figure 1-1.1.

Yeast is a living organism and therefore it should not properly be considered a raw material. Yeast is largely responsible for making beer.

However, to understand the yeast fermentation; we have to explain the previous stages before the fermentation.

1.1. BREWING BEFORE FERMENTATION

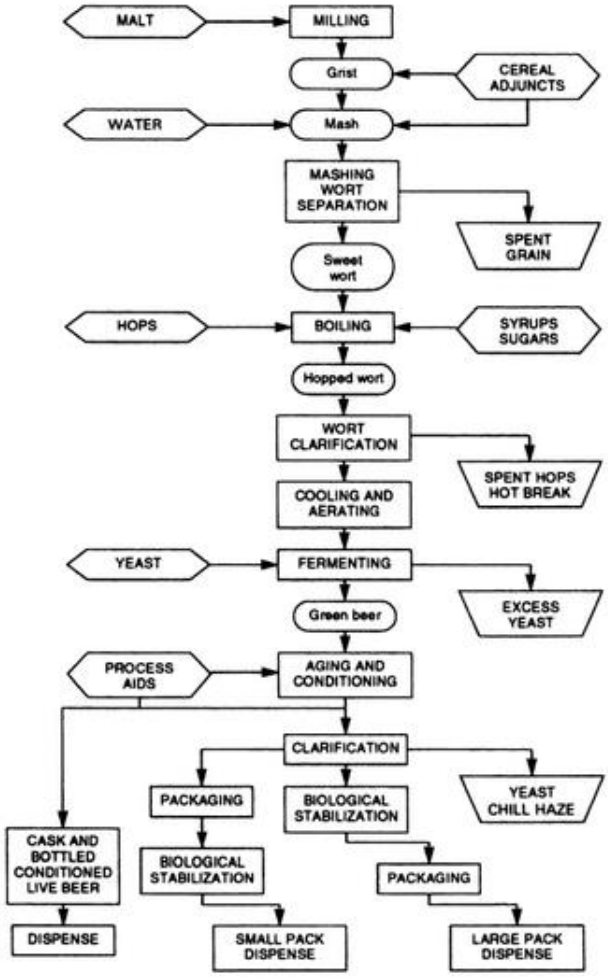


Figure 1.1-1: Diagram of general steps in brewing [1]

1.1.1. Malting

Malting is the process of germination of grain (barley) in well-controlled conditions. In the figure 1.1-2 the flow sheet of malt production is presented.

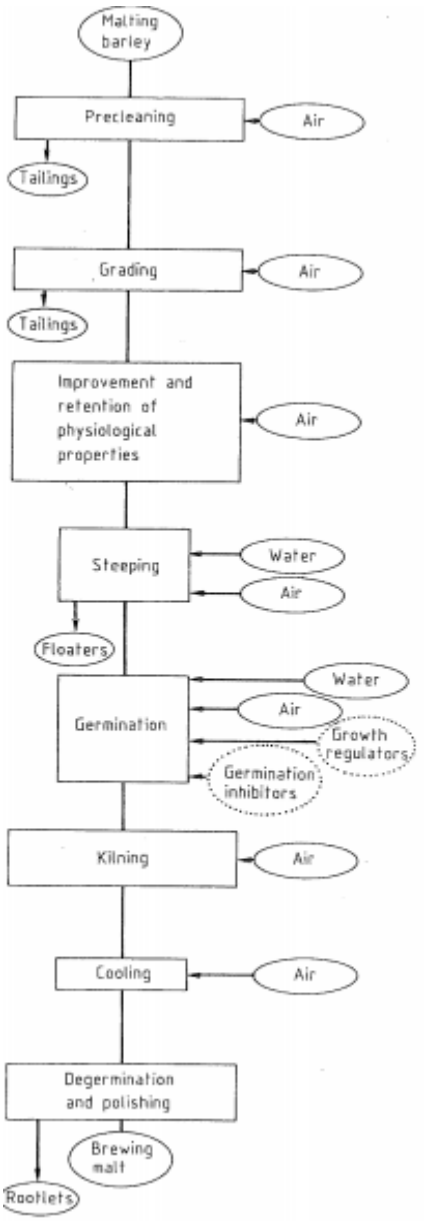


Figure 1.1-2: Flowsheet of malting. [2]

1.1.1.1. Steeping

It is the first step of malting after the properly preparation of barley for malting. Mainly it consists in wetting and aerating the grain (barley) to prepare it for the next stage: the germination. Soaking it in a deep tank with cool water, barley takes up water of vegetation and swells by one-third of its volume. The steeping takes on average between 40 and 50 hours [1]. Upon addition of water, steeping, germination of grain begins.

1.1.1.2. Germination

Germination is a physiological process during which the embryo develops rootlets. Germination consists in a cool aeration, during this time the shoot and rootlet grow and important enzymes are formed and act. The germination produces enzymes and changes the chemical structure of malt. The aim of controlled germination is to produce green malt of a definite composition. This malt germination takes 3 to 5 days [1].

1.1.1.3. Kilning

The grain is then dried by kilning with warm air which fixes the properties of the resultant malt and imbues malt with its unique flavour. This stage reduces the malt moisture from 40 -50 % to 3.5 % [2]. Also its objective is to stop the chemical and biological changes that take place during the germination. This can take up to 24 - 48 hours [1].

These three steps are basically how the malt is prepared. The flavour stabilizes with low moisture and the malt can be stored. The follow step is milling.

The high molecular mass substances of malt and other adjuncts must be solubilized by milling and mashing.

1.1.2. Milling

Malt contains everything for a successful manufactured beer except water and energy. Milling or grading is crushing malt keeping husk fairly intact. The main objective is to leave the malt husk as intact as possible in order to prevent the undesirable tannins, bitter compounds or

colouring substance. With milling the husk exposes the enzymes and the food polymers. Another objective is to produce crushed malt with the ideal spectrum of particles to extract production and recovery. That leaves the husk as filtration aid. This takes 1 to 2 hours and it operates in ambient temperature.

Milled malt is called grist. If water is added, the grist turns into mash. It is possible to add cereal adjuncts before the water is added or after.

1.1.3. Mashing and wort separation

During the mashing the solid particles are solubilized by the action of brewing water and the enzymes formed during the previous steps. The objectives of this step are to promote the enzyme action and solubilize extract malt solids. The following step is wort separation. There are two methods to do wort separation: lautering and mash filters. The main objectives are filtering wort and getting the maximum extract of desired fermentability. What comes out of this step is called sweet wort. These two steps take to 1 or 2 hours [1].

The raw materials on the previous steps are only malt, water and some cereal adjuncts. Just before boiling hops, one of the four principal raw materials, are added. Hops are kiln-dried cones and also there could be used hops with other forms like powdered hops, hops pallets or hop extract. Hops and hop-derived products have no other significant use but to impart flavour to beer. Bitterness is the primary flavour impact of hops though under some processing conditions their aroma character can survive into beer.

1.1.4. Boiling

In this point of brewing sweet wort is boiled in a kettle, which is obtained from the total process, with hops, and process aids, like syrups or sugars. Usually syrups are derived from maize, barley or wheat. Sugars are usually sucrose. When it is used in the kettle (copper), it is referred to as "copper sugar".

This mix boils in a kettle at a temperature above 100 °C and at a time between 30 minutes and 1 hour and 30 minutes [1]. One of the objectives is to extract the hop, to make new bitter substances. With the high temperature the wort is sterilized. Also the haze precursors (hot break) are precipitated. The hop essential oil is driven off. Another objective of boiling is to increase the characteristic beer colour.

After boiling, wort is called hopped wort.

1.1.5. Wort clarification

Wort clarification consists in straining, settling or centrifugation the hopped wort in order to remove the spent hops and hot break (trub) to clarify wort. The temperature of the clarification process is between 100 – 80 ºC and it takes less than one hour [1].

1.1.6. Cooling and aerating

The wort is cooled lowering the temperature from 100 to 12-18 °C [1]. The wort is cooled passing it through a heat exchanger and being aerated by injecting oxygen. The aeration is a useful step, because the oxygen added is absolutely necessary for the yeast propagation.

1.2. FERMENTATION

Fermentation (see in figure 1.2-1) is the key step to understand the origin of the aroma compounds. In general terms, fermentation is the metabolic process of converting sugar to alcohol, acids or/and gases by yeast or bacteria. In the brewing world three different fermentation methods exist: warm, cool and wild or spontaneous. The main products of fermentation are ethanol and carbon dioxide. Other reaction products include: higher alcohols, aromatic alcohols, esters, organic acids, carbonyl compounds, sulphur-containing compounds and polyhydric alcohols; all of which are important for the properties and the quality of the resulting beer. All the compounds formed have a different taste. Their combined contribution makes up or off-flavour beer.

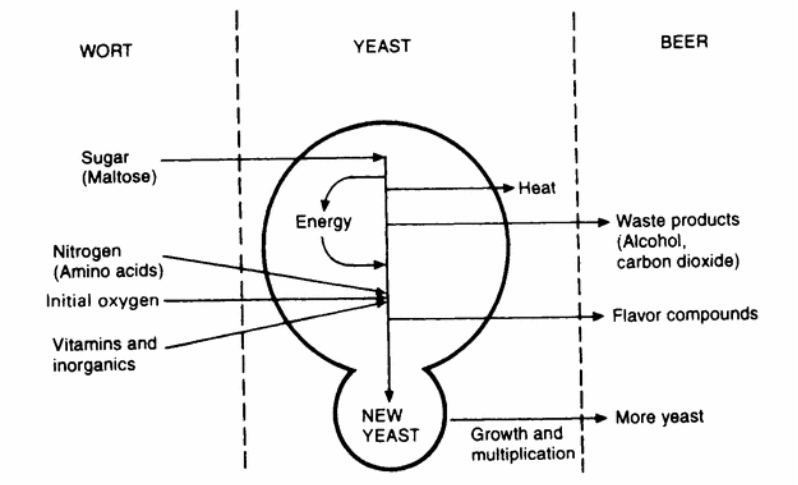


Figure 1.2-1: Overview of the yeast fermentation [1].

In the first part of fermentation yeast is added and this step is called pitching.

1.2.1. Yeast

Yeast is the microorganism responsible for producing fermented beverages. The growth and multiplication of this living organism are inseparable from the metabolic products which produce ethanol, carbon dioxide and the whole range of metabolic products which contribute to the flavour of the finished product. Yeast magnificently enhances and reveals the fundamental flavour of beer derived primarily from malt and hops.

To have a good fermentation it is good to choose a good yeast strain. There are numerous types of brewing yeast. Some examples of yeast types are: ale yeast, larger yeast, top yeast, bottom yeast, *Saccharomyces cerevisiae* (Ale yeast) or *S.carlsbergensis* (Lager yeast) [3].

Yeast can grow aerobically or anaerobically. In the former case the cells are said to be respiring and in the latter fermenting.

The cooled aerated wort made in the brew house is fermented by yeast to make an immature beer. During this process, yeast reproduces. The main fermentation (primary fermentation) in which green beer and yeast are produced is often followed by a slower process at lower

temperature in the presence of lesser amounts of yeast. This is referred to as a secondary fermentation, conditioning or even maturation.

For yeast to live and grow, wort must contain a sufficient supply of nutrients. Nutritional deprivation leads to incomplete and inadequate fermentation. Yeast needs fermentable carbohydrate, assimilable nitrogen, molecular oxygen, the vitamin biotin, sources of phosphorus and sulphur, calcium and magnesium ions and trace elements such as copper and zinc ions [3].

1.2.2. Pitching

The first step of fermentation is pitching. Pitching is simply a brewer's term meaning adding yeast to the fermenter. But, before the pitching you have to consider many things. Next, there is a short list of the most important issues to take into consideration:

- the temperature of the wort: The metabolism of yeast changes as the temperature changes;
- the concentration of the oxygen in the wort;
- the amount of nutrients in wort;
- the amount of yeast that will be pitched;
- the fermentation temperature;
- the starting specific temperature.

In the brewing process, to pitch the correct number of yeast cells is crucial to produce beer of a superior and constant quality. Pitching rates are governed by a number of factors, including yeast strain, fermentation capacity of yeast, yeast viability, flocculation characteristics, previous history of yeast, and desired beer flavour characteristics. Other considerations when choosing pitching rates include wort gravity, wort constituents, fermentation temperature, and the degree of wort aeration [4].

The pitching rates should be adjusted to account for the number of viable cells rather than the total number of cells. That concept is called viability. Dead cells can be determined by staining with methylene blue and counting with a hemacytometer, the most common method to determinate the viability [3].

1.2.3. Fermentation metabolism

Figure 1.2-1 describes the simplified overview of yeast fermentation. This project analyses the flavour compounds.

Once pitched into wort, yeast uses the nutrients to provide energy (ATP) and in doing so forms alcohol and carbon dioxide (CO_2). It produces reducing power (NADPH) for the synthesis of new yeast substance [1].

These pathways, whose main purpose is to generate energy, act so by chemical oxidation of substrates [1].

ADP + inorganic phosphate (Pi) → ATP

The energy transfer reactions are never 100 % efficient because waste energy (in the form of heat) is always dissipated.

The yeast cells consume and metabolize sugars. The fermentable sugars are in the wort; the most commons are maltose, maltotriose and lesser amounts of sucrose, glucose and fructose.

Figure 1.2-4 is a diagram which shows the process of the sugars metabolism.

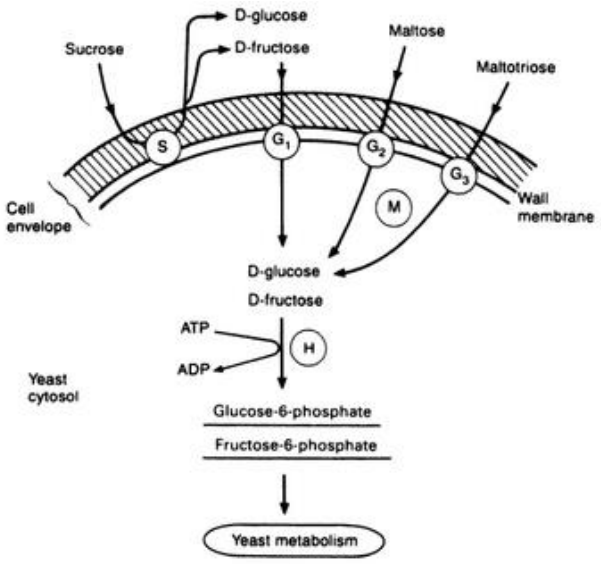


Figure 1.2-4 : Diagrammatic representation of a simplified view of the uptake of wort fermentable sugars by yeast. The cell envelope is the wall (hatched area) and membrane (single line). [1]

Yeast has the ability to adjust its metabolism to aerobic as well as to anaerobic conditions. Yeast doubles or triples its mass during fermentation. For the build-up of cell substance (proteins and enzymes) yeast needs mostly amino acids, which are taken from fermentation substrate or are synthesized by themselves. Besides proteins, lipids are also synthesized for yeast propagation because they are important components of the cell wall, and they are needed for the up-take of nutrients. For the synthesis of these lipids from acetyl CoA, molecular oxygen is needed. Finally, yeast also requires minerals for the stabilization of its enzyme systems [2].

The time of the fermentation is between 2 and 7 days depending on the fermentation type. [1]. After the fermentation time, yeast is removed for further fermentations.

1.3. BREWING AFTER FERMENTATION

1.3.1. Aging and conditioning

After fermentation, the product that has been obtained is green beer. The next step is Aging and conditioning beer. This process consists in passing beer to oxygen-free tanks and then chilling the beer. At this moment, process aids can be added to green beer. At this point beer matures and the flavour is modified. Also the carbon dioxide levels are adjusted and the yeast

and cold break settle down. After 7-21 days [1] of this step, that beer is stabilized. This is the right time to transfer live beer to package where the second fermentation will take place, if desired

1.3.2. Clarification

The beer type which will not fermented again, is centrifuged and filtered in order to remove yeast and suspended solids (cold break). After these steps, we obtain a bright beer.

1.3.3. Biological stabilization

In order to kill or remove any microbes the beer is pasteurized or flirted with sterile filter.

1.3.4. Packaging

The beer is made; the beer could be packed in several ways, in bottles, cans, kegs or casks.

2. ANALYSED COMPOUNDS

The used method analyses ten aromatic compounds in beer. These compounds are: acetaldehyde, ethyl acetate, isoamyl acetate, ethylhexanonate, ethyloctanoate, ethylbutyrat, n-Propanol, isobutanol, N-amyalcohol and dimethyl sulphur.

These ten compounds could be classified in four categories: aldehydes, esters, higher alcohols and sulphur compounds, but there is certainly an excess of 700 of such constituents in a beer [1].

The next table gives an indication of the range and concentration of the constituents of a typical beer. Most of these are present at levels just below those which are easily perceived. When these individual components are present at double levels or more which can easily be discerned, they exhibit specific flavour notes. These may be characteristic (therefore desirable flavour) or uncharacteristic of beer (and therefore undesirable, taints). For one beer one flavour will be a desirable flavour and for another it will be a taint.

Table 2-1: Some constituents of a typical beer [1]

Component	Typical concentration [g/l]
Ethanol	35
Carbon dioxide	5 (30 produced)
Organic acids	<0.1
Aldehydes	<0.1
Esters	<0.01
Higher alcohols	<0.01
Diketones	<0.0002
Sulfur compounds	<0.00005

2.1. ALDEHYDES

Aldehydes are derived from alcohols through dehydrogenation. They contain the radical –CHO, and their content is around 10-20 mg/L [5]. Next, there is a list of the most important aldehydes in beer.

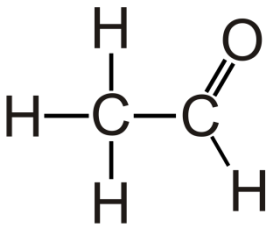
Table 2.1: Principal aldehydes constituents on a beer [6]

Aldehydes	Concentration [mg/l]	Flavour descriptors
Acetaldehyde	2-20	Green, Paint
Propanal	0.01-0.3	Green, fruity
Butanal	0.03-0.02	Melon, varnish
trans- 2-Butenal	0.003-0.02	Apple, almond
2-Methylpropanal	0.02-0.05	Banana, melon
C_5 Aldehydes	0.01-0.3	Grass, apple, cheese
Hexanal	0.003-0.07	Bitter, vinous

Several of the aldehydes are of yeast origin or other results of Strecker degradation of amino acids during the kettle boil and appear as a result of a random decarboxylation of organic acids. Acetaldehyde is the most common aldehyde in concentration, as it is shown in the above table. And for that reason acetaldehyde is the only aldehyde which is analysed in this method.

2.1.1. Acetaldehyde

Acetaldehyde (CH_3CHO) is a two-carbon aldehyde. Acetaldehyde is a component present in all beer. Its appearance is of a colourless liquid, pungent and with a fruity odour. It is a flammable liquid and soluble in water. Its boiling temperature is 20.2ºC, very low, compared to the other aroma compounds. Acetaldehyde has been identified as a toxic compound with mutagenic properties and has been demonstrated to exert a wide range of carcinogenic effects in vitro and in vivo. [7]



When studying the effect of acetaldehyde in beer flavour it must be distinguished two types of concentrations: low concentration, less than 15 mg / L, and high concentration, higher than 15 mg/L. [6] At low concentrations acetaldehyde is associated with green apple, bruised apple and in some occasions the flavour could be like emulsion paint, wine (white wine), and sherry flavour notes. At high concentrations acetaldehyde can contribute to 'harshness' in beer. This can affect drinkability, especially at warmer serving temperatures. In some beer types a medium concentration could be a positive contribution to the flavour.

The acetaldehyde is excreted into green beer by yeast during the first three days of fermentation and it is responsible for the green young beer flavour. In normal fermentation, acetaldehyde is a precursor to ethanol. It is noticed mostly in young beers where the yeast is not able to reabsorb or finish the conversion of glucose to pyruvic acid to acetaldehyde and finally to ethanol [8]. During fermentation, after the first three days, when its formation is faster than its reduction, acetaldehyde is reduced to ethanol but it can be oxidized to acetic acid, and this is how the amount of acetaldehyde decreases.

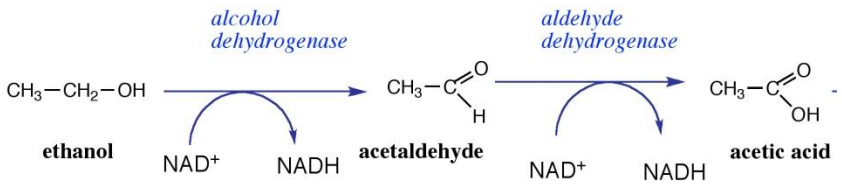


Figure 2.1-2: The oxidation of alcohol to acetaldehyde and then to acetic acid.

If beer has a high concentration of acetaldehyde it can be indicative of high O_2 levels in packaged beer, because ethanol oxidizes in acetaldehyde. A good solution is the minimization of dissolved oxygen concentrations in the bright beer and during the filling process.

The most important impact factor to the production of acetaldehyde is the contamination of wort with Zymomonas [3]. Another factor that changes the production of acetaldehyde is when the pressure of the fermenter. When this increased, also the production of acetaldehyde increases. It also increases the production the premature separation of yeast from beer.

Another factor that increases the production is the factors that inhibit the yeast activity.

2.2. ESTERS

All alcohols and acids present in beer are theoretically capable of esterification reaction, potentially forming almost 4000 esters [5]. In beer many esters have been found. The most common ester is ethyl acetate.

Table2.2: Principal esters constituents on a beer [6]

Esters	Concentration [mg/l]	Flavour descriptors
Ethyl acetate	10-6	Solvent-like, sweet
Isoamyl acetate	0.5-5.0	Banana, ester, solvent
Ethyl hexanoate	0.1-0.5	Apple, fruity, sweet
Ethyl octanoate	0.1-1.5	Apple, tropical fruit, sweet
2-Phenylethylacetate	0.05-2.0	Roses, honey, apple, sweet
Ethyl nicotinate	1.0-1.5	Grainy, perfume
Ethyl butyrate [9]	0.05-0.25	Tropical fruits, Mango, Tinned pineapple

The method analyses five esters: Ethyl acetate, Isoamyl acetate, Ethyl hexanoate, Ethyl octanoate and Ethylbutyrat.

Esters involve equilibrium between fatty acids and alcohol (usually ethanol). This balance leads to the esterification reaction in which a carboxylic acid and an alcohol react becoming an ester and water.

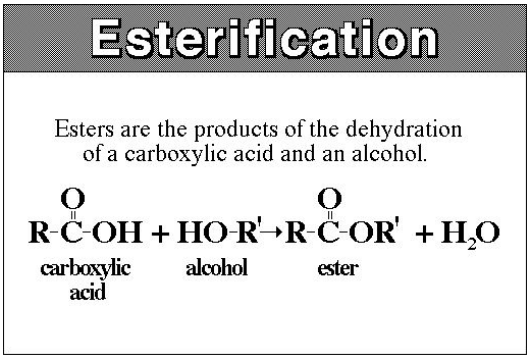


Figure 2.2-1: Esterification reaction

This shows the important role played by yeast in the biosynthesis of esters. Yeast strain can influence both the quantity and type of ester produced in beer and an abnormal increase in ethyl acetate is caused by wort with a high sugar concentration. Although some increase in the concentration of ethyl esters takes also place during the beer shelf life. The following diagram shows the way esters are formed in yeast.

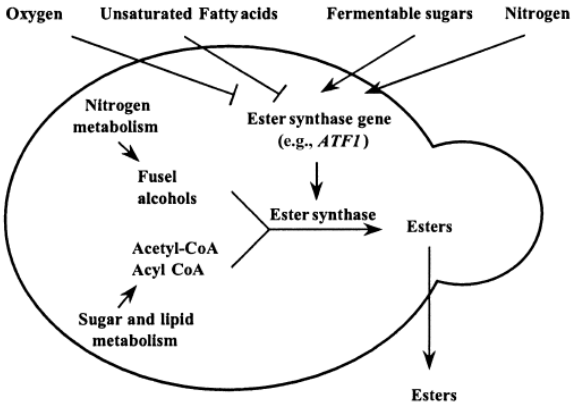
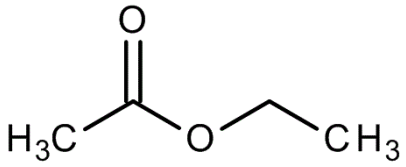


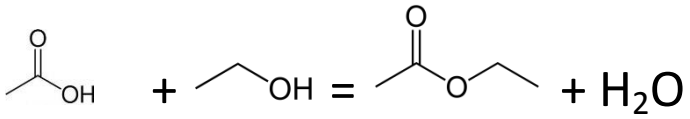
Figure 2.2-2: Formation of the esters in yeast metabolism.

All the esters have common factors that impact in their production. The factors that decrease are the factors that increase the yeast growth because acetyl CoA diverted to cell biomass rather than esterification of higher alcohols, and less higher alcohols, less esters. High oxygen, increase of the pressure on the fermenter or if the wort has a high concentration of lipids (dirty wort) are examples of the factors that decrease the production. The factor that increases the production is higher C:N ratio in wort.

2.2.1. Ethyl acetate

Ethyl acetate or ethyl ethanoate ($CH_3COOCH_2CH_3$) is the acetate ester of the acetic acid and ethanol. It is a colourless liquid with a characteristic sweet smell (similar to pear drops). It is soluble in water (8.3 g/100ml) and in ethanol. This is the most common ester in beer by weight, but not necessarily by flavour impact. The reason why ethyl acetate has the largest concentration in beer is the constant equilibrium of the reaction between acetic acid, the most common organic acid, and ethanol, the most common alcohol in beer. It smells of nail polish and solvent at high concentrations, but it can have a slightly fruity aroma at low levels. Its boiling temperature is 77.1ºC.

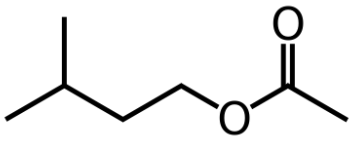




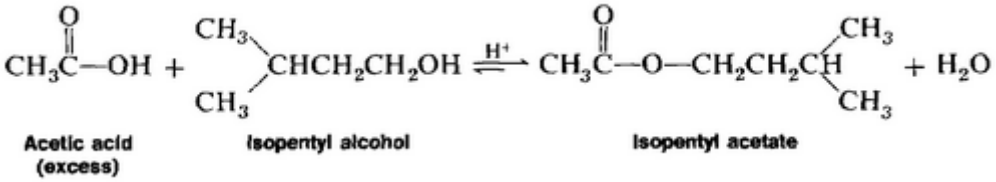
Acetic acid ethanol ethyl acetate

During the fermentation of sugars, ethanol has relatively high concentration and this is undesirable. In addition, the by-product ethyl acetate is also produced with the Acetyl (CoA). Ethyl acetate is the most abundant ester produced by yeast and it is particularly difficult to separate from ethanol by distillation. (Boiling temperature of ethanol is 78.4ºC and ethyl acetate is 77.1ºC).

2.2.2. Isoamyl acetate

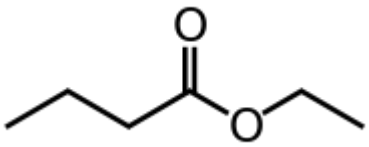


Isoamyl acetate or isopentyl acetate ($CH_3COOCH_2CH_2CH(CH_3)_2$) is an organic ester formed from isoamyl alcohol and acetic acid. It is only slightly soluble in water, but very soluble in most organic solvents. Its boiling point is 142ºC. It is very aromatic and fruity, it smells like banana or pear drop, but it could also smell like estery. In very low concentration (2ppm), the flavour of isoamylacetate can be easily perceived.

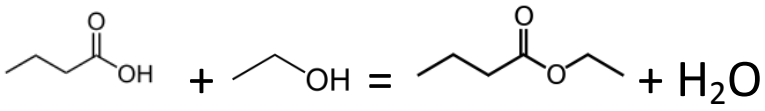


In some beers their characteristic flavour is a soft banana flavour, so it is desirable to increase the production of isoamyl acetate. The most common method to increase the isoamyl acetate production in beer is to do the fermentation at higher temperatures. Brewers choose to under-aerate their wort to influence the isoamyl acetate production. This method is more variable and less ideal than raising the temperature. The third option is under-pitching the yeast to make sure isoamyl acetate production is high, but again, it's not as predictable as choosing a known isoamyl acetate producing strain and letting it ferment at a high temperature. Additionally, under-pitching yeast can leave your brew open to bacterial infection, which is usually undesirable.

2.2.3. Ethyl butyrate

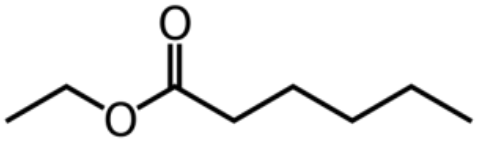


Ethyl butyrate or ethyl butanoate ($C_6H_{12}O_2$) is a volatile ester. It has a fruity odour, similar to pineapple or mango. Its boiling point is 120-121ºC. Ethyl butyrate contributes to a pleasant 'tropical fruit ester'. It is associated with the use of particular yeast strains and hop varieties. Ethyl butyrate is produced during the yeast fermentation. The amount produced depends on wort composition, yeast strain and fermentation conditions. The presence of ethyl butyrate in beer can be indicative of brew house hygiene problems, with the compound being formed during fermentation as a result of esterification of butyric acid produced by contaminant bacteria. The concentrations of ethyl ester decrease over time as an alcoholic beverage ages due to spontaneous hydrolysis [10]. Next it is shown the esterification reaction, the fatty acid is butanoic acid and the alcohol is ethanol [11].

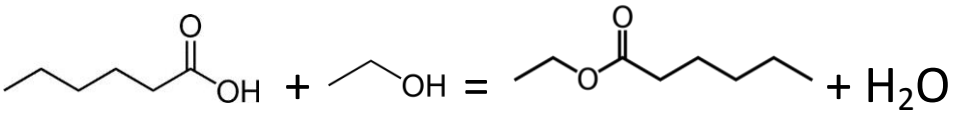


Butanoic acid Ethanol Ethyl butyrate

2.2.4. Ethyl hexanoate

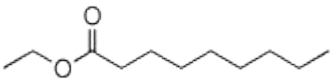


Ethyl hexanoate or caproate ($C_8H_{16}O_2$) is a volatile ester found in alcoholic beverages and produced during fermentation by yeast. Ethyl hexanoate is an ester which is present in all beers. Ethyl esters are formed by the reaction of ethanol with a fatty acid, hexanoic ester. Its boiling point is 168ºC. Ethyl hexanoate has an apple flavour. Ethyl hexanoate is responsible for flowery or fruity aromas [11]. Concentrations of ethyl hexanoate vary from beer to beer. Ethyl hexanoate is a key flavour impact character in some lagers and ales. The concentrations of ethyl hexanoate decrease over time as an alcoholic beverage ages due to spontaneous hydrolysis [10].

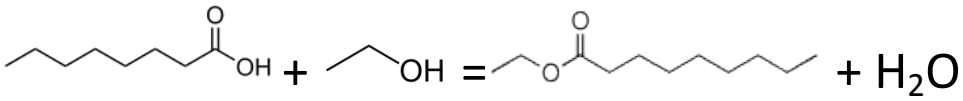


Hexanoic acid ethanol ethyl hexanoate

2.2.5. Ethyl octanoate



Ethyl octanoate or ethyl caprylate ($C_{10}H_{20}O_2$) is a volatile ester. It is a colourless liquid and its flavour is floral, fruity, like apricot, pear or pineapple. Its boiling point is 206 – 207 ºC. It is formed by the esterification reaction. The fatty acid is an octanoic acid and the alcohol is ethanol, the most common alcohol in beer.



Octanoic acid ethanol ethyl Octanoate

2.3. HIGHER ALCOHOLS

Besides ethanol beer contains several alcohols which are derived mainly from yeast fermentation. Also it can be formed by hops and malt, derived from the breakdown of malt and hops polyphenols. Many hops derived alcohols have a floral aroma, which can be a positive flavour in beer.

Most of the other alcohols are derived from yeast fermentations. They are called high alcohols, because their charger molecular weight, or fusel alcohols. In beer higher alcohols can content about 60-100 mg/L, but concentrations above 100mg/l can negatively affect the flavour of beer. Next, the table of some alcohols commonly present in beer.

Table 2.3-1: Principal esters constituents on a beer [6]

Alcohols	Concentration [mg/l]	Flavour descriptors
Ethanol	20000-80000	Alcoholic, strong
Methanol	0.5-3.0	Alcoholic, solvent
n-Propanol	3-16	Alcoholic
2-Propanol	3-6	Alcoholic
Isobutanol [12]	6-72	Alcoholic
Isoamylalcohol	8-30	Alcoholic, vinous, banana
Active amyl alcohol	30-70	Alcoholic, vinous, banana
2-Phenylethanol	8-35	Roses, bitter, perfumed

In our method, the higher alcohols, n-propanol, isobutanol and n-amyalcohol are analysed. N-amyl alcohol is not in the table because, n-amyalcohol can be present in beer but in very low quantities [13].

The amount of higher alcohols is correlated to the amount of alcohol. Beers with low percentage of alcohols have less content of higher alcohols and may be considerate a more agreeable beer with a better taste.

These alcohols are produced by yeast removing amino groups from the amino acids and replacing them with the –OH group. Therefore, the concentration of amino acids plays a very important role for the synthesis of higher alcohols.

Next, the formation reaction of higher alcohols is shown. In the generalized pathway R can be any amino acid side chain. This pathway also applies to the aldehydes. The second figure shows some pathways of the most common higher alcohols.

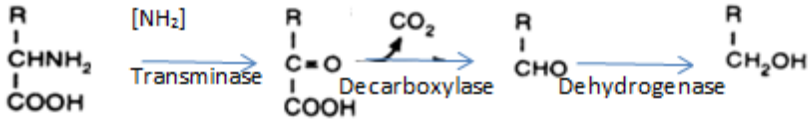


Figure 2.3-1: General pathway of formation of higher alcohols [1]

The most significant reason of the amount of higher alcohols is the yeast strain use. If the brewer uses ale strain, the beer has higher alcohols than if the brewer uses lager strain. Another thing that increases the amount of higher alcohols is the over oxygenation, the higher temperature or the low pressure in the fermentation.

The amount of assimilable nitrogen is really important in the production of higher alcohol. If the assimilable nitrogen is insufficient, the production increases due to the biosynthetic pathway. But if the wort has excess of assimilable nitrogen it increases the production too because of the Ehrlich pathway.

2.3.1. n-Propanol

n - Propanol or 1-Propanol ($CH_3CH_2CH_2OH$) is a primary alcohol. This colourless liquid has a boiling point of 97-98 ºC. n -Propanol is thought to be similar to ethanol in its effects on human body, but

2-4 times more powerful. The reaction of formation can be seen in the next figure.

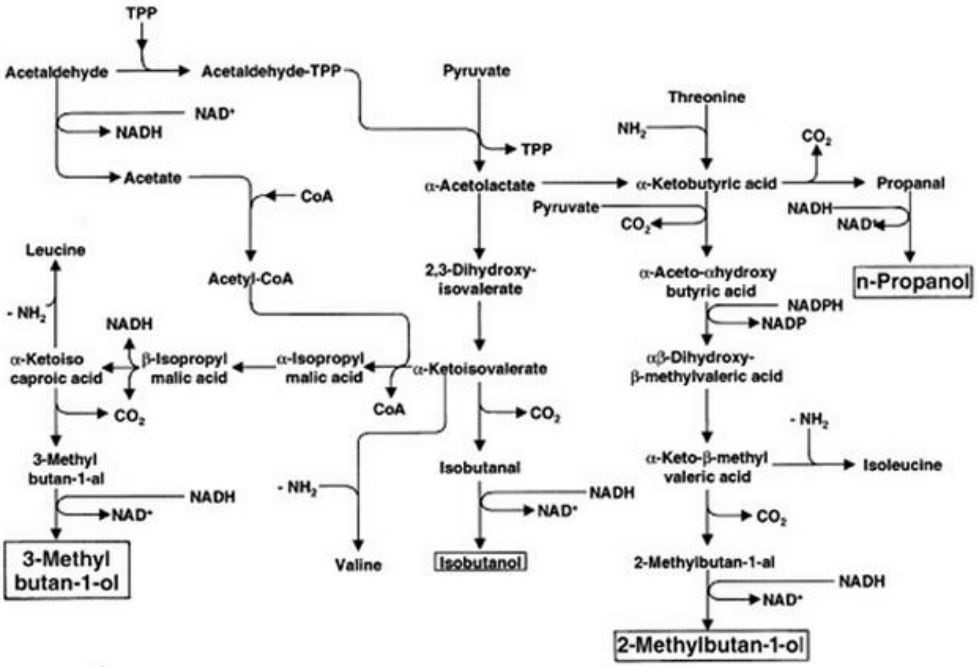
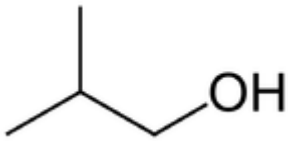


Figure 2.3-2: Biosynthetic routes for synthesis of some higher alcohols important to beer flavour and aroma. [14]

2.3.2. Isobutanol

Isobutanol or 2-methylpropan-1-ol ($(CH_3)_2CHCH_2OH$) is an organic higher alcohol present in beer. It is a colourless, flammable liquid with a characteristic smell. Its boiling point is 108ºC. The presence of isobutanol in beer is of great importance in the flavour profiles, as they generate desired fruity aromas in these products when produced in favourable amount. [15]



Isobutanol is a common by-product of yeast fermentation; it is the final product of the catabolism of valine in S.cerevisiae. However, isobutanol levels are very low and are dependent on the fermentation conditions. Next, it is the pathway of the valine degradation and the formation of the by-product isobutanol.

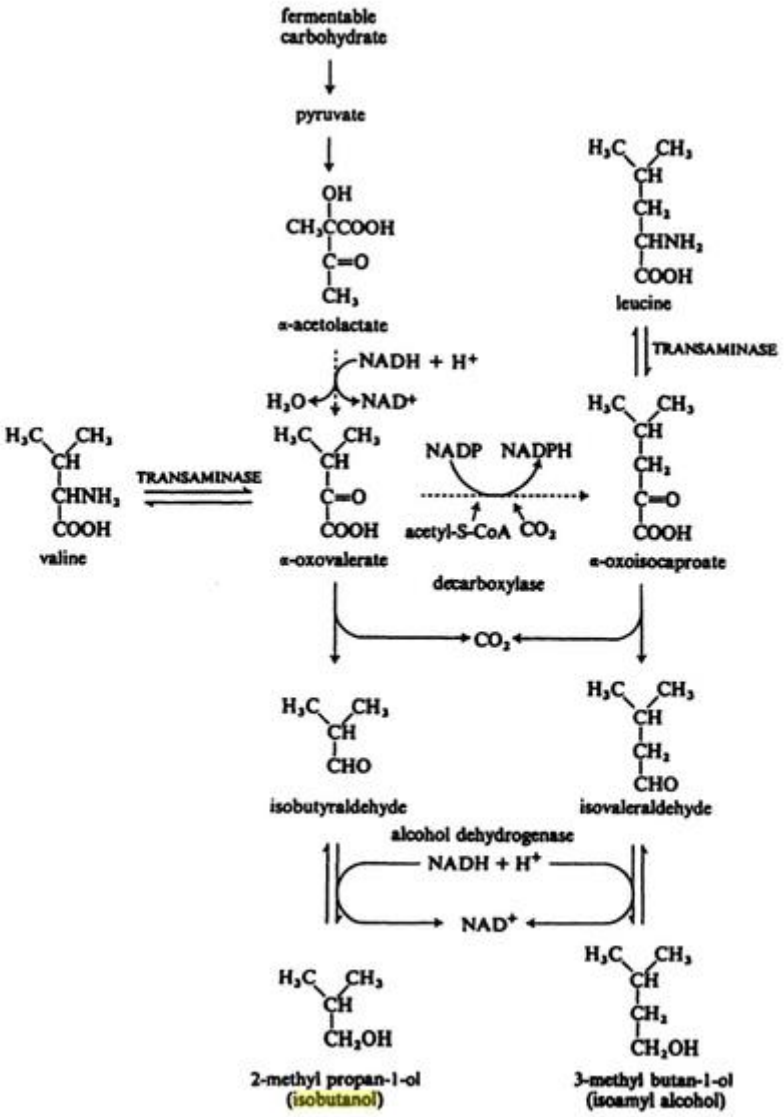


Figure 2.3-3: Formation of higher alcohols from carbohydrate metabolism and the biosynthesis of amino acid. [16]

2.3.3. N-Amyalcohol

Amyl alcohol is any of the alcohols with the formula $C_5H_{10}OH$. The most common amylalcohols in beer are the primary alcohols isoamyl alcohol and active amyl alcohol. In the method it is analysed the n-amylalcohol, also known as normal amyl alcohol or 1-Pentanol. It is a colourless liquid with unpleasant aroma. Its presence has been detected in trace amounts in yeast fermentation, but the biosynthetic pathway is not well understood. [17]

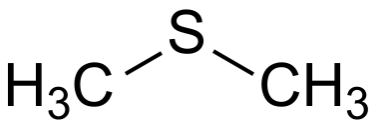
2.4. SULFUR COMPOUNDS

Sulfur compounds may be present in the raw materials, primarily in malt but they may also result from metabolism or from infecting microorganism.

Table 2.4-1: Principal sulfur compounds constituents on a beer [6]

Sulfur compounds	Concentration [µg/l]	Flavour descriptors
Dimethyl sulfide (DMS)	10-100	Sweet corn, boiled vegetables
Hydrogen sulfide	1-20	Sulfidic, rotten eggs
Sulfur dioxide	200-20000	Sufidic, burnt match

2.4.1. Dimethyl sulfide



Dimethyl sulfide or its abbreviation DMS ($(CH_3)_2S$) is an organosulphur compound. It is a water-insoluble flammable liquid. Its boiling point is 37ºC. It has a strong smell; it smells like the smell produced from cooking certain vegetables, notably maize, cabbage, beetroot and seafood. Opinion is divided if the DMS is desirable in beer. [18]

In general, however the level of DMS in beer is low and present under taste threshold of 50-60 ppb [5], but other brewers get paranoid if the level rises above 20 ppb, which is below the level of nose detection. The perception of DMS can be masked by phenylethanol.

DMS arises in beer via two routes. First, from S-methylmethionie (SMM) which decomposes to DMS on heating and, second, via the reduction of dimethyl sulphide (DMSO). The latter reaction is catalysed by yeast during fermentation. The common precursor of all DMS arising in beer is SMM and this derives from green malt.

In fermentation yeast reduces DMSO to DMS, but every yeast strain has different reducing capabilities. The DMSO reduction is inhibited by methionine sulphoxid (MetSO). If the pitching wort has a high pH this leads to more DMS production by yeast. If the fermenter vessel is evolved with CO_2, DMS have more volatilization. When the yeast doesn't have enough nitrogen, the DMSO reduction is higher. If the fermenter temperature is low, more DMSO converts into DMS. Usually DMS concentration in lager beer seems to be considerably higher than in ales because their malts are highly modified at very high temperatures that partly destroy the SMM. The lower DMS values in ales could also be explained by the higher temperatures used in top fermentation, which may give a more efficient carbon dioxide washout of volatiles during fermentation. [3]

Aside from the fermentation, other brewing steps influence the presence of DMS in the final product.

Where we can find the first factor that impacts the formation of DMS is in the grist. The only significant grist compound in the DMS production is the malted barley. A high concentration of nitrogen in barleys gives a high amount of SMM. Another factor that increases the SMM potential is to increase the vigour of barley during the storage.

In kilning, if the temperature is increased, the amount of SMM is reduced. At higher temperatures, SMM is partially converted into DMSO. It also produces MetSO. Therefore, ale malts contain more DMSO and MetSO and less SMM than lager malt.

In the mashing process if the temperature is insufficiently high it degrades much SMM. Also if during the sweet wort there is a contamination of *Enterobacter* it reduces the DMSO to DMS.

In boiling, SMM half-life at 100ºC is 38 minutes, every 6ºC decrease in temperature leads to double of its half-life. [3]The vigour of boiling impacts volatilization of DMS released from SMM.

Also another raw material contributes to increase the DMS: hop. It occurs when hop in oil form may have small quantities of DMS.

G. METHOD

Our reference to start working is Bavaria: its method “Quality Control: Inspection and testing beer. Aroma Compounds (Alcohols and esters) using gas chromatography”.

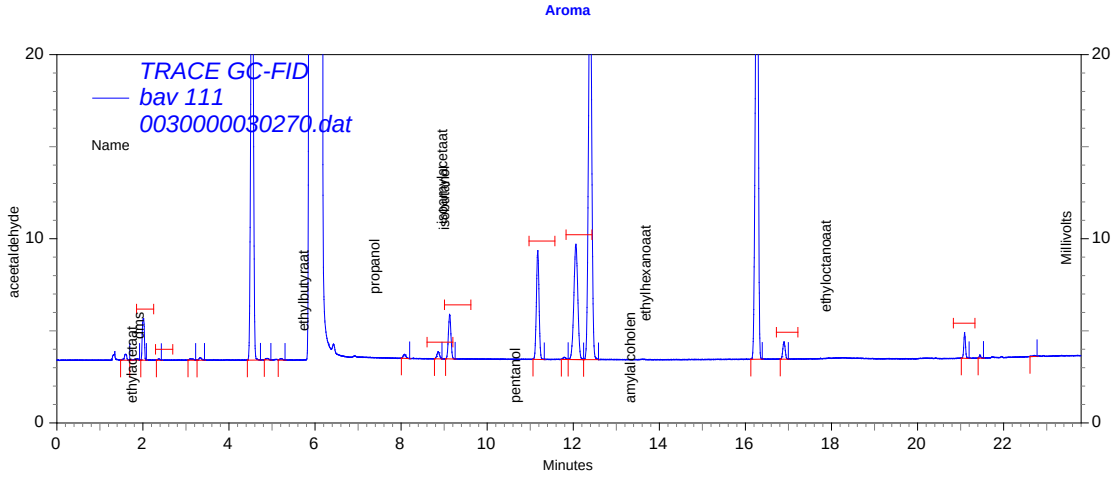
The aroma compounds are quantified using 2-pentanol as internal standard. The samples are first filtered for the removal of the yeast. 2 ml of the filtrate is pipetted into a vail of 8 ml. The sample is heated for 20 minutes at 60 º C. After reaching from a static balance using an autosampler a headspace sample is taken and is injected in the cromatograph. This is ultimately injected into the GC using a capillary column and a FID as detector. The components are separated and their concentrations are calculated using their peak ratios relative to the internal standard for the calibration is made of a standard addition method.

The difficulty of this method is that beer contains low concentrations of these compounds. This method permits to analyse all these ten compounds in only one chromatogram.

Compounds analysed

- Acetaldehyde
- Ethylacetal
- N-Propanol
- Isobutanol
- N-amyalcohol
- Ethylbutyrat
- Ethylhexanonate
- Ethyloctanoate
- Isoamyl acetate
- DMS

This is one example of the Bavaria chromatogram with all the compounds.



3. INSTRUMENTATION

The instrumentation of this analysis method is the Head Space sampling coupled with gas chromatography (HS – GC). This method is a widely used technique for the analysis of beer throughout the world. HS-GC is commonly used for quality control, to identify problems or changes occurring in the fermentation process.

The aromatic compounds, which we want to analyse, are extremely volatile. HS-GC is an ideal technique used for the concentration and analysis of volatile organic compounds, like the aroma compound we have to analyse.

One of the advantages of the headspace is that saves time and cost removing the non-volatile material, by directly sampling the volatile headspace from the container in which the sample is placed.

In GC, the mobile phase is a carrier gas, which could be nitrogen or helium. Both have the optimum characteristics of a mobile phase because they are inert and unreactive gases. The static phase is the material which the column is made of. In our case the column is made of polyethylene glycol (PEG). We use the column DB-WAX, which is one of the column types that has a lower upper temperature and a lower low temperature limit but exhibits better reproducibility and inertness. The gaseous compounds being analysed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound.

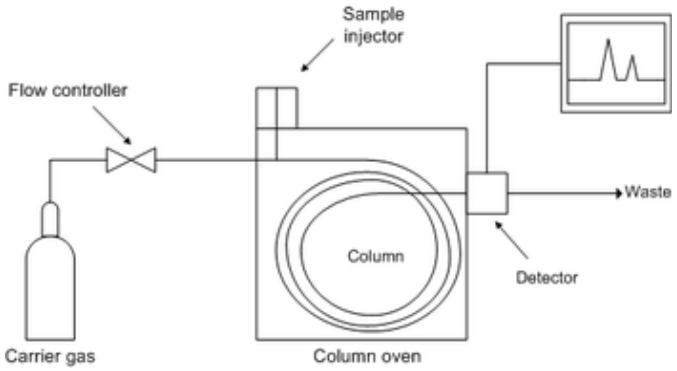


Figure 3-1: Diagram of gas chromatogaph

3.1. SAMPLE PREPARATION

Choosing the type of sample and how to be stored is a very important decision. To obtain a good chromatogram you have to consider a great number of things. The first thing to take into account is that samples must be prepared in such a manner as to maximize the concentration of the volatile sample compounds in the headspace while minimizing unwanted contamination from other compounds in the sample aroma mix. It is also good to know, that high-

concentration samples can produce ghost peaks in subsequent analyses due to carryover of sample from previous injections

For the analysis the sample is stored in vials. Sample vials should be selected to match the type and size of the sample being analysed. Always use pre-cleaned vials for sample preparation and storage. Vials that are not properly cleaned prior to packaging or that absorb contaminants during shipping can produce unknown chromatographic peaks, or “ghost peaks.” Ghost peaks that are the result of vial contamination can be identified by running method blanks.

3.2. Sample Vial Heater and Mixer

The compounds to be analysed by chromatography gases must be in gaseous state. Therefore, a liquid sample to be analysed by GC, must have the characteristic of maintaining its composition in aromatic compounds when it vaporizes. The vial sample is introduced in an oven to extract the volatile components from complex sample mixtures from non-volatile sample mixtures and isolated in the head-space or a gaseous phase in a sample vial. Both phases must have the same concentration in the volatile compounds, to have an accurate analysis. The gas phase (G in figure 3.2-1), commonly reared as the headspace, lies above the condensed sample phase. The liquid phase or also known as sample phase (S in figure 3.2-1) contains the compounds of interest and it is usually in the form of a liquid or solid in combination with a dilution solvent or a aroma mix modifier.

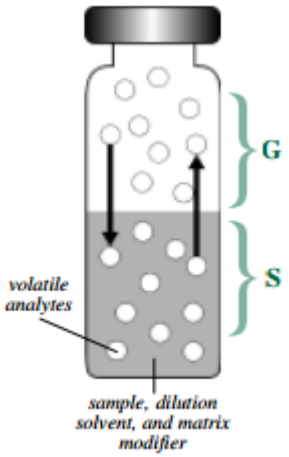


Figure 3.2-1 - Phases of a head space vial [19]

Temperature, time, and mixing can be used to improve the transfer of volatile analyses from the sample into the headspace of the vial. Adjusting the temperature of the sample will change the solubility in the analysis in the sample aroma mix and can be used to drive the equilibrium in favour of the gas phase. Shaking or vibrating the vial during heating can assist in achieving equilibrium.

3.3. SAMPLING

Before, the sample vial is heated in the oven, the headspace sample has to be injected in the column. There exist a few different methods of doing the injection, for example Gas - Tight Syringe injection, Balanced-Pressure System or Pressure-Loop System. We used the first one: Gas - Tight Syringe system.

The gas-tight syringe technique operates by initially thermostating the sample in an incubation oven at a given temperature and for a given time until it has reached a state of equilibrium (Figure 3.3-1, Step 1). Once the sample has reached an equilibrium, an aliquot is taken from the headspace using the gas-tight syringe (Figure 3.3-1, Step 2), and the aliquot is injected into the GC as if it were a liquid sample injection (Figure 3.3-1, Step 3).

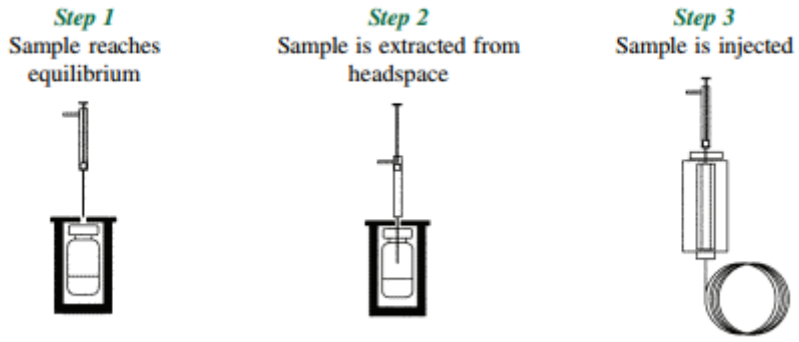


Figure 3.3-1 – Gas syringue system [19]

This technique has several problems. The sample is being transferred from a heated oven; the syringe also must be heated to ensure that the sample will not be recondensed in the syringe. There are also reproducibility issues because of possible sample loss. As the sample is transferred from the vial to the injection port, some of it may be lost because of the pressure differences between the vial and atmospheric conditions. To be sure that you are doing the injection correctly, you have to use a headspace auto samplers and the Leap Technology COMBI PAL. Also it helps to reduce problems to heat the syringe to a temperature comparable to the sample vial temperature. This minimizes pressure differences and condensation problems. To prevent carryover from inside the syringe, flush the syringe after each injection. Because gas-tight syringe samplers inject through the GC injection port septum, ensure the septum is well maintained to decrease the possibility of a leak

3.4. DETECTOR

The most commons detectors are FID and TCD. We have used FID, Flame Ionization detector. In this common detector electrodes are placed adjacent to a flame fuelled by hydrogen or air near the exit of the column, and when carbon containing compounds exit the column they are pyrolyzed by the flame. This detector works only for organic compounds. FID has low detection limits. FID compatible carrier gasses include nitrogen, helium, and argon

.

3.5. CHROMATOGRAPH CONDITIONS IN OUR METHOD

3.5.1. Chromatograph

First we used Focus GC but we had some problems with this chromatograph and for that reason we changed it for another chromatograph in the middle of the study in order to obtain better results. We changed it for Varian CP-3380 and this chromatograph brought about a huge improvement in the investigation.

3.5.2. Column

In our chromatograph we used the column DB-WAX with the following parameters: 60mx0.53mm 0.5µm. This is the most important change in this study compared to Bavarian method. Bavarian method uses the same column but with a different length, 30 m. This meant that our retention time was not the same as Bavaria method.

3.5.3. Gas carrier

First we used nitrogen as a carrier gas, but the results were not correct and the retention time was too different from Bavaria method. For that reason it is a possible to change the carrier gas for hydrogen like Bavaria method. But we still using nitrogen as a gas carrier.

3.5.4. Oven temperature

In this method the oven temperature increases during the chromatograph progress. There are some little changes in our method from Bavaria method and those differences are shown in the following tables.

- **Bavarian method**

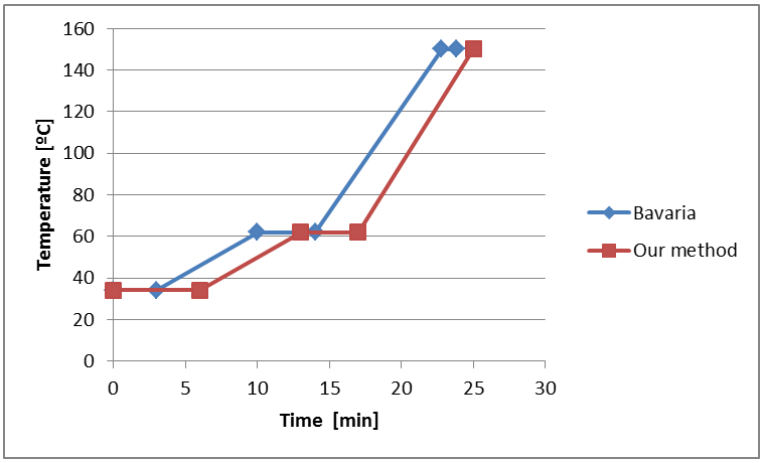
	Rate	Temperature	Hold time
	ºC/min	ºC	Min
Initial		34	3
Ramp 1	4	62	4
Ramp 2	10	150	1
Total			23,8

- **Our method**

	Rate	Temperature	Hold time
	ºC/min	ºC	Min
Initial		34	6
Ramp 1	4	62	4
Ramp 2	11	150	0
Total			25

Following there is a graphic which compares the Bavaria oven temperature in all the process and our method. It could be observed that the temperatures change a little Bavaria chose the correct temperature for each peak compound in the correct time. The new temperature program was chosen to obtain more or less the same retention time for ethanol as in the

Bavaria method. It is optional to investigate in the future other time programs to get a better separation between certain compounds.



3.5.5. FID

Our FID temperature is 250ºC and it is higher than 170ºC from Bavaria method. We changed the FID detector in order not to have problems with noise or spiking.

3.5.6. Autosampler

The Bavaria method indicates to preheat the samples in the autosampler oven at 60ºC, 20 minutes with agitation 500 r.p.m. We followed this indication but the COMBIPAL broke down in the middle of the study and for that reason we did the injection manually. By injecting the sample with this technic it is possible to have some human error. Not all the samples were heated during the same amount of time and this meant that possibly not every sample had the same amount of head space phase. Also with manual injection we did not have the same precision as when it was done with automatic injection, and we did not inject always the same volume of sample in the chromatograph.

3.5.7. Observations

When you work with gas chromatography there are many parameters to consider. When you start a method for the first time, you spent long time during the first few months searching the cause of the failings and trying to fix them. Our first attempts were a disaster and until we realized that the problem was the syringe, we could not progress efficiently.

4. Standard Addition Method

In the standard addition method, the analysis of the original sample is followed by the analysis of the sample to which known amounts of the analyte are added; all measurements are carried out under identical conditions. This method is used in situations where sample aroma mix also contributes to the analytical signal, a situation known as the aroma mix effect, thus making it impossible to compare the analytical signal between sample and standard using the traditional calibration curve approach.

To determinate the concentration of the original sample, first you have to analyse one sample without the aroma mix solution but adding the internal standard. In this chromatogram it will be seen the original concentration of the beer.

The following step is analysing the beer with internal standard and with some amount of the aroma mix solution. In the different standards, it will be increased the amount of the aroma mix solution in order to do a calibration line and finally find the original concentration. In the chromatograms there are several peaks belonging to different components, and knowing the area of each peak in each sample a calibration curve for each component can be created, so we can finally find all concentrations.

Next, I will show a theoretical example in order to show how calibration curves have been created.

4.1. Theoretical example

In this example two compounds and one internal standard will be analysed. The sample is beer, like our method, and that is why solutions are made in ethanol solution.

Retention Time

- Compound A: 0.5 min
- Compound B: 1.3 min
- Internal standard: 1.6 min

Aroma mix concentration

- Compound A: 2 ppm
- Compound B: 0.5 ppm

Samples composition

Standard	Beer sample [ml]	Internal standard [ml]	Aroma mix [ml]	Ethanol solution [ml]
1	1	0.4	0	1.6
2	1	0.4	0.3	1.3
3	1	0.4	0.6	1.0
4	1	0.4	0.9	0.7
5	1	0.4	1.2	0.4

All the vials have to have the same volume. In this example all the vials have 2 ml.

Then, the following step is to calculate each concentration, of compound A and B.

Standard	Concentration Compound A [ppm]	Concentration Compound B [ppm]
1	0	0
2	0,3	0,075
3	0,6	0,15
4	0,9	0,225
5	1,2	0,3

- Standard 1

$$\frac{0.000\ ml\ matrix}{2.000\ ml\ total} \cdot \frac{2\ mg}{1000\ ml} = 0.000\ ppm$$

- Standard 2
  - Compound A

$$\frac{0.3\ ml\ matrix}{2.000\ ml\ total} \cdot \frac{0.5\ mg}{1000\ ml} = 0.075\ ppm$$

After this previous calculation, the analyses could be done. It is good to duplicate the analyses with each standard to make sure the results are correct.

Results

Standard	Compound A		Compound B		Internal Standard	
	Time [min]	Peak Area	Time [min]	Peak Area	Time [min]	Peak Area
1	0,48	300	1,28	1600	1,59	1500
2	0,51	650	1,31	3000	1,6	2250
3	0,5	750	1,3	3300	1,61	2025
4	0,49	650	1,29	2700	1,58	1400
5	0,5	900	1,29	3500	1,61	1600

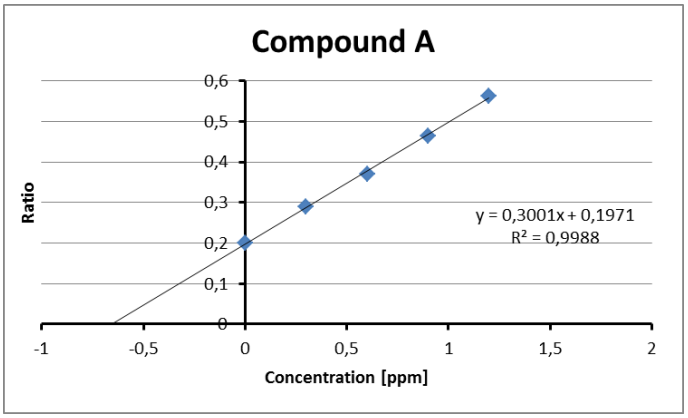
These are the results and now to create the calibration curves the peak area of the compound has to be divided by the peak area of internal standard.

$$ratio = \frac{Peak\ Area\ compound}{Peak\ Area\ Intenal\ Standard}$$

Calculation

Compound A

	Compound A	
Standard	Concentration [ppm]	Ratio
1	0	0,2
2	0,3	0,28888889
3	0,6	0,37037037
4	0,9	0,46428571
5	1,2	0,5625



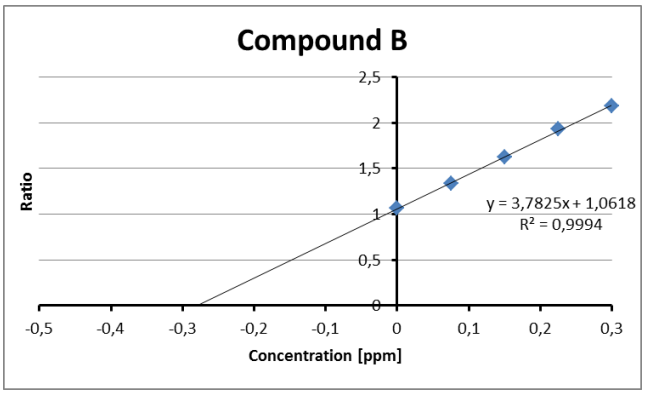
Linear regration: Y= 0.3001x +0.1971

Concentration beer: 0=0.3001· [concentration] + 0.1971

→ Concentration = 0.1971/0.3001=**0.657 ppm**

Compound B

	Compound B	
Standard	Concentration [ppm]	Ratio
1	0	1,06666667
2	0,075	1,33333333
3	0,15	1,62962963
4	0,225	1,92857143
5	0,3	2,1875



Linear regration: Y= 3.7825x+1.0618

Concentration beer: 0=3.7825· [concentration] + 1.0618

→ Concentration = 1.0618/3.7825=**0.281 ppm**

5. DETERMINATION OF RETENTION TIMES

The first step of validation of this method is to determinate the retention times of all the compounds which have to be analysed in the method.

To determinate the concentration times we analyse a vial with only one compound each in ethanol solution. We are using ethanol solution in order to recreate the beer solution.

These analyses also are used to check whether the conditions of the chromatograph are correct and whether the chromatograph detects all the compounds. We will duplicate these analyses to be sure that the retention time is right. It is very important that this data is reliable because later we will use it to identify each peak.

The vial concentration to determinate the retention time is right in the middle of the range of concentration we need in the standards to be more useful and have the size peaks we will need later. If the peaks are not good, they are too small or they do not appear in the chromatogram, then we have to look for where is the problem and solve it.

5.1. PREPARATION OF SAMPLES FOR DETERMINATE THE RETENTION TIMES

5.1.1. Reagents

- Acetaldehyde 780 – 784 g/l 99%
- Ehylacetate 901-904g/l 99.5% Emsure (Merk) 607-022-00-5
- N-Propanol 804g/l 99.5% Sigma-Aldrich 3359
- Iso-butanol 803-804 g/l 99% Emsure (Merk) 603-108-00-1
- N-Amylalcohol (1-Pentanol) 810 g/l Merk 973
- Ethyl butyrat 878-879 g/l 98% Merk 800500.0100
- Ethyl hexanonate 869 g/l 99% Acros- Organics 16499500
- Ethyl octanoate 878 g/l 99%Acros- Organics 14,37784
- Iso-amylacetate 720-730 g/l 98% Merk 1231

- DMS 99% 1100 g/L
- 2-Pentanol

5.1.2. Vials

A) Preparation of the flasks.

- *Acetaldehyde*

  Flask 1C: 200 µL Acetaldehyde in 50.00 mL Et-OH 50%

  Flask 1D: 200 µL Flask 1C in 100.00 mL Et-OH 5% (6.2088 ppm)

- *Ethyl acetate*

  Flask 2C: 400 µL Ethylacetate in 50.00 mL Et-OH 50%

  Flask 3D: 200 µL Flask 2C in 100.00 mL Et-OH 5% (14.368 ppm)

- *n-propanol*

  Flask 3C: 200 µL n-propanol in 50.00 mL Et-OH 50%

  Flask 3D: 200 µL Flask 3C in 100.00 mL Et-OH 5% (6.400 ppm)

- *Isobutanol*

  Flask 4C: 200 µL Isobutanol in 50.00 mL Et-OH 50%

  Flask 4D: 200 µL Flask 4C in 100.00 mL Et-OH 5% (14.368 ppm)

- *n-amylalcohol*

  Flask 5C: 2000 µL n-amylalcohol in 50.00 mL Et-OH 50%

  Flask 5D: 200 µL Flask 5C in 100.00 mL Et-OH 5% (32.076 ppm)

- *Esters solution*

  Flask A (Esters initial solution)

  100.00ml Et-OH 50%
  - 0.4 gr. Ethylbutyrat → 0.464 mL
  - 0.4 gr. Ethylhexanonate → 0.465 mL
  - 0.4 gr. Ethyloctanoate → 0.460 mL

  Flask 6C: 500 µL Flask A in 50.00 mL Et-OH 50%

  Flask 6D: 200 µL Flask 6C in 100.00 mL Et-OH 5% (0.08ppm)

- *DMS*

  Flask C: 250 µL Isoamylacetate in 100.00 mL Et-OH 50%

  Flask 8C: 800 µL Flask B in 50.00 mL Et-OH 50%

  Flask 8D: 200 µL Flask 7C in 100.00 mL Et-OH 5% (0.014ppm)

- *Isoamylacetate*

  Flask C: 4000 µL Isoamylacetate in 100.00 mL Et-OH pure

  Flask 8C: 500 µL Flask C in 50.00 mL Et-OH 50%

  Flask 8D: 200 µL Flask 8C in 100.00 mL Et-OH 5% (0.682ppm)

B) Preparation of the vials

In every vial we put 2000 µL of the flask D (1D, 2D, 3D, 4D…) and 2000 µL of 2-Pentanol

5.1.3. Results: Retention times

Identification peak			Avarege [min]
	1D1	1D2	
Et-OH	5,805	5,787	
Acetaldehyde	3,502	3,493	3,4975
	2D1	2D2	
Et-OH	5,826	5,648	
Ethylacetate	4,913	4,783	4,848
	3D1	3D2	
Et-OH	5,818	5,825	
N-propanol	8,811	8,813	8,812
	4D1	4D2	
Et-OH	5,835	5,825	
Isobutanol	10,936	10,903	10,9195
	5D1	5D2	
Et-OH	5,812	5,787	
N-amylalcohol	18,247	18,205	18,226
	6D1	6D2	
Et-OH	5,83	5,887	
Ethybutyrat	8,702	8,693	8,6975
Ethylhexanoat	17,424	17,402	17,413
Ethyloctanoat	23,628	23,618	23,623
	7D1	7D2	
Et-OH	5,812	5,821	3,6725
DMS	3,674	3,671	
	8D1	8D2	
Et-OH	5,77	5,802	
Isoamylacetate	11,94	12,005	11,9725
	Is.1	Is.2	
I.S	12,005	12,007	12,006

6. STANDARDS PREPARATION

When the retention times are determined, then it is the moment to start the analyses.

6.1. AROMA MIX PREPARATION

In a volumetric flask of 50.00 ml with Et-OH (50%). Take out 3.800 mL. Cool till -18ºC

- 0.200 ml Acetaldhyde
- 0.400 ml Ethylacetate
- 0.200 ml n-propanol
- 0.200 ml isobutanol
- 1.000 ml n-amylalcohol
- 0.500 ml Solution with esters (Flask A)

- 0.800 ml DMS solution (Flask B)
- 0.500 ml Isoamylacetate (Flask C)

6.2. *Internal Standard Aroma Preparation*

- 2.000 L make 50% Et-OH. Take out 2.500 mL and add 2.500 ml of 2-Pentanol. To store internal standard aroma, put each time 6.000 ml of this I.S solution Aroma in vials of 10 mL and store at -18ºC.

6.3. *Standards Preparation*

When aroma mix and internal standard aroma is made, you are ready to prepare the standard vials which are the vials to be analysed in the method. In each vial there has to be the same amount of beer of which we want to know the aromatic compounds concentration. Also in each vial there is the same volume of internal standard to calculate the ratio. The only paramater that changes is the amount of aroma mix because then it is possible to create the calbritation curves. In this study the vials have been prepared as the next table shows.

For the preparation, beer is been cooled in the fridge, before introducing it in the vial, it has been filtred in order to remove CO_2. It has been filtred in 597 ½ SaS Ø150 mm filter.

Standard	Beer [µl]	Aroma mix Internal Standard [µL]	5 % Et-OH [µL]	Aroma mix [µL]
1	2000	100	200	0
2	2000	100	180	20
3	2000	100	160	40
4	2000	100	140	60
5	2000	100	120	80
6	2000	100	100	100
7	2000	100	80	120

All the vials have the same volume, 2.3 mL. This parametre changes from Bavaria Method because Bavaria method use vials with 2.0 mL, and this means that this method concentration is a little different from Bavaria method.

Next, the calculation of the concentration of each standard is shown.

1- First we have to know the concentration of flask A (Esters solution), Flask B (DMS solution) and Flask C (Isomaylacetate solution)

	Density [g/L]	ml in 100 ml	Purity [%]	g/L
DMS	850	0,25	99	2,10375
Isoamylacetate	870	4	99	34,452
		g in 100 mL		
Ethylbutyrate	-	0,4	-	4
Ethylhexanoate	-	0,4	-	4
Ethyloctanoate	-	0,4	-	4

The Ester solution information has been given in mass units, which is the only exception since all other information for preparation is in volumetric unities.

1- The second step is calculating the concentration of aroma mix. This concentration is made in a 50.00 mL flask.

Compounds	density [g/L]	ml in mix aroma	Purity %	Concentration mg/L
Acetaldhyde	782	0,2	99	309,672
Ethylacetate	902,5	0,4	99,5	718,39
N-propanol	800	0,2	99	316,8
Isobutanol	802,5	0,2	99	317,79
N-amylalcohol	810	1	99	1603,8
	Concentration [g/L]			
Ethylbutyrate	4	0,5	-	40
Ethylhexanoate	4			40
Ethyloctanoate	4			40
DMS	2,10375	0,8	-	33,66
Isoamylacetate	34,452	0,5	-	344,52

2- In the last table the concentrations in the standard vials are calculated. This information is used afterwards to create the calibration curves.

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standar 6	Standard 7
Beer µl	2000	2000	2000	2000	2000	2000	2000
I.S µl	100	100	100	100	100	100	100
Et-OH 5%	200	180	160	140	120	100	80
Aroma mix	0	20	40	60	80	100	120
Concentration ppm							
Acetaldehyde	0,000	2,693	5,386	8,078	10,771	13,464	16,157
Ethylacetate	0,000	6,247	12,494	18,741	24,987	31,234	37,481
N-propanol	0,000	2,755	5,510	8,264	11,019	13,774	16,529
Isobutanol	0,000	2,763	5,527	8,290	11,054	13,817	16,580
N-amylalcohol	0,000	13,946	27,892	41,838	55,784	69,730	83,677
Ethylbutyrate	0,000	0,348	0,696	1,043	1,391	1,739	2,087
Ethylhexanoate	0,000	0,348	0,696	1,043	1,391	1,739	2,087
Ethyloctanoate	0,000	0,348	0,696	1,043	1,391	1,739	2,087
DMS	0,000	0,293	0,585	0,878	1,171	1,463	1,756
Isoamylacetate	0,000	2,996	5,992	8,987	11,983	14,979	17,975

This is an example how the standard calculation has been made. It is for the acetaldehyde, but for other compounds, the calculation procedure is identical.

- Standard 1

$$\frac{0.000\ ml\ Aroma\ mix}{2.300\ ml\ total} \cdot \frac{309.672\ mg}{1000\ ml} = 0.000\ ppm$$

-
- Standard 2

$$\frac{0.020\ ml\ Aroma\ mix}{2.300\ ml\ total} \cdot \frac{309.672\ mg}{1000\ ml} = 2.693\ ppm$$

- Standard 3

$$\frac{0.040\ ml\ Aroma\ mix}{2.300\ ml\ total} \cdot \frac{309.672\ mg}{1000\ ml} = 5.386\ ppm$$

- Standard 4

$$\frac{0.060\ ml\ Aroma\ mix}{2.300\ ml\ total} \cdot \frac{309.672\ mg}{1000\ ml} = 8.076\ ppm$$

- Standard 5

$$\frac{0.080\ ml\ Aroma\ mix}{2.300\ ml\ total} \cdot \frac{309.672\ mg}{1000\ ml} = 10.771\ ppm$$

- Standard 6

$$\frac{0.100\ ml\ Aroma\ mix}{2.300\ ml\ total} \cdot \frac{309.672\ mg}{1000\ ml} = 13.464\ ppm$$

- Standard 7

$$\frac{0.120\ ml\ Aroma\ mix}{2.300\ ml\ total} \cdot \frac{309.672\ mg}{1000\ ml} = 16.157\ ppm$$

H. RESULTS AND DISCUSSION

When we got a proper chromatogram, we decided to do four rounds, analysing all the standard twice, with samples of beer. The beer sample was the Belgian beer Jupiler and we prepared all the standards following the protocol.

Firstly, we prepared the first fourteen standards, twice the seven standards, of the first bottle. We called those samples 'beer 1'. Then, on a different day we extracted the sample from another bottle, but also it was a Jupiler beer, with the second bottle we did forty-two standards, fourteen of 'beer 2.1', fourteen of 'beer 2.2' and fourteen of 'beer 2.3'. With all these analyses we tried to verify the method.

Next is it a list of the main problems we had of identification and determination of peak areas:

- Due to the manual injection, sometimes retention times were not as accurate as they should be for identifying peaks. Sometimes it caused some confusion.
- When the acetaldehyde and DMS are in low concentrations it may be difficult to do the peak identification because they are surrounded by other peaks that are considerate discardable.
- The ethylbutyrate and n-propanol peaks are really close and sometimes are impossible to divide the two peaks to calculate the area.
- Some chromatograms have an unknown and unexpected big peak around 16 minutes.
- In my opinion, the biggest problem is that isoamylacetate and internal standard have a very similar retention time, most of the times there was only one peak. This caused a lot of problems because we were using an internal standard method to do the calculation, and if we are not sure to be able to determine the internal standard area all the concentrations will be wrong.

All the figures and chromatograms are in the annex, but here it is shown the calibration curves of the first three compounds as an example of all aromatic compounds.

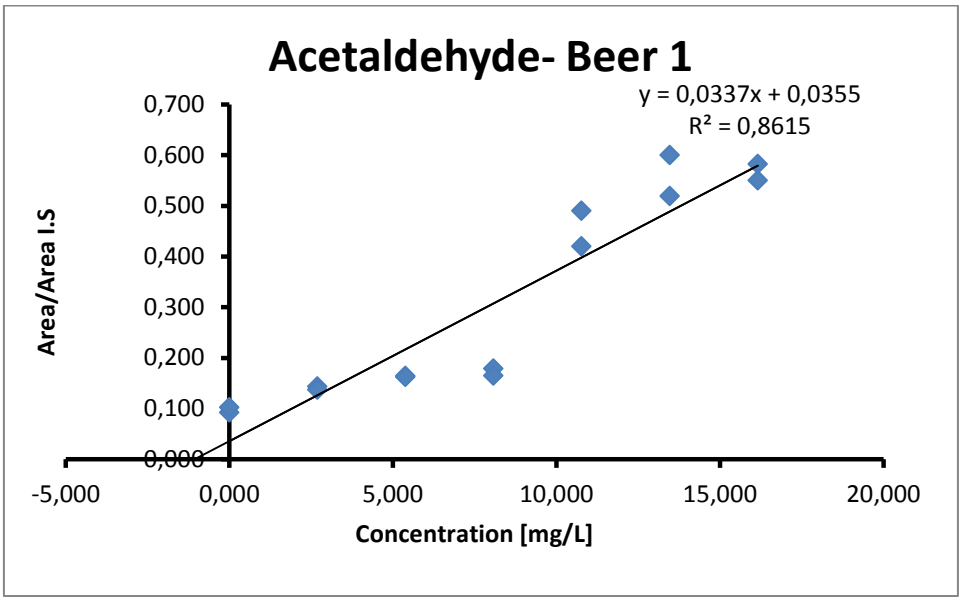
7. *ACETALDEHYDE CALIBRATION CURVE*

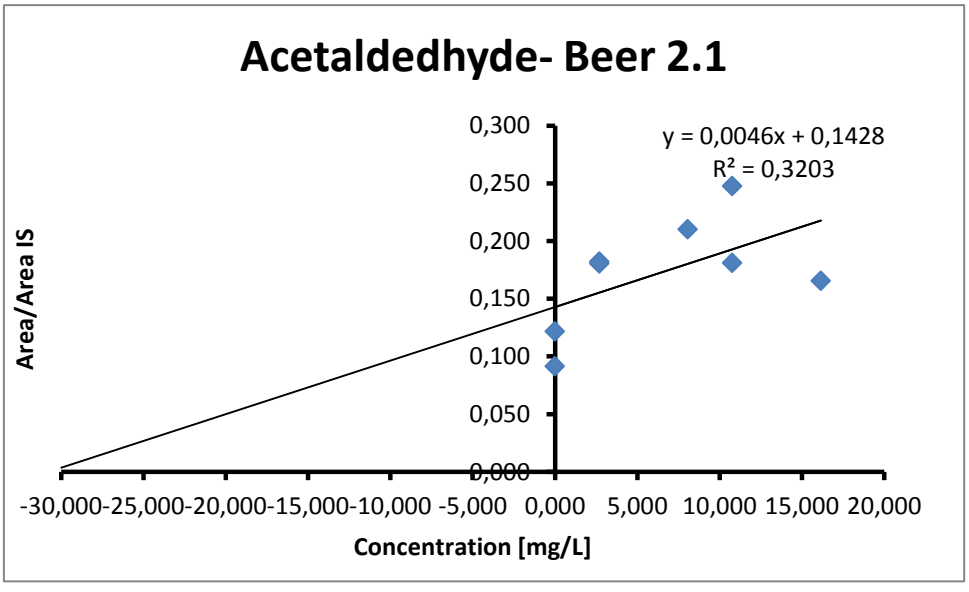
In the following table it is shown all the ratios of all standards. We used this table to create the calibration curves. We removed some figures in order to have an acurate calibration curve. The removed figures are also in the annexes.

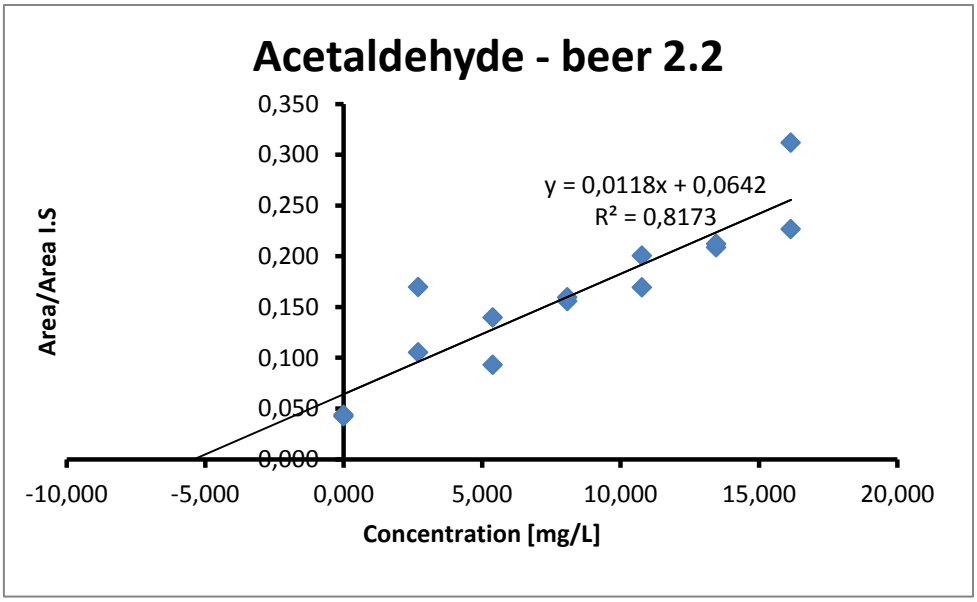
		Ratio: Area/Area I.S			
Standard	Concentration [mg/L]	beer 1	beer 2.1	beer 2.2	beer 2.3
1.1	0	0,09196	0,12155982	0,0421757	0,04698409
1.2	0	0,102	0,09134656	0,04380432	0,04499144
2.1	2,6928	0,14319	0,18218346	0,16983103	0,07813468
2.2	2,6928	0,13702104	0,18049089	0,10523842	0,07291367
3.1	5,3856	0,164	-	0,13983676	0,1025886
3.2	5,3856	0,162	-	0,09302025	0,13995813
4.1	8,0784	0,1785	0,21	0,15589204	0,25788946

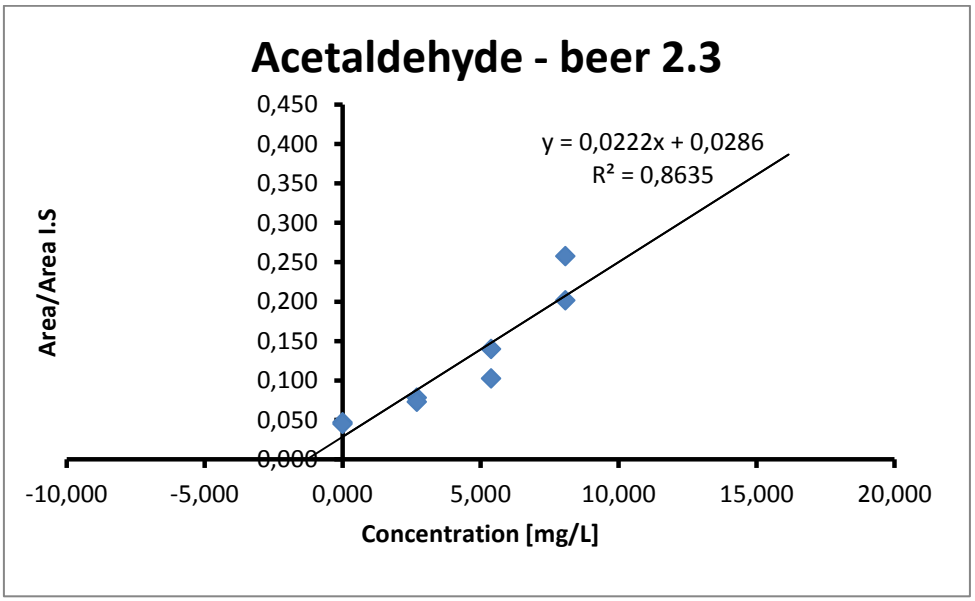
4.2	8,0784	0,1644	-	0,15972128	0,20147447
5.1	10,7712	0,49	-	0,20061871	-
5.2	10,7712	0,42	0,1810938	0,1693701	-
6.1	13,464	0,6	-	0,20871763	-
6.2	13,464	0,5188	-	0,21217666	-
7.1	16,1568	0,55	0,16543975	0,22672527	-
7.2	16,1568	0,582	0,3	0,31191268	-

Next, it is the four calibration curves of acetaldehyde.









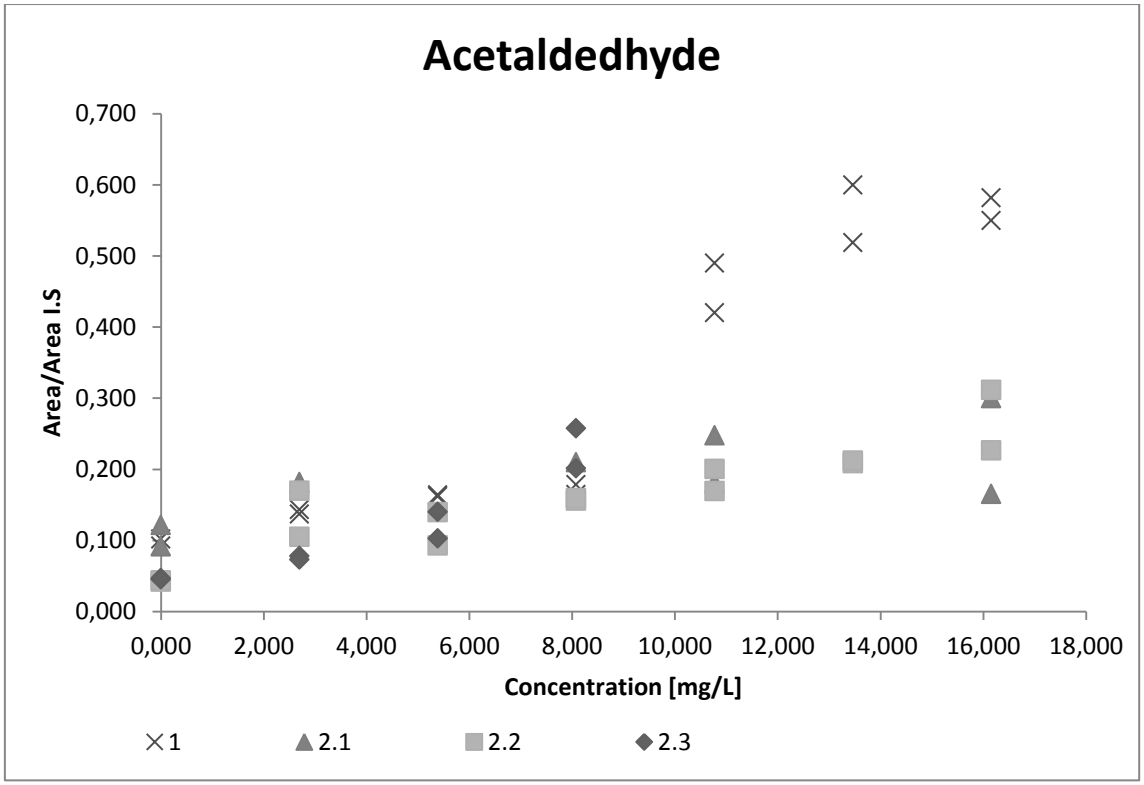
With all the calibration curves we designed the following table.

$$y = a \cdot x + b: Concentration = b/a$$

Beer	A	B	R^2	Concentration [ppm]
1	0,0337	0,0355	0,8615	1,053
2.1	0,0068	0,1428	0,3202	21,000
2.2	0,0198	0,0642	0,8173	3,242
2.3	0,0222	0,0286	0,8635	1,288

The regression coefficients are really low, and we had to reject several figures to create these calibration curves. The concentrations are very different and for these results we are not sure what is the acetaldehyde concentration in Juplier beer.

Next, it is shown a graphic with all the ratios points. In this graphic you can see all the variaty of ratios in each concentration.

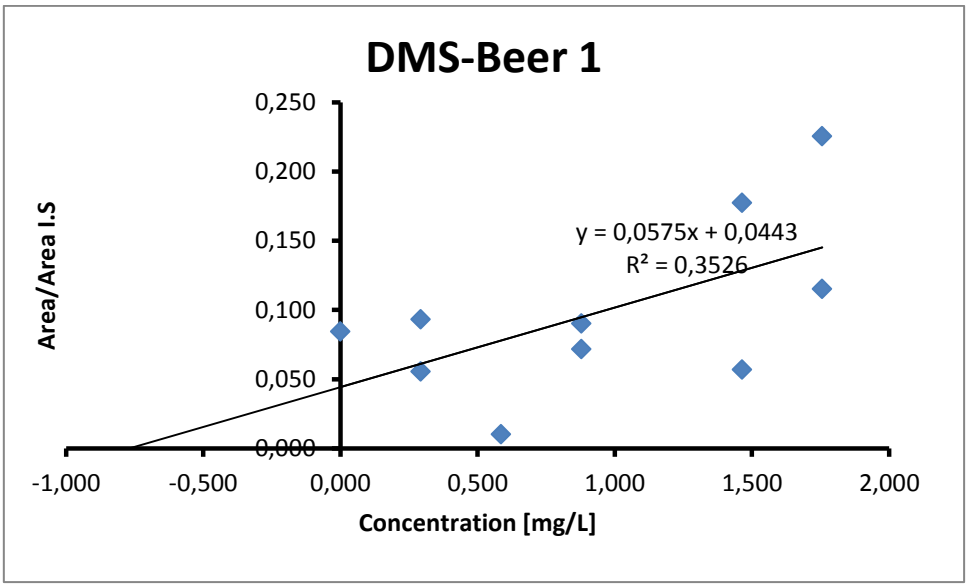


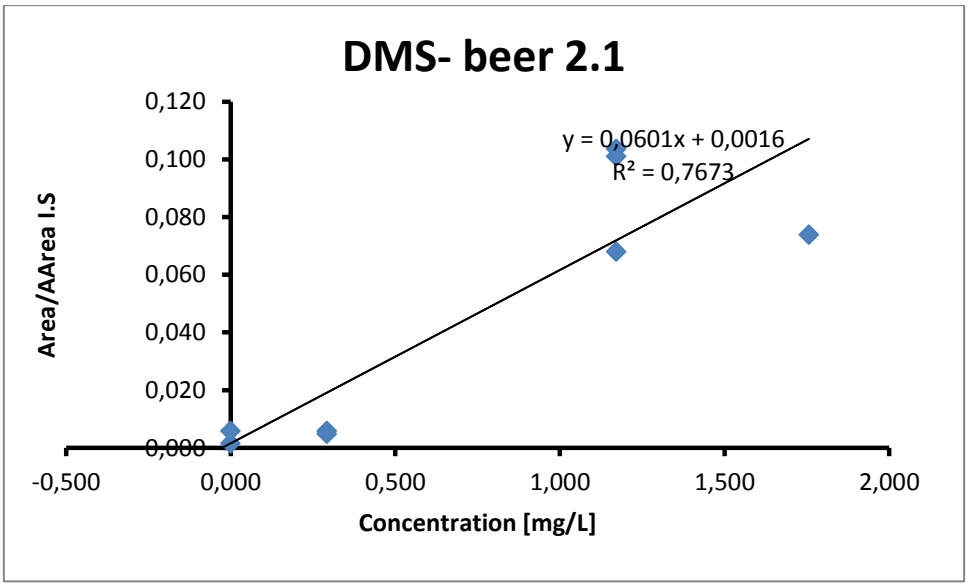
8. *DMS CALIBRATION CURVE*

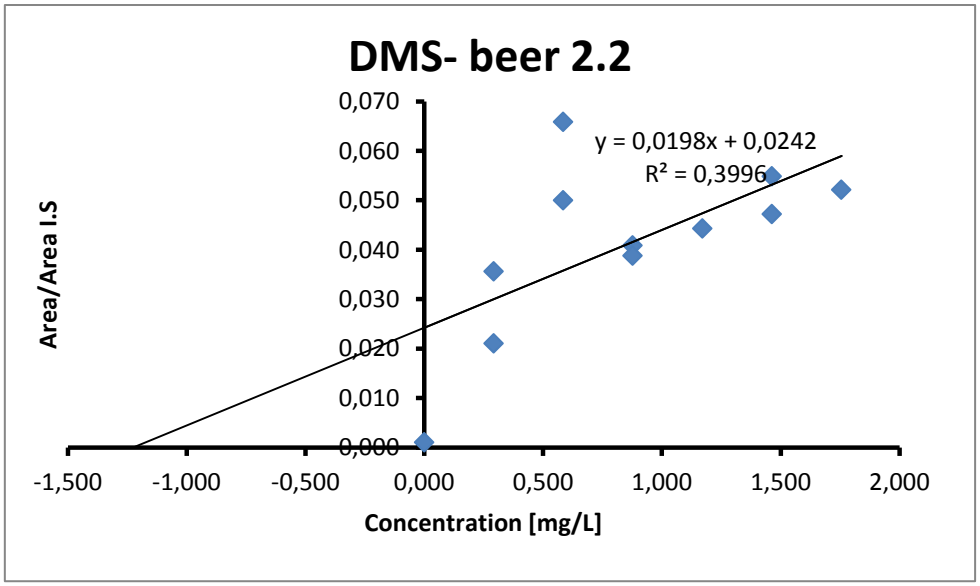
In the following table it is shown all the ratios of all standards. We used this table to create the calibration curves. We removed some figures in order to have an acurate calibration curve. The removed figures are in the annexes.

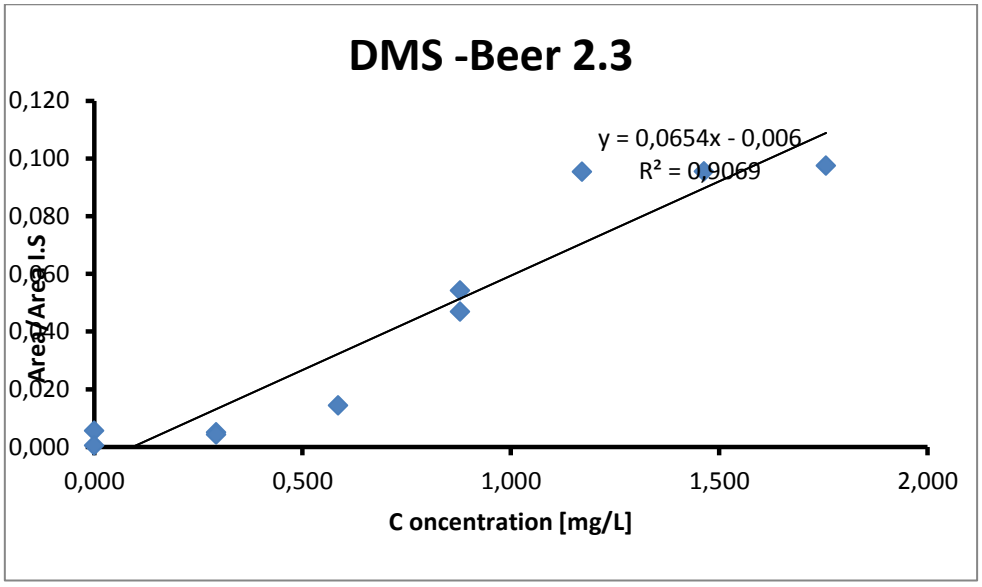
		Ratio: Area/Area I.S			
Standard	Concentration [mg/L]	beer 1	beer 2.1	beer 2.2	beer 2.3
1.1	0,000	0,085	0,001		0,006
1.2	0,000	0,069	0,006	0,001	0,000
2.1	0,293	0,094	0,006	0,036	0,005
2.2	0,293	0,094	0,005	0,021	0,004
3.1	0,585	0,116		0,066	0,014
3.2	0,585	0,106			
4.1	0,878	0,102		0,041	0,047
4.2	0,878	0,106		0,039	0,054
5.1	1,171	0,195	0,104		0,095
5.2	1,171	0,204	0,068	0,044	
6.1	1,463	0,170		0,047	0,095
6.2	1,463	0,160		0,055	
7.1	1,756	0,226	0,074	0,052	0,097
7.2	1,756	0,228			

Next, it is the four calibration curves of DMS.









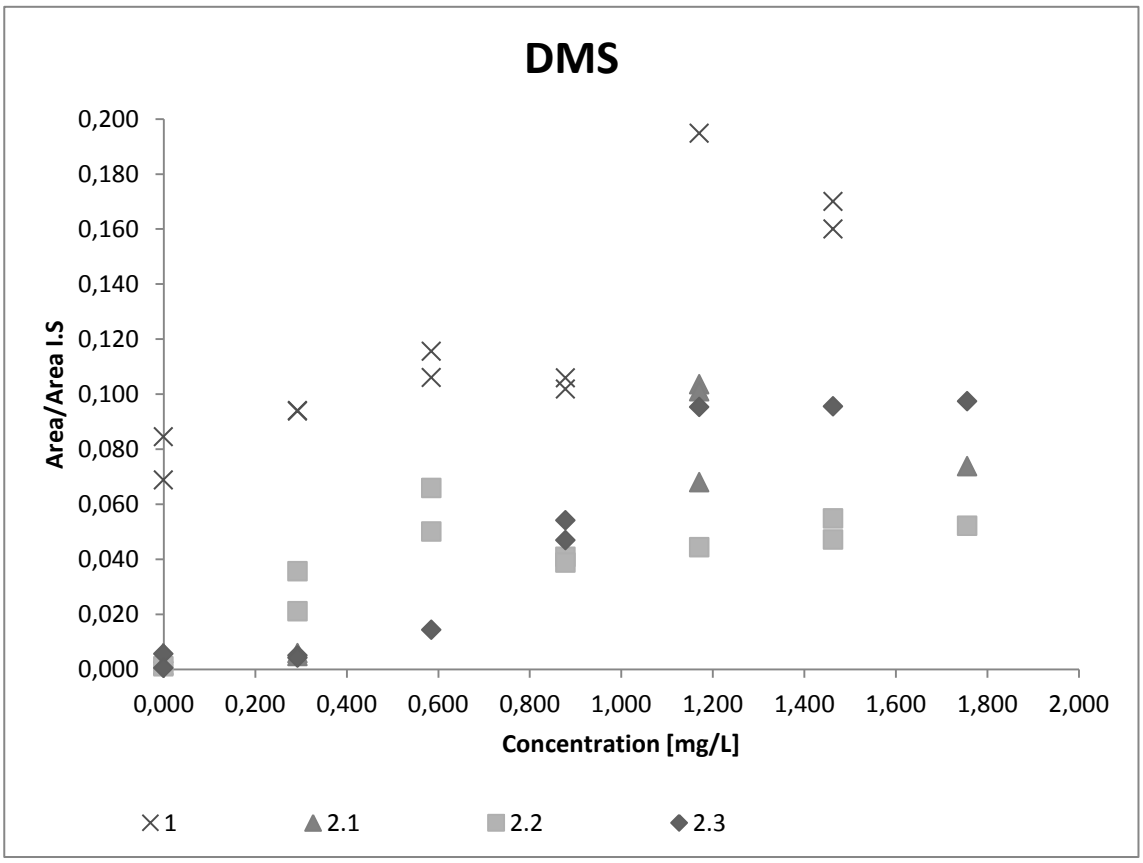
With all the calibration curves we designed the following table.

$$y = a \cdot x + b: Concentration = b/a$$

Beer	a	B	R^2	Concentration
1	0,0575	0,0443	0,8615	0,77043478
2.1	0,0601	0,0016	0,7673	0,0266223
2.2	0,0198	0,00242	0,3996	0,12222222
2.3	0,0654	-0,06	0,906	-0,91743119

The regression coefficients are really low, and we had to reject several figures to create these calibration curves. The DMS peak areas are very low, and the lower the area peak, the bigger the mistake. The concentrations are very different, for thess calibration curves we are not sure what is the DMS concentration in Juplier beer. The 'beer 2.3' is a good calibration curve, but the concentration result is negative.

Next, it is shown a graphic with all the ratios points. In this graphic you can see all the variaty of ratios in each concentration.



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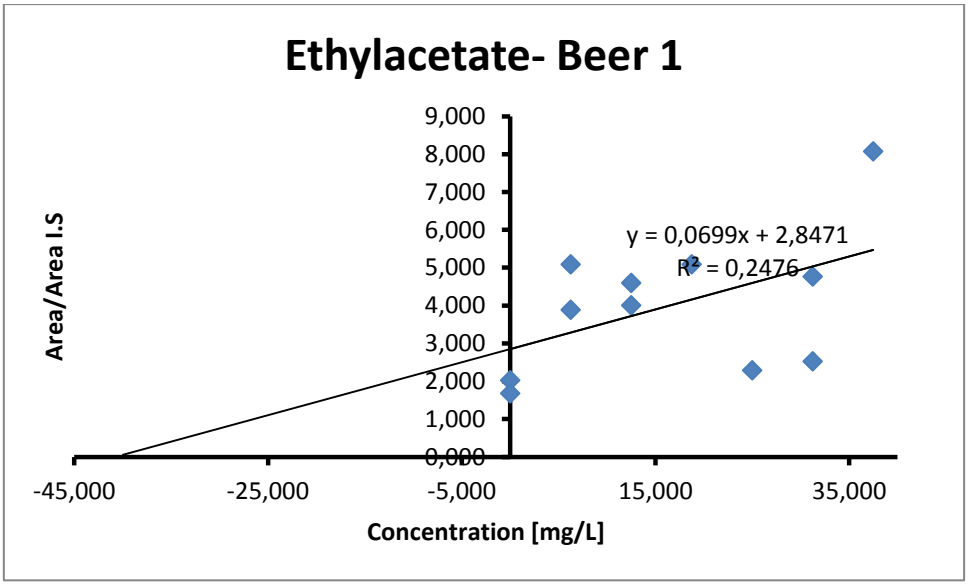
9. *ETHYLACETATE CALIBRATION CURVE*

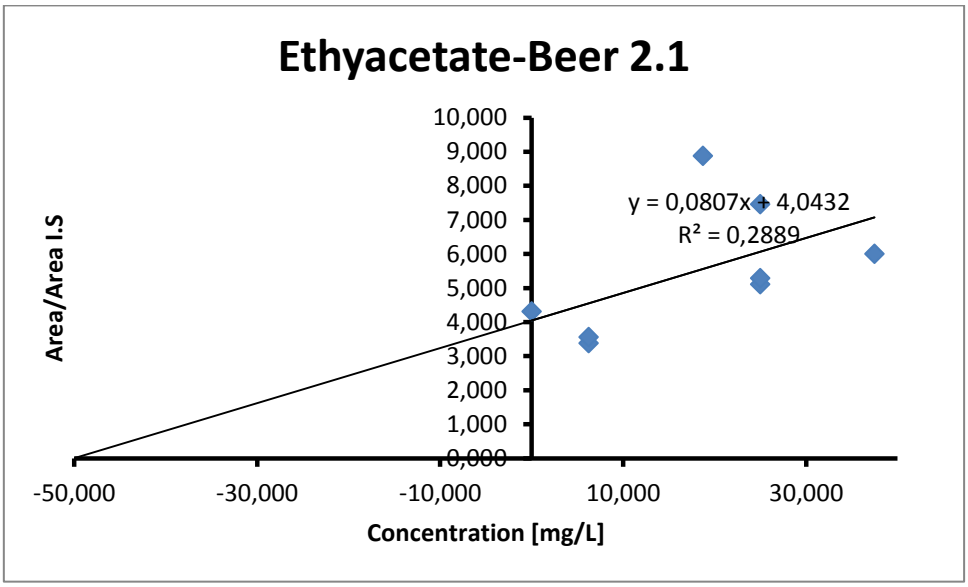
In the following table it is shown all the ratios of all standards. We used this table to create the calibration curves. We removed some figures in order to have an acurate calibration curve. All the removed figures are in the annexes.

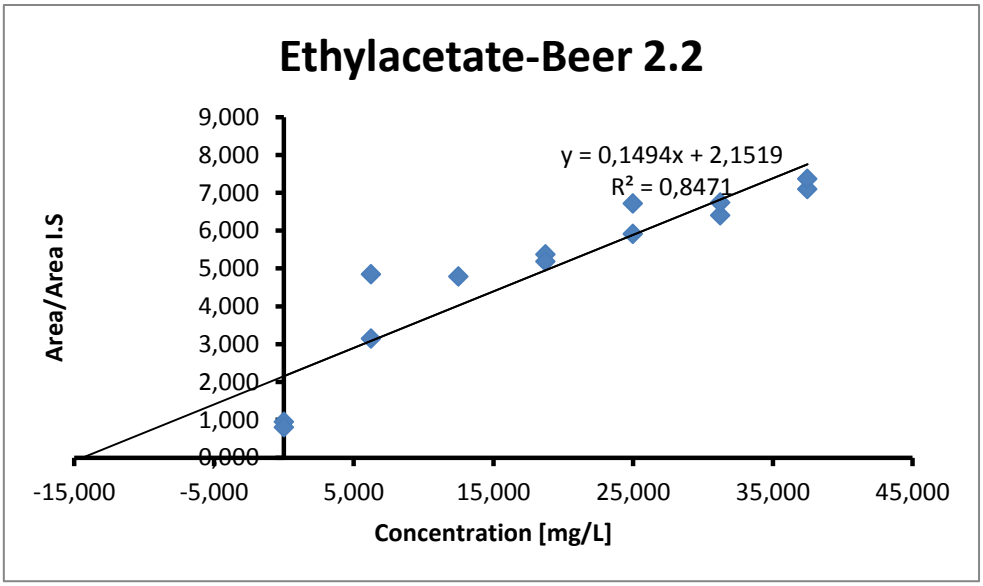
		Ratio:Area/Area I.S			
Standard	Concentration [mg/L]	beer 1	beer 2.1	beer 2.2	beer 2.3
1.1	0	2,018	4,30975044	0,94464003	
1.2	0	1,67934		0,80386678	0,30795966
2.1	6,24686957	4,9535	3,37092913	4,8470076	0,76970831
2.2	6,24686957	3,88118	3,54935829	3,1438194	1,39174513
3.1	12,4937391	4,589		4,78557002	3,00961345
3.2	12,4937391	3,9995			3,39508137
4.1	18,7406087	5,085	8,87667941	5,37413258	6,89484054
4.2	18,7406087	4,33		5,18327676	5,63668493
5.1	24,9874783	3,989	5,10311804	6,71313778	10,6108593
5.2	24,9874783	5,38	5,2819072	5,91012277	19,3358421
6.1	31,2343478	5,459		6,40261296	17,9243848

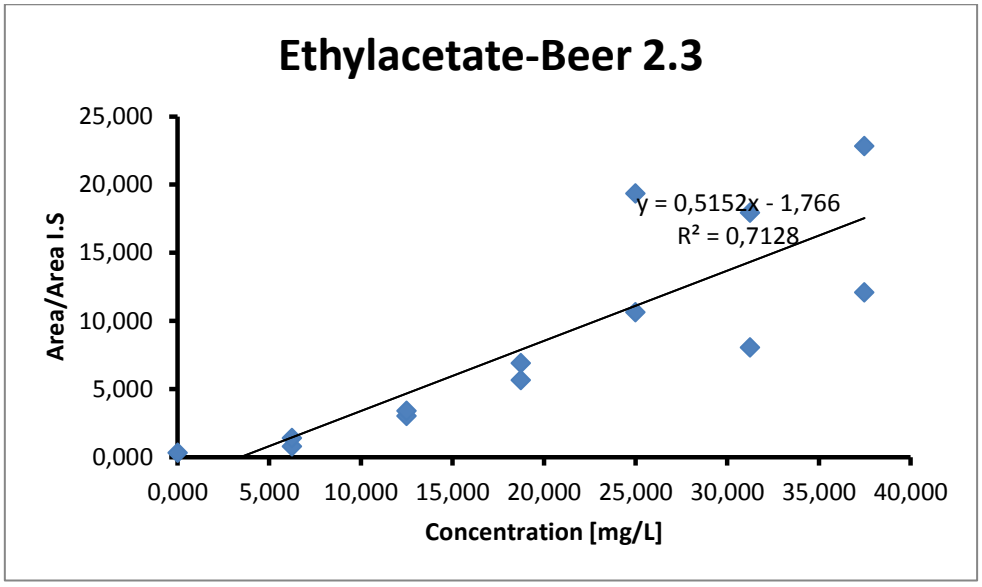
6.2	31,2343478	6,707		6,74152371	8,03515121
7.1	37,4812174	8,06	6,00059688	7,09866101	22,8184434
7.2	37,4812174	7,52		7,36712866	12,0902503

Next, it is the four calibration curves of Ethylacetate.









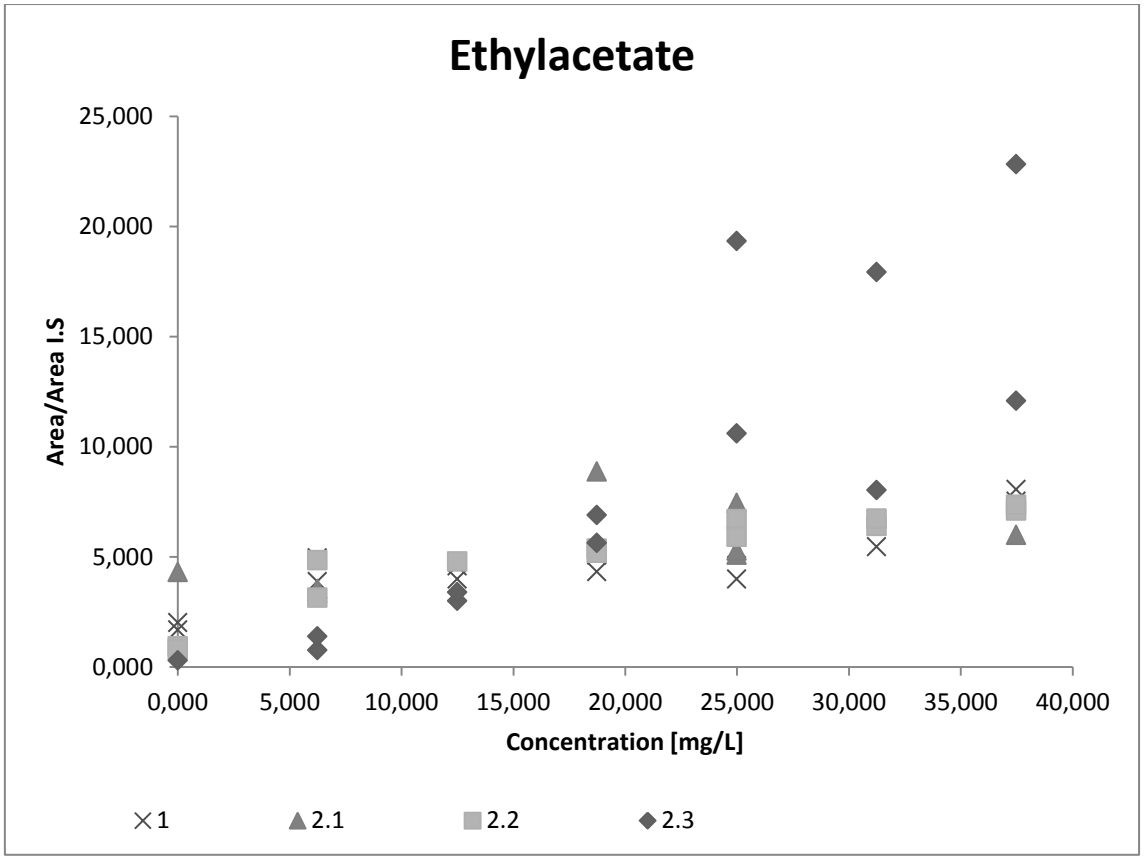
With all the calibration curves we designed the following table.

$$y = a \cdot x + b: Concentration = b/a$$

Beer	a	b	R^2	Concentration
1	0,0699	2,8471	0,8615	40,73104435
2.1	0,0807	4,0432	0,2889	50,1016109
2.2	0,1494	2,159	0,8471	14,45113788
2.3	0,5152	-1,776	0,7128	-3,447204969

The regression coefficients are really low, and we had to reject several figures to create these calibration curves. The concentrations are very different and for these results we cannot determine what the ethylacetate concentration in Juplier beer is. The 'beer 2.3' is a good calibration curve, but the concentration result is negative.

Next, it is shown a graphic with all the ratios points. In this graphic you can see all the variaty of ratios in each concentration



5. RESULTS DISCUSSION.

In all curves we have really different values, we are on the right track because all the calibration curves have positive slops, but the validation method failed. Some things to considerate in the following steps are:

- Use automatic injection to have a good and precise injection.
- Do the aroma mix without isoamylacetate to avoid the interference, with internal standard

The rest of calibration curves are in the annex.

Analysing with GC-HS is really slow and delicate. Any little change in the chromatograph impacts on the result chromatogram and finally on the concentration calculation. For that reason it is really important to determine the good chromatograph conditions and know where the problems are and solve them.

I. CONCLUSIONS

The aromatic compounds have a very important role in fermentation. Knowing these concentrations is important to improve the fermentation and finally the beer. Many factors affect the concentration of these compounds and when one of these concentrations change it is a signal that something has changed in the brewing process.

A good way to determine the aromatic compounds is using Head Space – Gas Chromatography. We tried to apply this method in our laboratory. In that time we improved this method, but not enough to have good results.

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J. ANNEX- CALIBRATION CURVES

1- CALIBRATION CURVES BEER 1

		Sample 1.1			Sample 1.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		4,548			6,026	
IS	**12,000**	8460	12,000		12044	10,814	
Acetaldehyde	**3,498**	478	3,134	0,057	428	3,265	0,036
DMS	**3,673**	715	3,328	0,085	2530	3,746	0,210
Ethyacetate	**4,848**	17075	4,011	2,018	20226	4,881	1,679
Ethylbutyrate	**8,698**	59503	6,026	7,033		6,026	0,000
N-Propanol	**8,812**	1158	6,403	0,137	14463	8,766	1,201
Isobutanol	**10,920**	940	7,926	0,111	1365	7,926	0,113
Isoamylacetate	**11,973**	922	8,399	0,109			0,000
Ethylhexanoate	**17,413**	210	17,494	0,025	78706	17,301	6,535
N-Amylalcohol	**18,226**				,		
Ethyloctanoate	**23,623**	2294	23,249	0,271	855	23,562	0,071

		Sample 2.1			Sample 2.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		6,026			5,802	
IS	**12,000**	23116	12,007		21296	12,000	
Acetaldehyde	**3,498**		3,266		2918	3,402	0,137
DMS	**3,673**	2157	3,673	0,09331199	1185	3,672	0,056
Ethyacetate	**4,848**	117505	4,903	5,08327565	82642	4,902	3,881
Ethylbutyrate	**8,698**				3749	8,678	0,176
N-Propanol	**8,812**	4383	8,817	0,18960893	3389	8,812	0,159
Isobutanol	**10,920**	6567	10,863	0,28408894	5650	10,882	0,265
Isoamylacetate	**11,973**	106	11,674	0,00458557			
Ethylhexanoate	**17,413**	7558	17,402	0,32695968	5257	17,402	0,247
N-Amylalcohol	**18,226**				15039	18,246	0,706
Ethyloctanoate	**23,623**	160	23,041	0,00692161	1987	23,615	0,093

		Sample 3.1			Sample 3.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,604	0	0	5,777	
IS	**12,000**	38974	11,61	0	55418	11,941	
Acetaldehyde	**3,498**	6402	3,459	0,16426336	0	3,256	
DMS	**3,673**	402	3,844	0,01031457	28886	3,659	0,52123859

Ethyacetate	**4,848**	178875	4,756	4,58959819	221644	4,873	3,99949475
Ethylbutyrate	**8,698**	2879	8,322	0,07386976	400	8,419	0,00721787
N-Propanol	**8,812**	6578	8,485	0,16877919	10799	8,759	0,19486448
Isobutanol	**10,920**	13077	10,8822	0,33553138	,		
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	6231	16,861	0,15987581	6722	17,322	0,12129633
N-Amylalcohol	**18,226**	32129	17,833	0	53930	18,181	0,97314952
Ethyloctanoate	**23,623**	3526	23,313	0,41678487	5408	23,567	0,09758562

		Sample 4.1			Sample 4,2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**					5,286	
IS	**12,000**	77803	12,015		16292	11,057	
Acetaldehyde	**3,498**	11889	3,477	0,15280902	826	3,747	0,05069973
DMS	**3,673**	7025	3,659	0,09029215	1170	4,534	0,07181439
Ethyacetate	**4,848**	395652	4,898	5,08530519	256008	4,534	15,7137245
Ethylbutyrate	**8,698**	4950	8,68	0,06362223	986	7,526	0,0605205
N-Propanol	**8,812**	13200	8,813	0,16965927	14691	7,925	0,90173091
Isobutanol	**10,920**	30276	10,813	0,38913667	26602	10,925	1,63282593
Isoamylacetate	**11,973**				40797		2,50411245
Ethylhexanoate	**17,413**	8258	17,408	0,10613987	6569	16,292	0,40320403
N-Amylalcohol	**18,226**	86191	18,249	1,10781075	127895	17,487	7,85017186
Ethyloctanoate	**23,623**	6371	23,615	0,0818863	7840	23,246	0,48121778

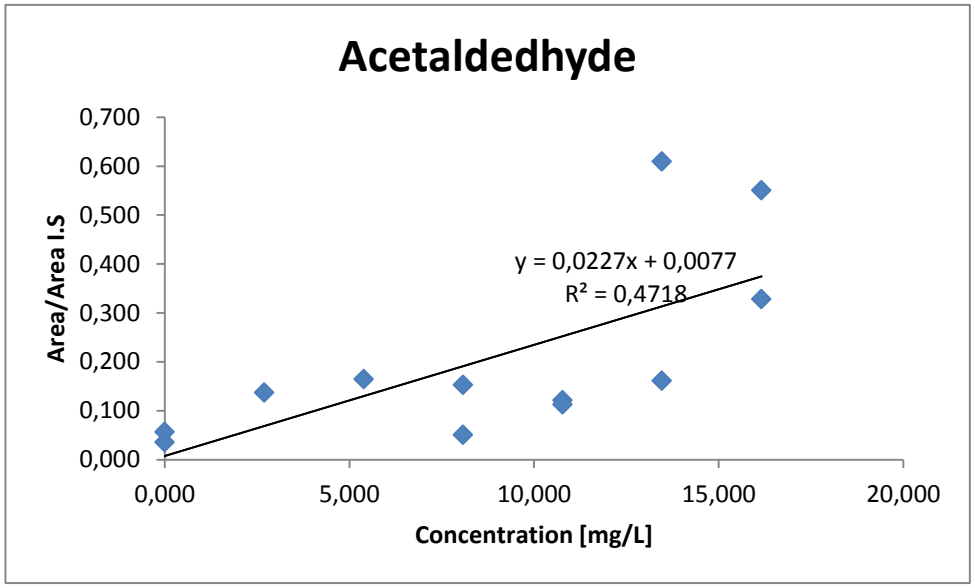
		Sample 5.1			Sample 5.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,787				
IS	**12,000**	5641	12,017		969		
Acetaldehyde	**3,498**	683	3,252	0,12107782	109		0,1124871
DMS	**3,673**	1999	3,663	0,35436979			
Ethyacetate	**4,848**	84505	84505	14,9804999	2215		2,28586171
Ethylbutyrate	**8,698**				764		0,78844169
N-Propanol	**8,812**	2062	8,817	0,36553803			
Isobutanol	**10,920**	3039	10,822	0,53873427	858		0,88544892
Isoamylacetate	**11,973**	.					
Ethylhexanoate	**17,413**	1535	16,008	0,27211487			
N-Amylalcohol	**18,226**	359	17,402	0,0636412	5493		5,66873065
Ethyloctanoate	**23,623**	794	23,613	0,14075519	142		0,14654283

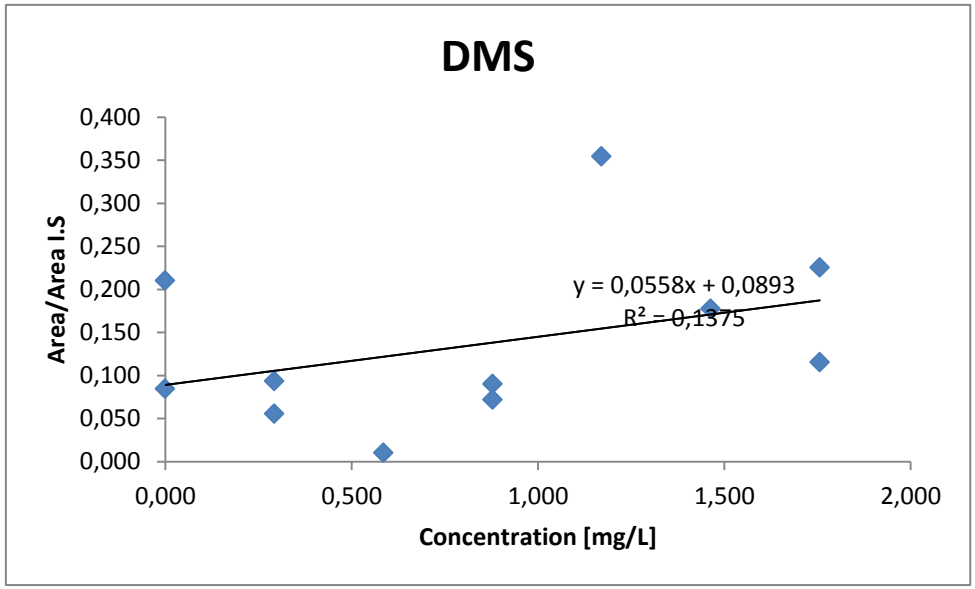
		Sample 6.1			Sample 6.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,707				
IS	**12,000**	3839			1912		
Acetaldehyde	**3,498**	2339		0,60927325	308		0,16108787
DMS	**3,673**	681		0,17738995	109		0,05700837
Ethyacetate	**4,848**	18280		4,76165668	4825		2,52353556
Ethylbutyrate	**8,698**	1134		0,29538942	.		
N-Propanol	**8,812**				1596		0,83472803
Isobutanol	**10,920**	1963		0,51133108	2201		1,15115063
Isoamylacetate	**11,973**				.		
Ethylhexanoate	**17,413**	463		0,12060432	.		
N-Amylalcohol	**18,226**	10584		2,75696796	11127		5,81956067
Ethyloctanoate	**23,623**	896		0,23339411	414		0,2165272

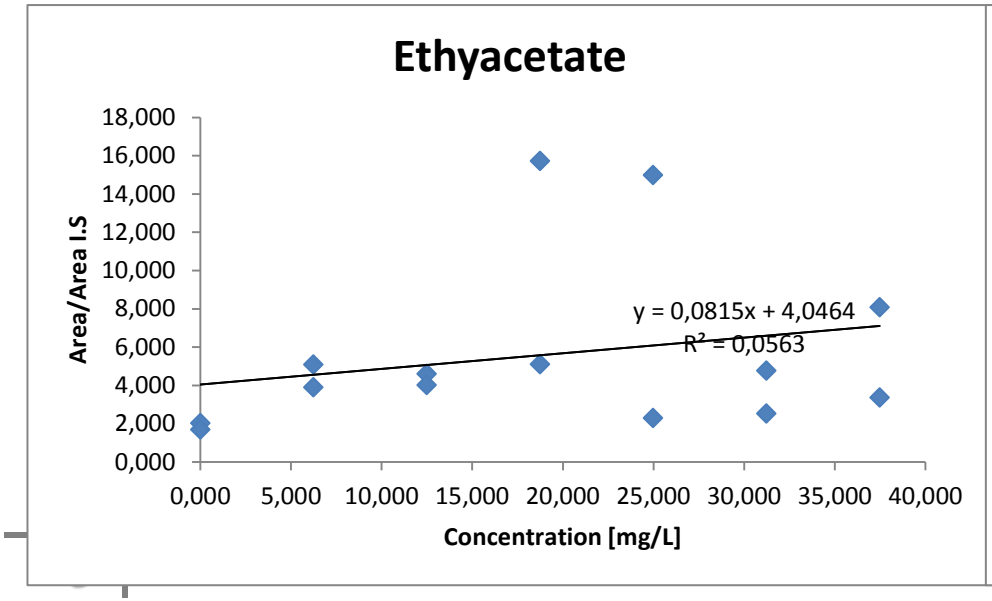
		Sample 7.1			Sample 7.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**						
IS	**12,000**	2283			2402		
Acetaldehyde	**3,498**	1256		0,55015331	788		0,32805995
DMS	**3,673**	515		0,22558038	277		0,11532057
Ethyacetate	**4,848**	18420		8,06833114	8063		3,35678601
Ethylbutyrate	**8,698**						
N-Propanol	**8,812**						
Isobutanol	**10,920**				263		0,10949209
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	2932			382		0,15903414
N-Amylalcohol	**18,226**				3053		1,27102415
Ethyloctanoate	**23,623**	430		0,18834866	459		0,19109076

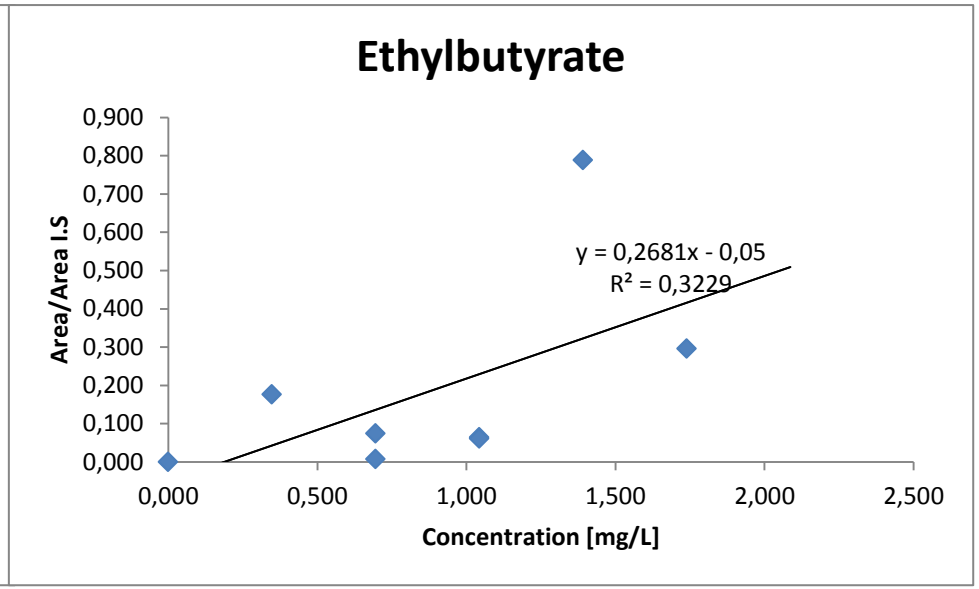
	Acetaldedhyde		DMS		Ethyacetate		Ethylbutyrate		N-Propanol	
Standard	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S
1.1	0,000	0,057	0,000	0,085	0,000	2,018	0,000	7,033	0,000	0,137
1.2	0,000	0,036	0,000	0,210	0,000	1,679	0,000	0,000	0,000	1,201
2.1	2,693		0,293	0,093	6,247	5,083	0,348		2,755	0,190
2.2	2,693	0,137	0,293	0,056	6,247	3,881	0,348	0,176	2,755	0,159
3.1	5,386	0,164	0,585	0,010	12,494	4,590	0,696	0,074	5,510	0,169
3.2	5,386		0,585	0,521	12,494	3,999	0,696	0,007	5,510	0,195
4.1	8,078	0,153	0,878	0,090	18,741	5,085	1,043	0,064	8,264	0,170
4.2	8,078	0,051	0,878	0,072	18,741	15,714	1,043	0,061	8,264	0,902
5.1	10,771	0,121	1,171	0,354	24,987	14,980	1,391		11,019	0,366
5.2	10,771	0,112	1,171		24,987	2,286	1,391	0,788	11,019	
6.1	13,464	0,609	1,463	0,177	31,234	4,762	1,739	0,295	13,774	
6.2	13,464	0,161	1,463	0,057	31,234	2,524	1,739		13,774	0,835
7.1	16,157	0,550	1,756	0,226	37,481	8,068	2,087		16,529	
7.2	16,157	0,328	1,756	0,115	37,481	3,357	2,087		16,529	

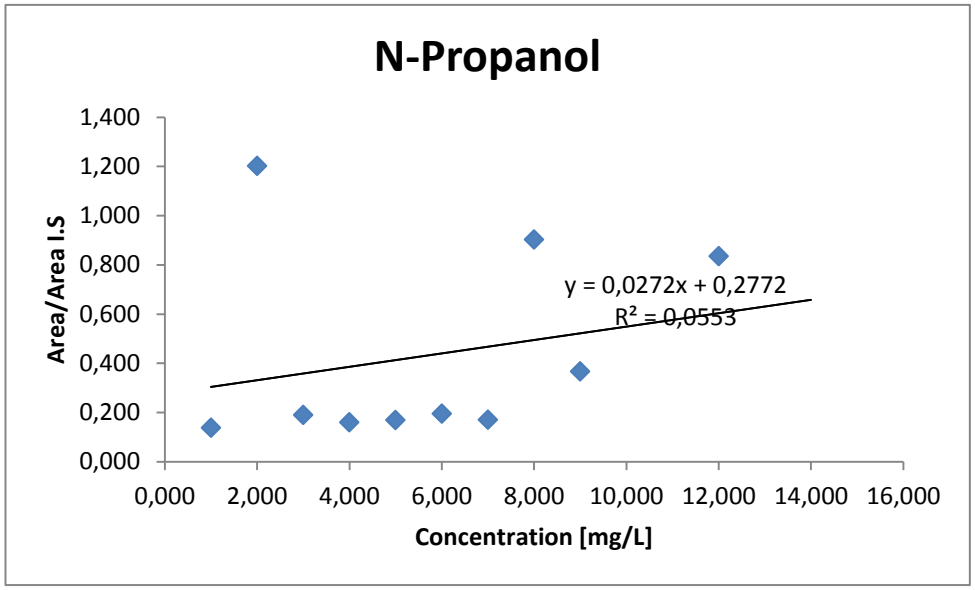
	Isobutanol		Isoamylacetate		Ethylhexanoate		N-Amylalcohol		Ethyloctanoate	
Standard	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S	Concentration [mg/L]	Area/Area I.S
1.1	0,000	0,111	0,000	0,109	0,000	0,025	0,000		0,000	0,271
1.2	0,000	0,113	0,000	0,000	0,000	6,535	0,000		0,000	0,071
2.1	2,763	0,284	2,996	0,005	6,247	0,327	13,946		0,348	0,007
2.2	2,763	0,265	2,996		6,247	0,247	13,946	0,706	0,348	0,093
3.1	5,527	0,336	5,992		12,494	0,160	27,892	0,000	0,696	0,417
3.2	5,527		5,992		12,494	0,121	27,892	0,007	0,696	0,098
4.1	8,290	0,389	8,987		18,741	0,106	41,838	0,064	1,043	0,082
4.2	8,290	1,633	8,987	2,504	18,741	0,403	41,838	0,061	1,043	0,481
5.1	11,054	0,539	11,983		24,987	0,272	55,784		1,391	0,141
5.2	11,054	0,885	11,983		24,987		55,784	0,788	1,391	0,147
6.1	13,817	0,511	14,979		31,234	0,121	69,730	0,295	1,739	0,233
6.2	13,817	1,151	14,979		31,234		69,730		1,739	0,217
7.1	16,580		17,975		37,481	1,284	83,677		2,087	0,188
7.2	16,580	0,109	17,975		37,481	0,159	83,677		2,087	0,191

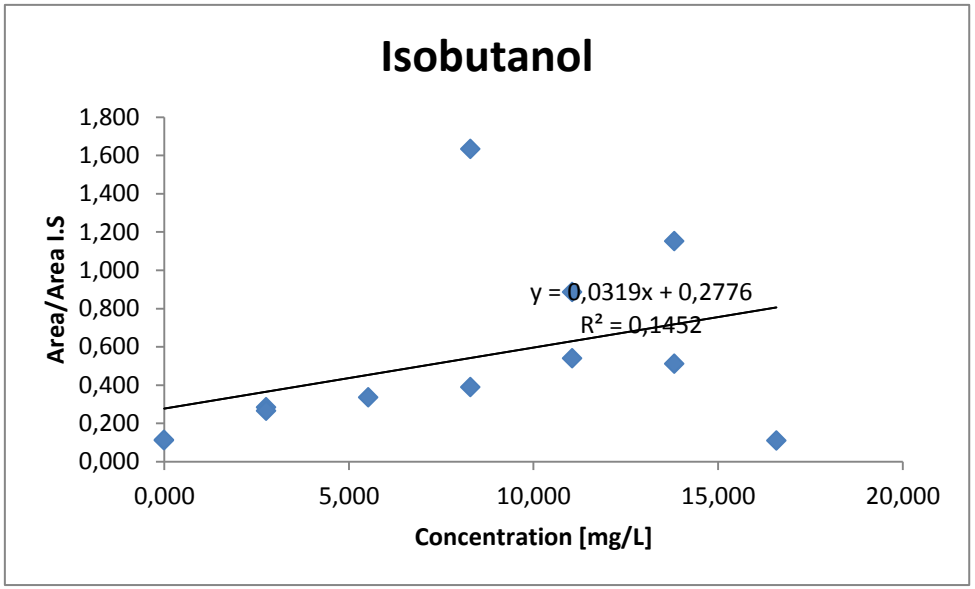

Acetaldedhyde
Area/Area I.S
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
y = 0,0227x + 0,0077
R² = 0,4718
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]

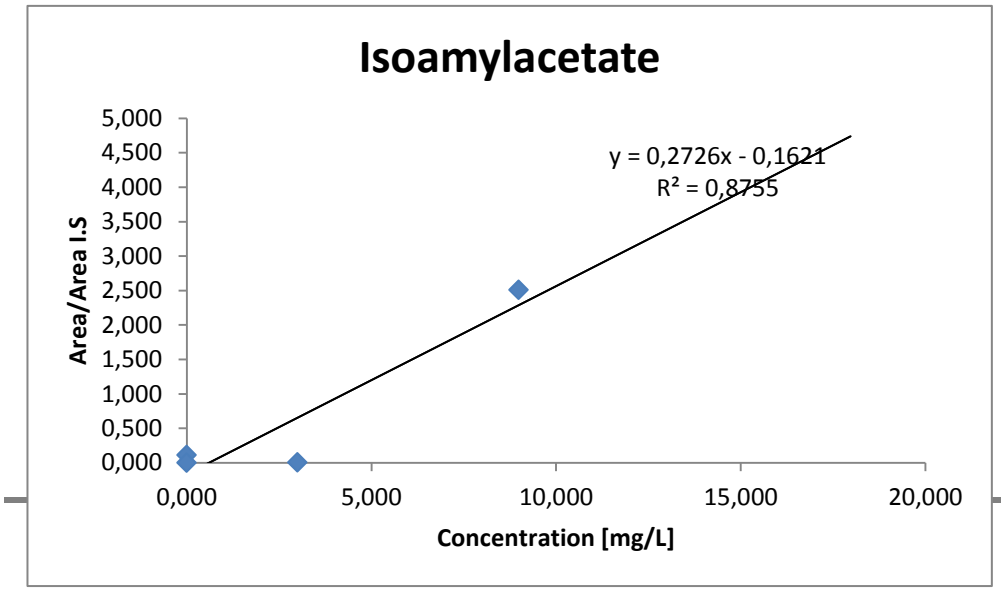

DMS
Area/Area I.S
0,400
0,350
0,300
0,250
0,200
0,150
0,100
0,050
0,000
y = 0,0558x + 0,0893
R² = 0,1375
0,000
0,500
1,000
1,500
2,000
Concentration [mg/L]

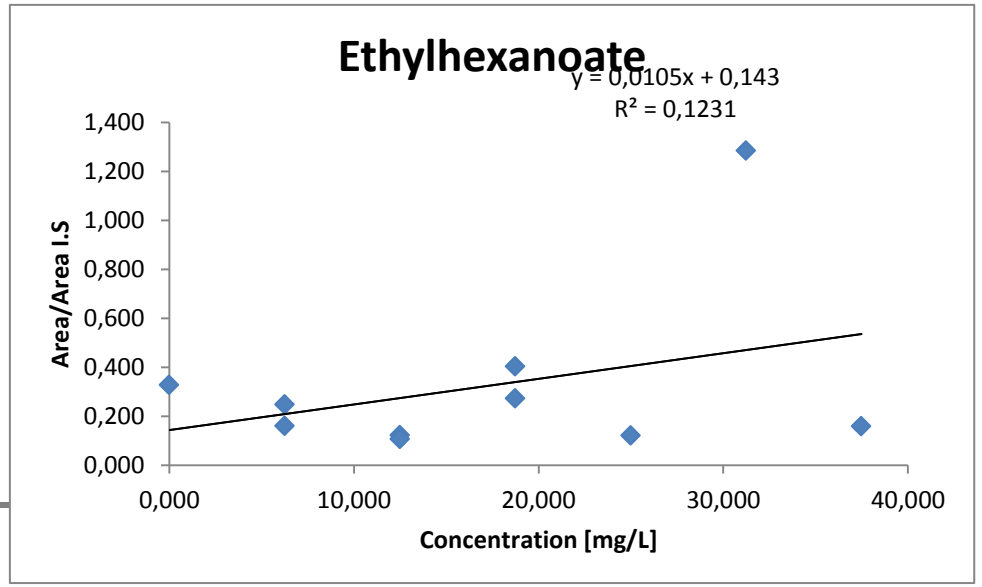

Ethyacetate
Area/Area I.S
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0,000
y = 0,0815x + 4,0464
R² = 0,0563
0,000
5,000
10,000
15,000
20,000
25,000
30,000
35,000
40,000
Concentration [mg/L]

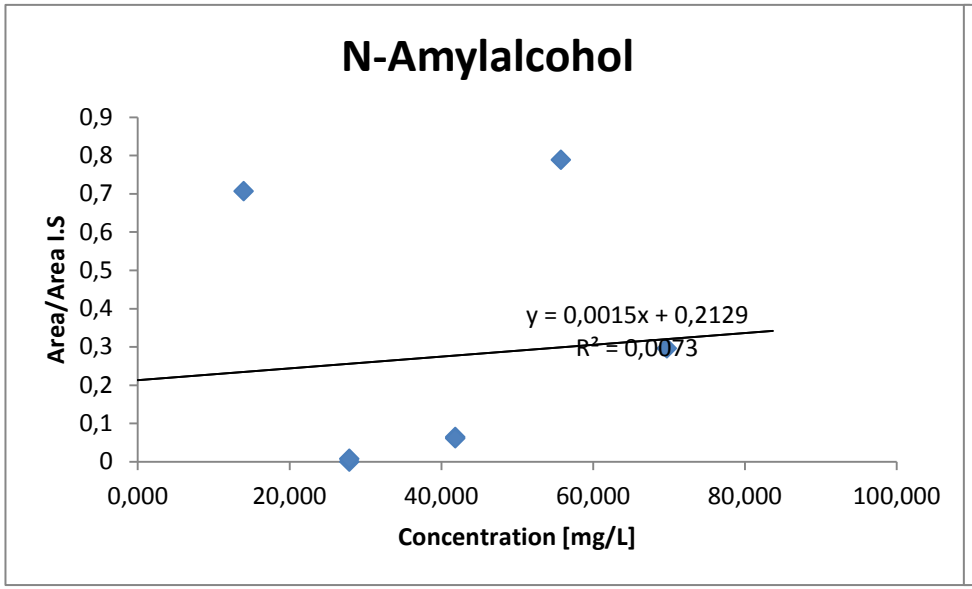

Ethylbutyrate
Area/Area I.S
0,900
0,800
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
y = 0,2681x - 0,05
R² = 0,3229
0,000
0,500
1,000
1,500
2,000
2,500
Concentration [mg/L]

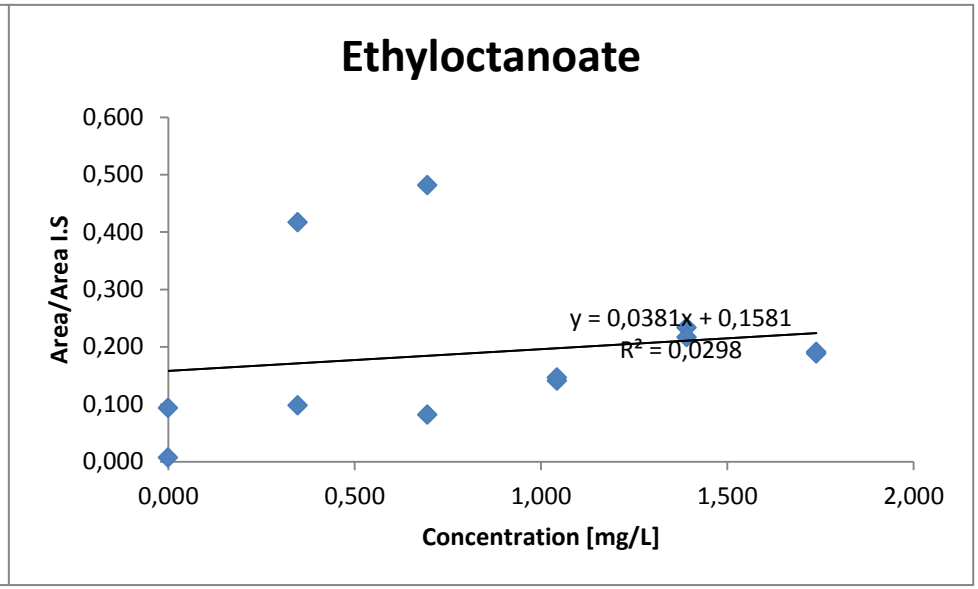

N-Propanol
Area/Area I.S
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
2,000
4,000
6,000
8,000
10,000
12,000
14,000
16,000
Concentration [mg/L]
y = 0,0272x + 0,2772
R² = 0,0553


Isobutanol
Area/Area I.S
1,800
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]
y = 0,0319x + 0,2776
R² = 0,1452


Isoamylacetate
Area/Area I.S
5,000
4,500
4,000
3,500
3,000
2,500
2,000
1,500
1,000
0,500
0,000
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]
y = 0,2726x - 0,1621
R² = 0,8755


Ethylhexanoate
Area/Area I.S
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
10,000
20,000
30,000
40,000
Concentration [mg/L]
y = 0,0105x + 0,143
R² = 0,1231


N-Amylalcohol
Area/Area I.S
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
y = 0,0015x + 0,2129
R² = 0,0073
0,000
20,000
40,000
60,000
80,000
100,000
Concentration [mg/L]


Ethyloctanoate
Area/Area I.S
0,600
0,500
0,400
0,300
0,200
0,100
0,000
y = 0,0381x + 0,1581
R² = 0,0298
0,000
0,500
1,000
1,500
2,000
Concentration [mg/L]

2- CALIBRATION CURVES BEER 2.1

		Sample 1.1			Sample 1.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-Oh	**5,802**						
IS	**12,000**	193304	11,185		187856	12,343	
Acetaldehyde	**3,498**	23498	3,41	0,12155982	17160	3,497	0,09134656
DMS	**3,673**	288	3,772	0,00148988	1097	4,109	0,00583958
Ethyacetate	**4,848**	833092	4,474	4,30975044	259988	4,9	1,38397496
Ethylbutyrate	**8,698**	11726	7,511	0,06066093	4151	8,689	0,02209671
N-Propanol	**8,812**	204028	7,793	1,05547738	160320	8,851	0,85341964
Isobutanol	**10,920**	359953	10,085	1,86210839	268752	11,33	1,43062771
Isoamylacetate	**11,973**	182067	10,534	0,94186877	7516	11,841	0,04000937
Ethylhexanoate	**17,413**	29759	15,802	0,15394922	15312	17,26	0,08150924
N-Amylalcohol	**18,226**	1707012	17,467	8,83071225	1470646	18,342	7,82858147
Ethyloctanoate	**23,623**	53044	23,206	0,27440715	37708	23,575	0,20072822

		Sample 2.1			Sample 2.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**						
IS	**12,000**	106519	11,993		105889	11,58	
Acetaldehyde	**3,498**	19406	3,481	0,18218346	19112	3,421	0,18049089
DMS	**3,673**	619	3,662	0,00581117	507	3,566	0,00478803
Ethyacetate	**4,848**	359068	4,891	3,37092913	375838	4,542	3,54935829
Ethylbutyrate	**8,698**	2350	8,664	0,02206179	2259	7,723	0,02133366
N-Propanol	**8,812**	34213	8,807	0,32119152	38165	7,943	0,3604246
Isobutanol	**10,920**	80582	10,85	0,75650353	84165	9,895	0,79484177
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	5093	17,391	0,04781307	3743	16,313	0,03534834
N-Amylalcohol	**18,226**	283213	18,247	2,65880265	313511	17,471	2,96075135
Ethyloctanoate	**23,623**	21547	23,615	0,20228316	11496	23,243	0,10856652

		Sample 3.1			Sample 3.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**						
IS	**12,000**						
Acetaldehyde	**3,498**						
DMS	**3,673**						
Ethyacetate	**4,848**	661	4,908				
Ethylbutyrate	**8,698**						

N-Propanol	**8,812**	277	8,805				
Isobutanol	**10,920**						
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**						
N-Amylalcohol	**18,226**						
Ethyloctanoate	**23,623**						

		Sample 4.1			Sample 4.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**						
IS	**12,000**	98621	12,142		529		
Acetaldehyde	**3,498**	56585	3,49	0,57376218	200	3,146	0,37807183
DMS	**3,673**	741	3,671	0,00751361			
Ethyacetate	**4,848**	875427	4,904	8,87667941	795		1,50283554
Ethylbutyrate	**8,698**	3140	8,674	0,03183906			
N-Propanol	**8,812**	64771	8,821	0,65676681	540		1,02079395
Isobutanol	**10,920**	122543	11,12	1,24256497	670		1,26654064
Isoamylacetate	**11,973**	22144	11,913	0,22453636			
Ethylhexanoate	**17,413**	1806	17,327	0,01831253	327		0,61814745
N-Amylalcohol	**18,226**	519897	18,276	5,27166628	3783		7,15122873
Ethyloctanoate	**23,623**	5297	23,594	0,05371067	91		0,17202268

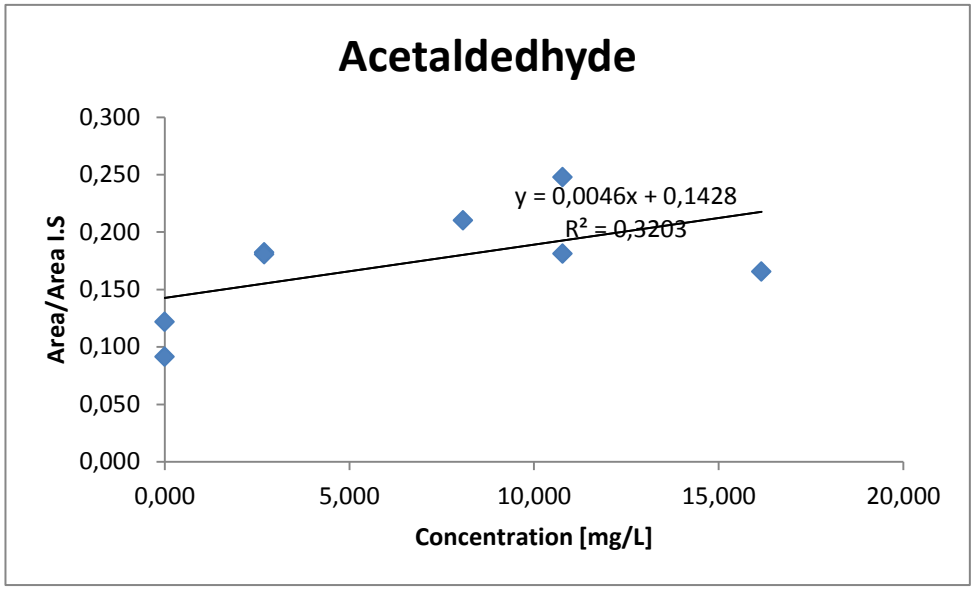
		Sample 5.1			Sample 5.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,78			5,297	
IS	**12,000**	4490	12,012		108557	10,989	
Acetaldehyde	**3,498**	19659	3,419	4,37839644	19659	3,419	0,1810938
DMS	**3,673**	465	3,674	0,10356347	7374	3,563	0,06792745
Ethyacetate	**4,848**	22913	4,904	5,10311804	573388	4,537	5,2819072
Ethylbutyrate	**8,698**				7614	7,715	0,07013827
N-Propanol	**8,812**	102	8,804	0,02271715	12008	7,925	0,1106147
Isobutanol	**10,920**	114	10,812	0,02538976	28840	9,863	0,26566688
Isoamylacetate	**11,973**			0			
Ethylhexanoate	**17,413**				13802	16,326	0,12714058
N-Amylalcohol	**18,226**	6363	18,25	1,41714922	78048	17,471	0,7189587
Ethyloctanoate	**23,623**	270	23,63	0,06013363	13693	23,242	0,1261365

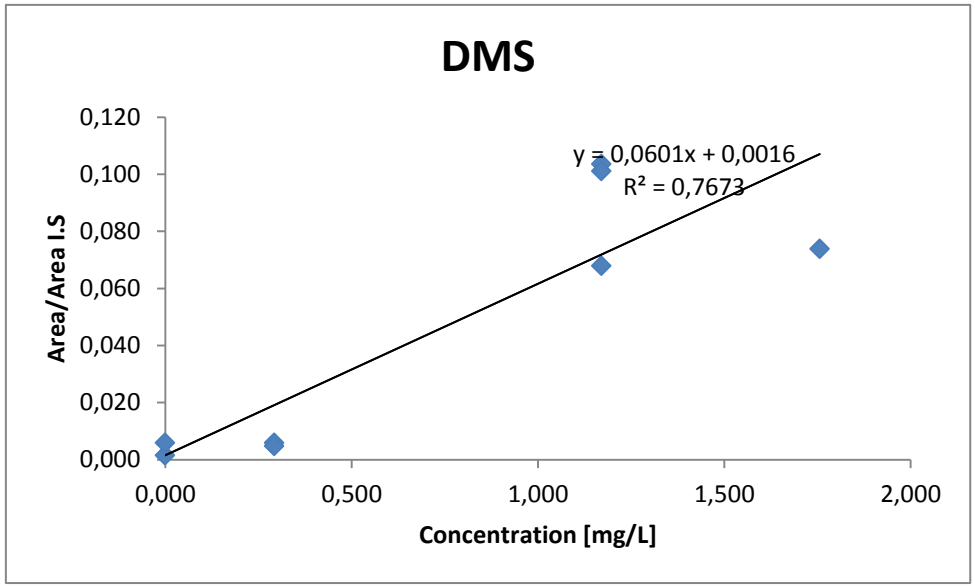
		Sample 6.1			Sample 6.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,788				
IS	**12,000**	1220	12		797	12,03	
Acetaldehyde	**3,498**	150	3,504	0,12295082			
DMS	**3,673**						
Ethyacetate	**4,848**	2815	4,909	2,30737705	828		1,03889586
Ethylbutyrate	**8,698**						
N-Propanol	**8,812**	963	8,819	0,78934426			
Isobutanol	**10,920**	1227	10,853	1,0057377			
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**						
N-Amylalcohol	**18,226**	8951	18,243	7,33688525	3733	18,256	4,6838143
Ethyloctanoate	**23,623**	1120	23,611	0,91803279	425	23,636	0,53324969

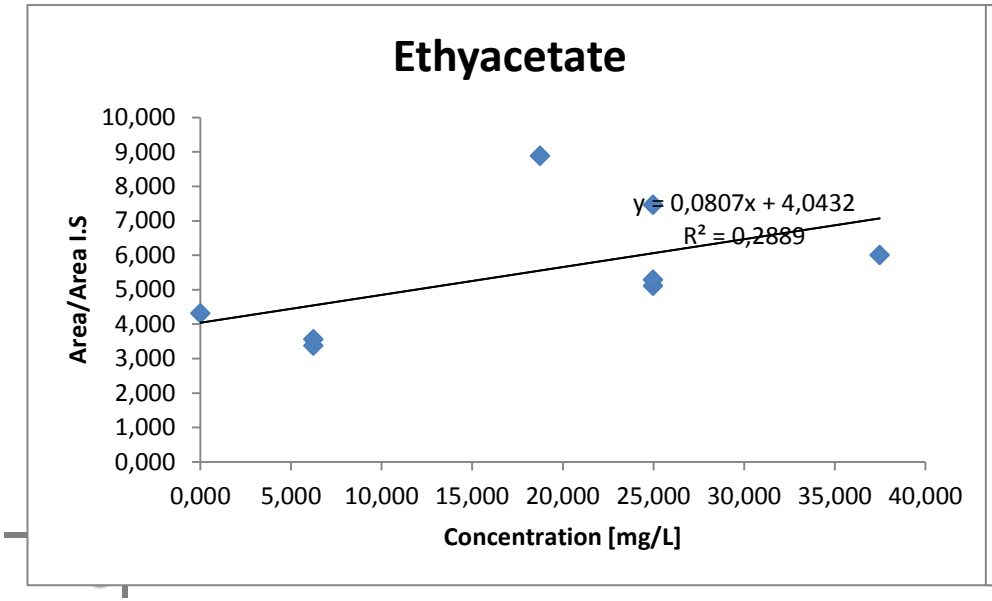
		Sample 7.1			Sample 5,1*		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**					5,841	
IS	**12,000**	381988	12,021		86258	12,006	
Acetaldehyde	**3,498**	63196	3,493	0,16543975	21361	3,492	0,2476408
DMS	**3,673**	28197	3,674	0,07381645	8715	3,673	0,10103411
Ethyacetate	**4,848**	2292156	4,912	6,00059688	642923	4,903	7,45348837
Ethylbutyrate	**8,698**	25673	8,683	0,06720892	7034	8,678	0,08154606
N-Propanol	**8,812**	75868	8,823	0,19861357	18910	8,814	0,21922604
Isobutanol	**10,920**	191615	10,838	0,50162571	37975	10,8886	0,44024902
Isoamylacetate	**11,973**			0			0
Ethylhexanoate	**17,413**	44861	17,409	0,11744086	8193	17,395	0,09498249
N-Amylalcohol	**18,226**	582704	18,26	1,52545106	102634	28,249	1,18984906
Ethyloctanoate	**23,623**	66452	23,619	0,17396358	5443	23,61	0,06310139

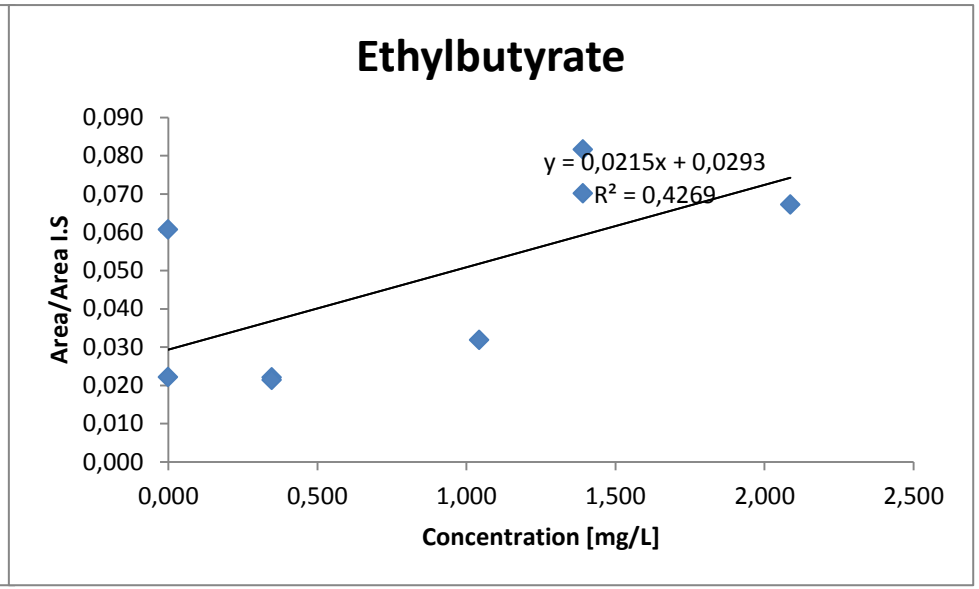
	Acetaldedhyde		DMS		Ethyacetate		Ethylbutyrate		N-Propanol	
Standard	Concentratic	Area/Area I.	Concentratic	Area/Area I.	Concentratic	Area/Area I.	Concentratic	Area/Area I.	Concentratic	Area/Area I.S
1.1	0,000	0,122	0,000	0,001	0,000	4,310	0,000	0,061	0,000	1,055
1.2	0,000	0,091	0,000	0,006	0,000		0,000	0,022	0,000	0,853
2.1	2,693	0,182	0,293	0,006	6,247	3,371	0,348	0,022	2,755	0,321
2.2	2,693	0,180	0,293	0,005	6,247	3,549	0,348	0,021	2,755	0,360
3.1	5,386		0,585		12,494		0,696		5,510	
3.2	5,386		0,585		12,494		0,696		5,510	
4.1	8,078	0,210	0,878		18,741	8,877	1,043	0,032	8,264	0,657
4.2	8,078		0,878		18,741		1,043		8,264	1,021
5.1	10,771		1,171	0,104	24,987	5,103	1,391		11,019	
5.2	10,771	0,181	1,171	0,068	24,987	5,282	1,391	0,070	11,019	0,111
6.1	13,464		1,463		31,234		1,739		13,774	0,789
6.2	13,464		1,463		31,234		1,739		13,774	
7.1	16,157	0,165	1,756	0,074	37,481	6,001	2,087	0,067	16,529	0,199
5.1*	10,771	0,248	1,171	0,101	24,987	7,453	1,391	0,082	11,019	0,219

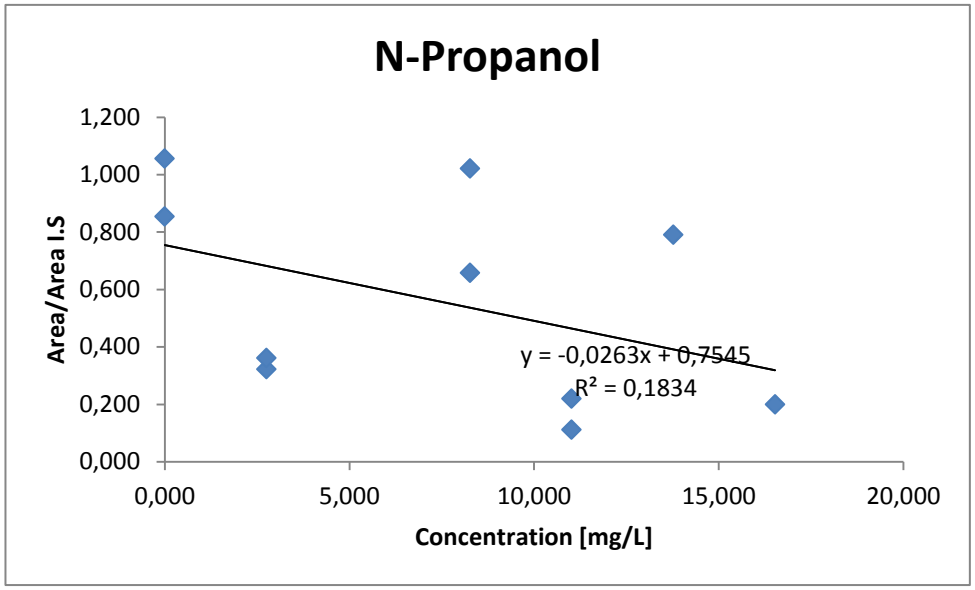
	Isobutanol		Isoamylacetate		Ethylhexanoate		N-Amylalcohol		Ethyloctanoate	
Standard	Concentratic	Area/Area I.	Concentratic	Area/Area I.	Concentratic	Area/Area I.	Concentratic	Area/Area I.	Concentratic	Area/Area I.S
1.1	0,000	1,862	0,000	0,942	0,000	0,154	0,000	8,831	0,000	0,274
1.2	0,000	1,431	0,000	0,040	0,000	0,082	0,000	7,829	0,000	0,201
2.1	2,763	0,757	2,996		6,247	0,048	13,946	2,659	0,348	0,202
2.2	2,763	0,795	2,996		6,247		13,946	2,961	0,348	0,109
3.1	5,527		5,992		12,494		27,892		0,696	
3.2	5,527		5,992		12,494		27,892		0,696	
4.1	8,290	1,243	8,987		18,741	0,018	41,838	0,032	1,043	0,054
4.2	8,290	1,267	8,987		18,741	0,618	41,838		1,043	0,172
5.1	11,054		11,983		24,987		55,784		1,391	0,060
5.2	11,054	0,500	11,983		24,987	0,127	55,784	0,070	1,391	0,126
6.1	13,817	0,540	14,979		31,234		69,730		1,739	
6.2	13,817		14,979		31,234		69,730		1,739	0,400
7.1	16,580	0,502	17,975		37,481	0,117	83,677	0,067	2,087	0,174
5.1*	11,054	0,44024902	11,983		24,987	0,09498249	55,784	1,18984906	1,391	0,06310139

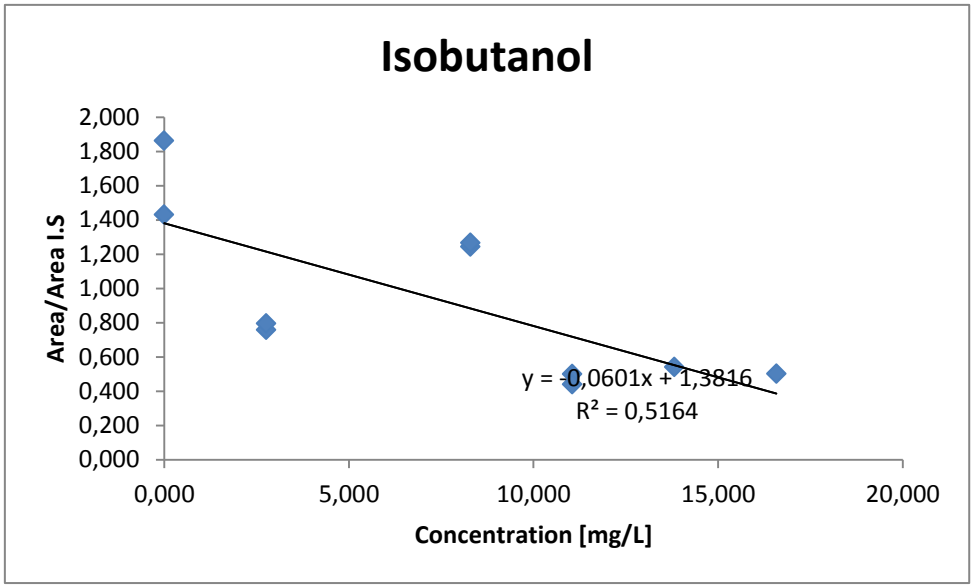

Acetaldedhyde
0,300
0,250
0,200
0,150
0,100
0,050
0,000
Area/Area I.S
y = 0,0046x + 0,1428
R² = 0,3203
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]

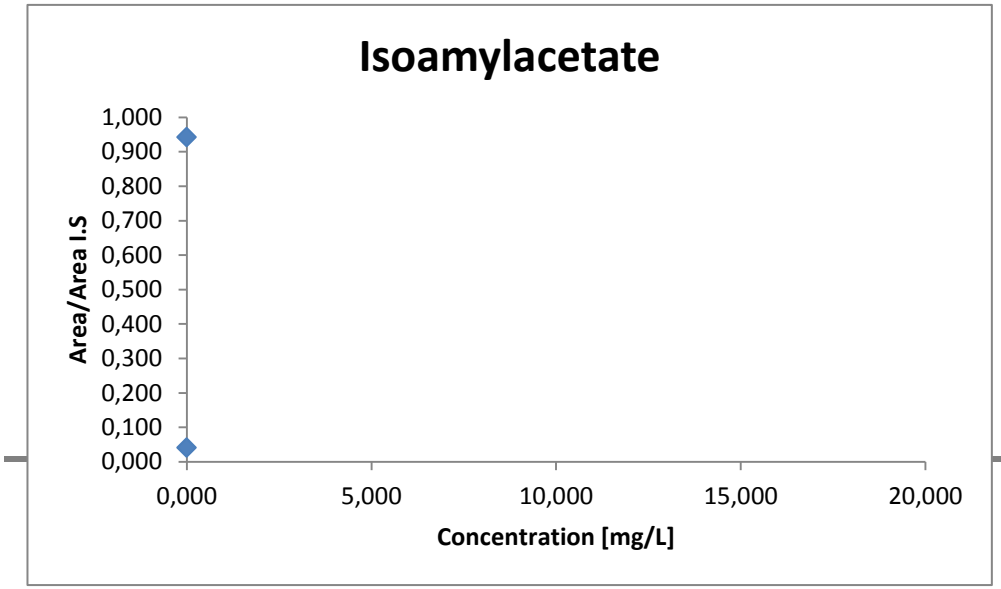

DMS
0,120
0,100
0,080
0,060
0,040
0,020
0,000
Area/Area I.S
y = 0,0601x + 0,0016
R² = 0,7673
0,000
0,500
1,000
1,500
2,000
Concentration [mg/L]

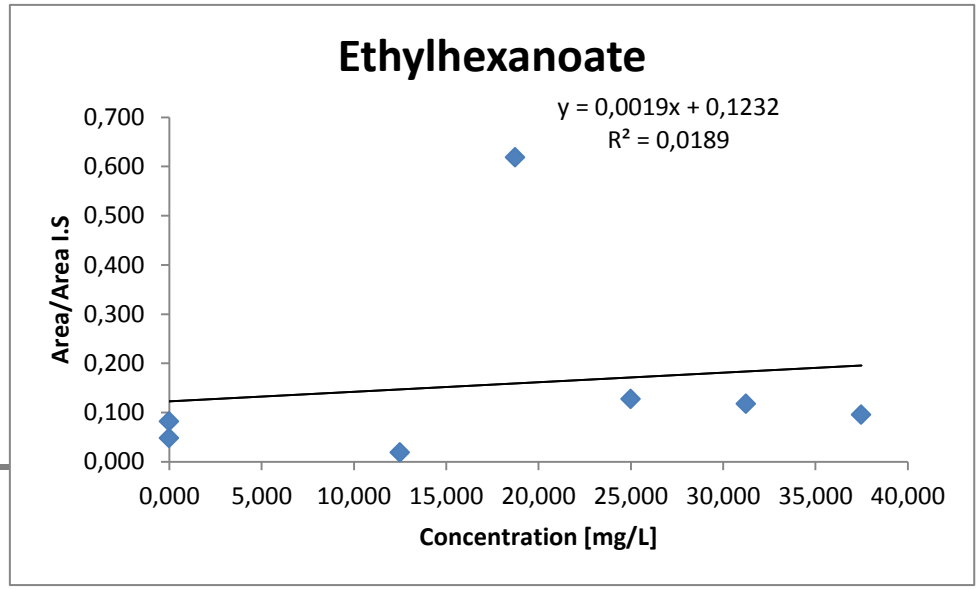

Ethyacetate
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0,000
Area/Area I.S
y = 0,0807x + 4,0432
R² = 0,2889
0,000
5,000
10,000
15,000
20,000
25,000
30,000
35,000
40,000
Concentration [mg/L]

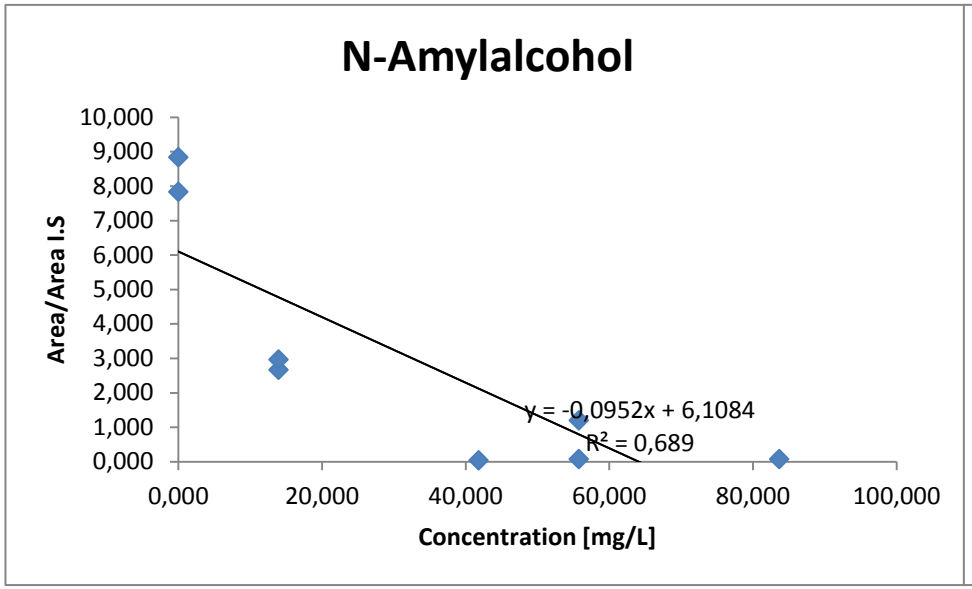

Ethylbutyrate
0,090
0,080
0,070
0,060
0,050
0,040
0,030
0,020
0,010
0,000
Area/Area I.S
y = 0,0215x + 0,0293
R² = 0,4269
0,000
0,500
1,000
1,500
2,000
2,500
Concentration [mg/L]

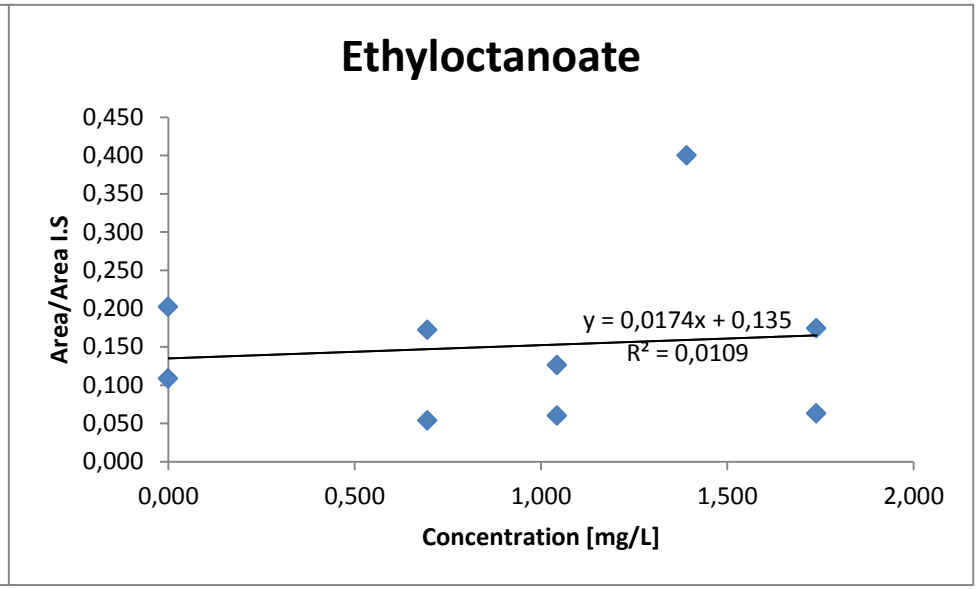

N-Propanol
Area/Area I.S
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]
y = -0,0263x + 0,7545
R² = 0,1834


Isobutanol
Area/Area I.S
2,000
1,800
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]
y = -0,0601x + 1,3816
R² = 0,5164


Isoamylacetate
Area/Area I.S
1,000
0,900
0,800
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]


Ethylhexanoate
Area/Area I.S
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
0,000
5,000
10,000
15,000
20,000
25,000
30,000
35,000
40,000
Concentration [mg/L]
y = 0,0019x + 0,1232
R² = 0,0189


N-Amylalcohol
Area/Area I.S
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0,000
0,000
20,000
40,000
60,000
80,000
100,000
Concentration [mg/L]
y = -0,0952x + 6,1084
R² = 0,689


Ethyloctanoate
Area/Area I.S
0,450
0,400
0,350
0,300
0,250
0,200
0,150
0,100
0,050
0,000
0,000
0,500
1,000
1,500
2,000
Concentration [mg/L]
y = 0,0174x + 0,135
R² = 0,0109

3- CALIBRATION CURVES BEER 2.2

		Sample 1.1			Sample 1.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**					5,803	
IS	**12,000**	85784	11,672		97342	11,955	
Acetaldehyde	**3,498**	3618	3,468	0,0421757	4264	3,492	0,04380432
DMS	**3,673**	2790	4,035	0,03252355	103	3,672	0,00105812
Ethyacetate	**4,848**	81035	4,775	0,94464003	78250	4,901	0,80386678
Ethylbutyrate	**8,698**	906	8,371	0,01056141	915	8,667	0,00939985
N-Propanol	**8,812**	19388	8,533	0,22600951	17419	8,822	0,1789464
Isobutanol	**10,920**	34495	10,534	0,40211461	26977	10,847	0,27713628
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	2914	16,935	0,03396904	95884	16,002	0,98502188
N-Amylalcohol	**18,226**	170220	17,878	1,98428611	136245	18,242	1,39965277
Ethyloctanoate	**23,623**	10959	23,339	0,1277511	6385	23,605	0,06559347

		Sample 2.1			Sample 2.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**					5,816	
IS	**12,000**	102443	12,14		108525	12,001	
Acetaldehyde	**3,498**	17398	3,49	0,16983103	11421	3,493	0,10523842
DMS	**3,673**	3647	3,669	0,03560029	2285	3,913	0,02105506
Ethyacetate	**4,848**	496542	4,894	4,8470076	341183	4,905	3,1438194
Ethylbutyrate	**8,698**	5621	8,66	0,05486954	3835	8,681	0,03533748
N-Propanol	**8,812**	38857	8,814	0,37930361	19926	8,824	0,18360746
Isobutanol	**10,920**	64535	11,125	0,62996008	41825	10,844	0,38539507
Isoamylacetate	**11,973**	65209	11,893	0,63653934	246	11,705	0,00226676
Ethylhexanoate	**17,413**	118877	16,048	1,16042092	68979	16,011	0,6356047
N-Amylalcohol	**18,226**	312265	18,261	3,04818289	159498	18,254	1,46968901
Ethyloctanoate	**23,623**	16039	23,58	0,15656511	9653	23,618	0,08894725

		Sample 3.1			Sample 3.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**					4,515	
IS	**12,000**	264394	12,012		77424	13,417	
Acetaldehyde	**3,498**	36972	3,493	0,13983676	7202	3,305	0,09302025
DMS	**3,673**	17415	3,674	0,06586761	6384	3,902	0,08245505
Ethyacetate	**4,848**	1265276	4,908	4,78557002	772397	3,97	9,97619601

Ethylbutyrate	**8,698**	15738	8,682	0,0595248	2087	5,808	0,02695547
N-Propanol	**8,812**	30622	8,829	0,11581957	25294	5,994	0,32669456
Isobutanol	**10,920**	72660	10,87	0,27481713	94822	8,323	1,22471068
Isoamylacetate	**11,973**				68729	8,781	0,88769632
Ethylhexanoate	**17,413**	63757	16,017	0,2411439	13736	14,466	0,17741269
N-Amylalcohol	**18,226**	202489	18,256	0,7658608	287635	16,172	3,71506251
Ethyloctanoate	**23,623**	40688	23,614	0,15389154	22883	23,213	0,29555435

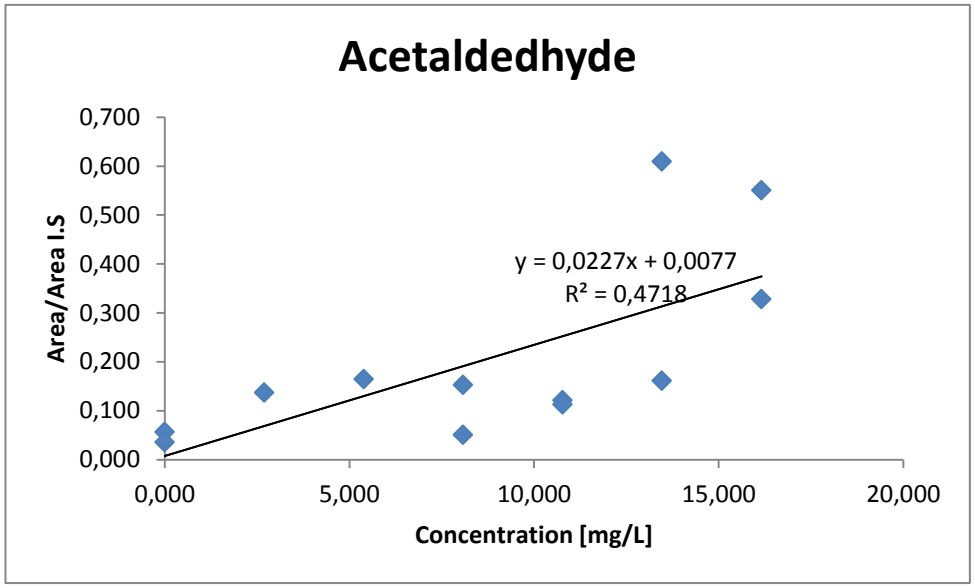
		Sample 4,1			Sample 4.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**					5,807	
IS	**12,000**	312710	11,982		250430	11,967	
Acetaldehyde	**3,498**	48749	3,486	0,15589204	39999	3,485	0,15972128
DMS	**3,673**	12777	3,666	0,04085894	9709	3,664	0,03876932
Ethyacetate	**4,848**	1680545	4,897	5,37413258	1298048	4,889	5,18327676
Ethylbutyrate	**8,698**	18007	8,658	0,0575837	13930	8,646	0,05562433
N-Propanol	**8,812**	63995	8,801	0,20464648	57401	8,792	0,22920976
Isobutanol	**10,920**	161393	10,825	0,51611077	144056	10,814	0,5752346
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	29824	17,367	0,09537271	23793	17,351	0,09500859
N-Amylalcohol	**18,226**	518267	18,226	1,65734067	481703	18,212	1,92350357
Ethyloctanoate	**23,623**	43725	23,594	0,13982604	38569	23,585	0,1540111

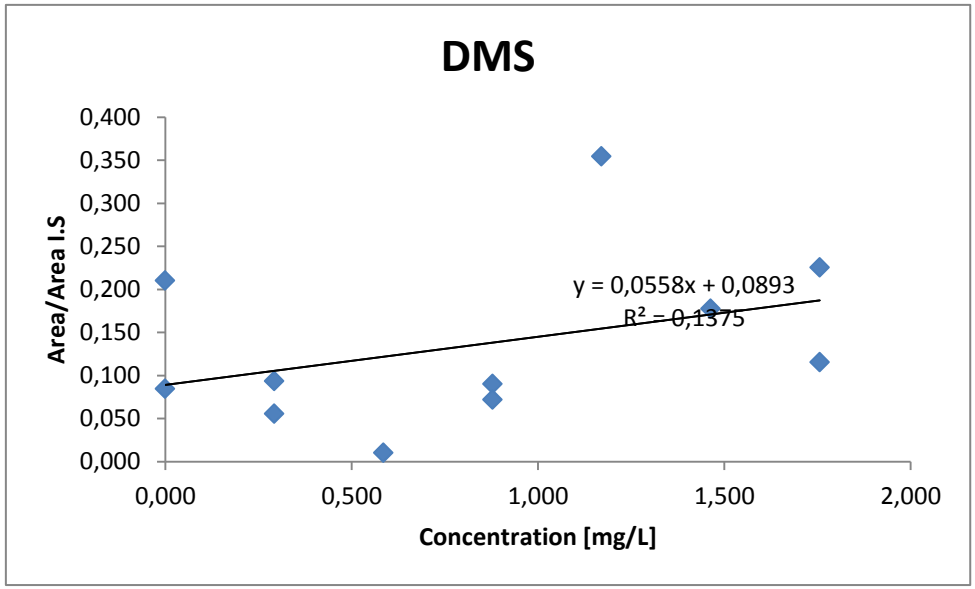
		Sample 5.1			Sample 5.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**					5,776	
IS	**12,000**	338124	11,973		251532	11,97	
Acetaldehyde	**3,498**	67834	3,488	0,20061871	42602	3,485	0,1693701
DMS	**3,673**	3903	3,668	0,0115431	11148	3,664	0,0443204
Ethyacetate	**4,848**	2269873	4,894	6,71313778	1486585	4,888	5,91012277
Ethylbutyrate	**8,698**	23582	8,644	0,06974364	15306	8,644	0,0608511
N-Propanol	**8,812**	54597	8,78	0,16147035	64950	8,785	0,25821764
Isobutanol	**10,920**	145954	10,792	0,43165821	160972	10,794	0,63996629
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	38269	17,347	0,11318037	25051	17,349	0,09959369
N-Amylalcohol	**18,226**	372269	18,202	1,10098366	436475	18,21	1,73526629
Ethyloctanoate	**23,623**	49355	23,581	0,14596716	41341	23,585	0,16435682

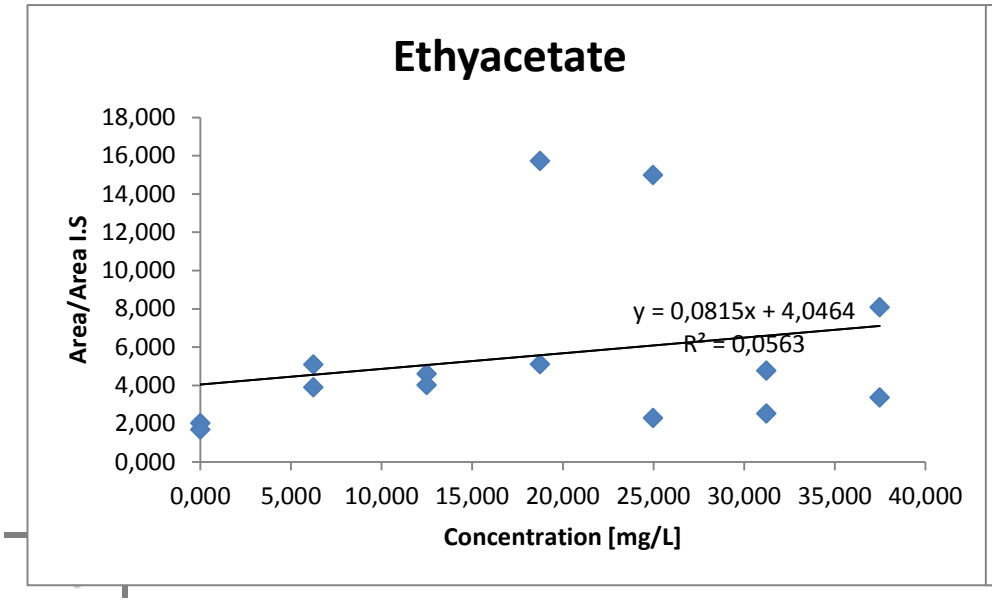
		Sample 6.1			Sample 6.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,586			5,8	
IS	**12,000**	266288	11,578		359031	11,988	
Acetaldehyde	**3,498**	55579	3,462	0,20871763	76178	3,486	0,21217666
DMS	**3,673**	12568	3,628	0,04719702	19693	3,666	0,05485042
Ethyacetate	**4,848**	1704939	4,754	6,40261296	2420416	4,898	6,74152371
Ethylbutyrate	**8,698**	18429	8,304	0,06920702	23520	8,66	0,06550966
N-Propanol	**8,812**	46816	8,471	0,17580965	95061	8,801	0,26477101
Isobutanol	**10,920**	122092	10,48	0,45849606	238281	10,819	0,66367807
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	31177	16,831	0,11708	37489	17,373	0,10441717
N-Amylalcohol	**18,226**	348256	17,813	1,3078171	662548	18,232	1,84537825
Ethyloctanoate	**23,623**	45052	23,302	0,16918524	55155	23,596	0,15362183
		Sample 7.1			Sample 7,2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,848			5,298	
IS	**12,000**	234804	12,002		281893	10,951	
Acetaldehyde	**3,498**	53236	3,479	0,22672527	87926	3,505	0,31191268
DMS	**3,673**	12244	3,661	0,05214562	305	3,643	0,00108197
Ethyacetate	**4,848**	1666794	4,9	7,09866101	2076742	4,535	7,36712866
Ethylbutyrate	**8,698**	16010	8,67	0,06818453	21306	7,718	0,07558187
N-Propanol	**8,812**	54920	8,807	0,23389721	58814	7,931	0,20863945
Isobutanol	**10,920**	142034	10,818	0,60490452	143509	9,879	0,50909033
Isoamylacetate	**11,973**						
Ethylhexanoate	**17,413**	25836	17,386	0,1100322	32065	16,299	0,11374883
N-Amylalcohol	**18,226**	416184	18,236	1,77247406	340252	17,447	1,20702536
Ethyloctanoate	**23,623**	38229	23,602	0,16281239	38861	23,227	0,13785727

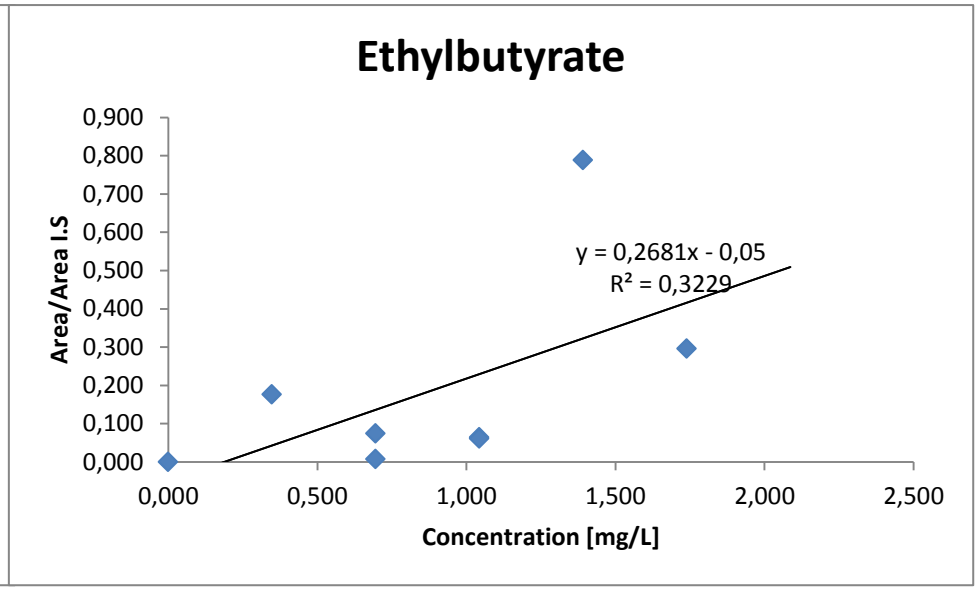
	Acetaldedhyde		DMS		Ethyacetate		Ethylbutyrate		N-Propanol	
Standard	Concentration [mg/L]	Ratio	Concentration [mg/L]	Ratio	Concentration [mg/L]	Ratio	Concentration [mg/L]	Ratio	Concentration [mg/L]	Ratio
1.1	0,000	0,042	0,000		0,000	0,945	0,000	0,011	0,000	0,226
1.2	0,000	0,044	0,000	0,001	0,000	0,804	0,000	0,009	0,000	0,179
2.1	2,693	0,170	0,293	0,036	6,247	4,847	0,348		2,755	0,379
2.2	2,693	0,105	0,293	0,021	6,247	3,144	0,348	0,035	2,755	0,184
3.1	5,386	0,140	0,585	0,066	12,494	4,786	0,696		5,510	0,116
3.2	5,386	0,093	0,585	0,050	12,494		0,696	0,027	5,510	0,327
4.1	8,078	0,156	0,878	0,041	18,741	5,374	1,043	0,058	8,264	0,205
4.2	8,078	0,160	0,878	0,039	18,741	5,183	1,043	0,056	8,264	0,229
5.1	10,771	0,201	1,171		24,987	6,713	1,391	0,070	11,019	0,161
5.2	10,771	0,169	1,171	0,044	24,987	5,910	1,391	0,061	11,019	0,258
6.1	13,464	0,209	1,463	0,047	31,234	6,403	1,739	0,069	13,774	0,176
6.2	13,464	0,212	1,463	0,055	31,234	6,742	1,739	0,066	13,774	0,265
7.1	16,157	0,227	1,756	0,052	37,481	7,099	2,087	0,068	16,529	0,234
7.2	16,157	0,312	1,756		37,481	7,367	2,087	0,076	16,529	0,209

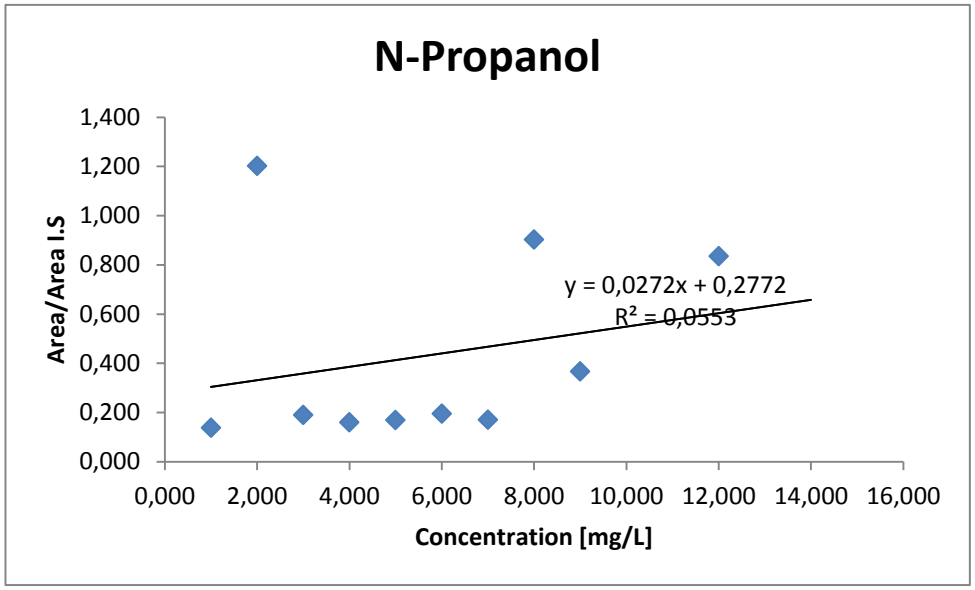
	Isobutanol		Isoamylacetate		Ethylhexanoate		N-Amylalcohol		Ethyloctanoate	
Standard	Concentration	Ratio	Concentration	Ratio	Concentration	Ratio	Concentration	Ratio	Concentration	Ratio
1.1	0,000	0,402	0,000		0,000	0,034	0,000	1,984	0,000	0,128
1.2	0,000	0,277	0,000		0,000	0,985	0,000	1,400	0,000	0,066
2.1	2,763	0,630	2,996	0,637	6,247	1,160	13,946	3,048	0,348	0,157
2.2	2,763	0,385	2,996		6,247	0,636	13,946	1,470	0,348	0,089
3.1	5,527	0,275	5,992		12,494	0,241	27,892	0,766	0,696	0,154
3.2	5,527	1,225	5,992	0,888	12,494	0,177	27,892	0,027	0,696	0,296
4.1	8,290	0,516	8,987		18,741	0,095	41,838	0,058	1,043	0,140
4.2	8,290	0,575	8,987		18,741	0,095	41,838	0,056	1,043	0,154
5.1	11,054	0,432	11,983		24,987	0,113	55,784	0,070	1,391	0,146
5.2	11,054	0,640	11,983		24,987	0,100	55,784	0,061	1,391	0,164
6.1	13,817	0,458	14,979		31,234	0,117	69,730	0,069	1,739	0,169
6.2	13,817	0,664	14,979		31,234	0,104	69,730	0,066	1,739	0,154
7.1	16,580	0,605	17,975		37,481	0,110	83,677	0,068	2,087	0,163
7.2	16,580	0,509	17,975		37,481	0,114	83,677	0,076	2,087	0,138

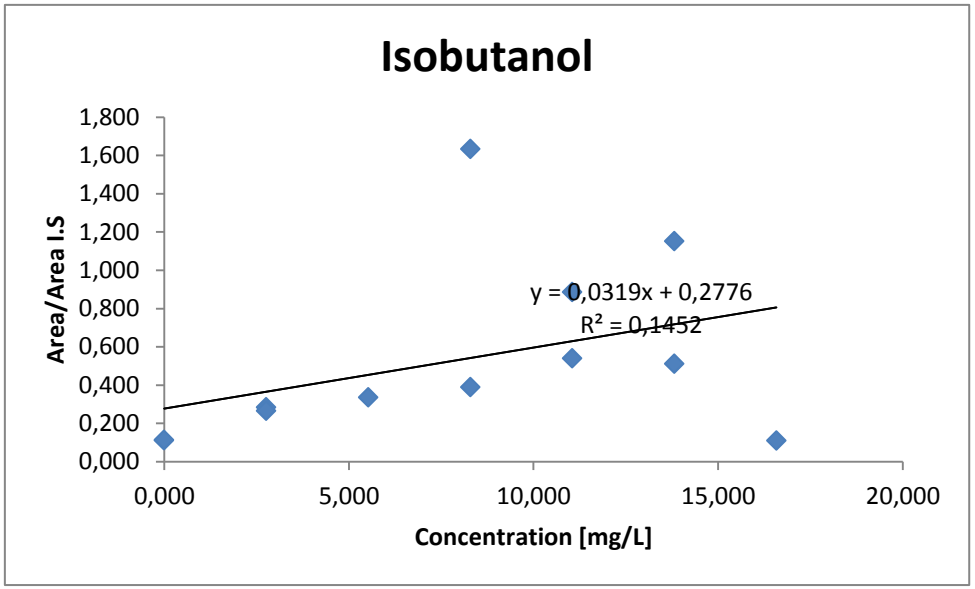

Acetaldedhyde
Area/Area I.S
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
y = 0,0227x + 0,0077
R² = 0,4718
0,000
5,000
10,000
15,000
20,000
Concentration [mg/L]

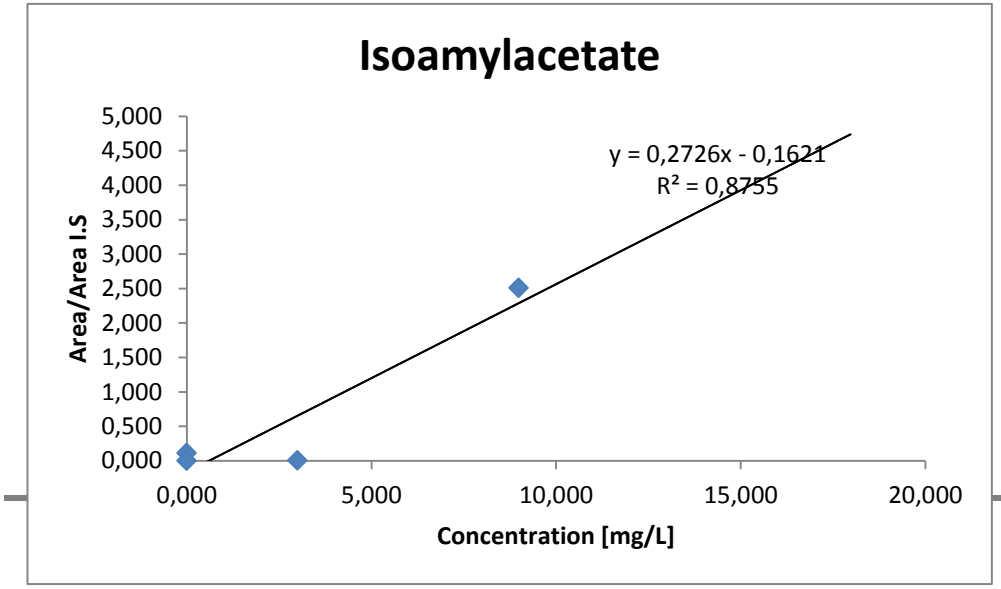

DMS
Area/Area I.S
0,400
0,350
0,300
0,250
0,200
0,150
0,100
0,050
0,000
y = 0,0558x + 0,0893
R² = 0,1375
0,000
0,500
1,000
1,500
2,000
Concentration [mg/L]

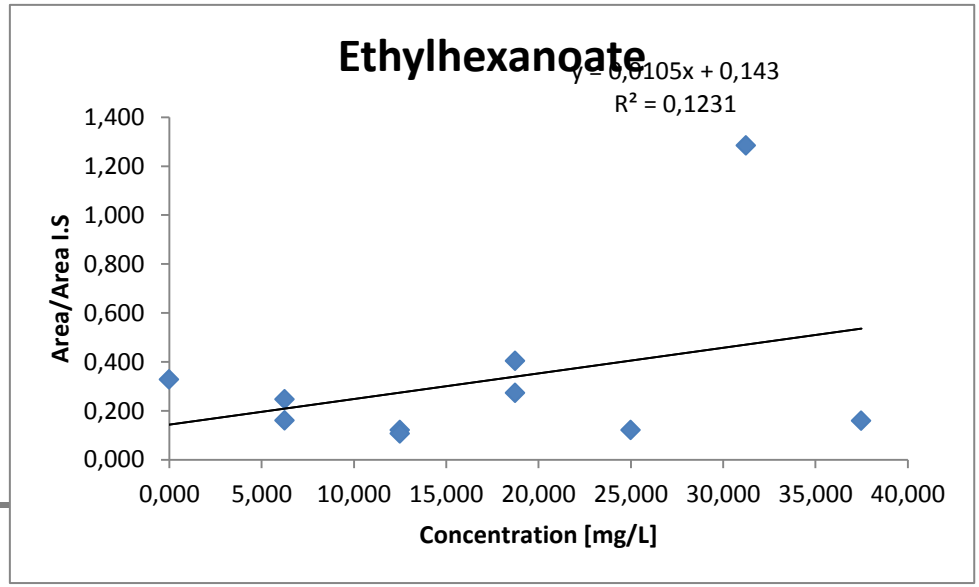

Ethyacetate
Area/Area I.S
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0,000
y = 0,0815x + 4,0464
R² = 0,0563
0,000
5,000
10,000
15,000
20,000
25,000
30,000
35,000
40,000
Concentration [mg/L]

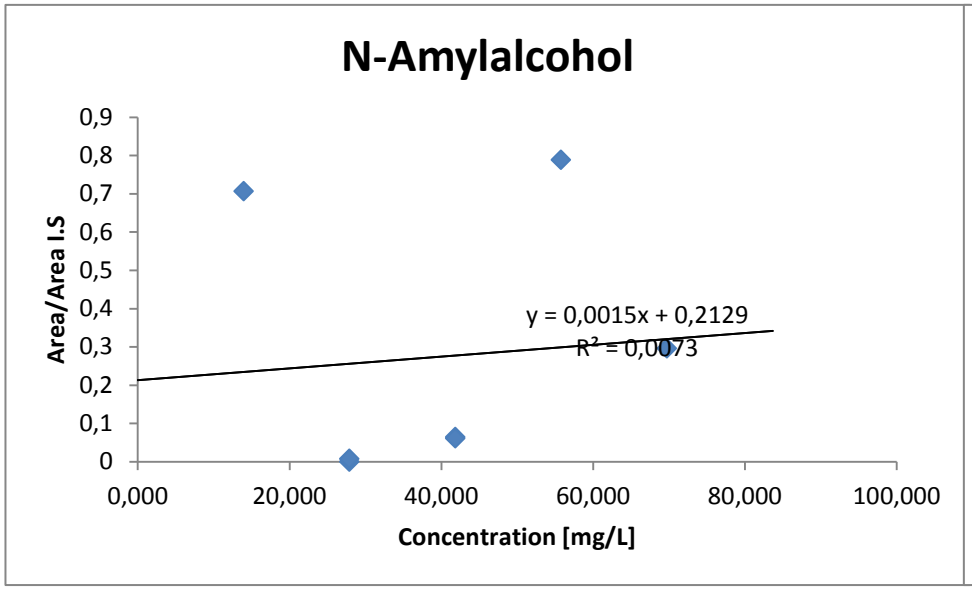

Ethylbutyrate
Area/Area I.S
0,900
0,800
0,700
0,600
0,500
0,400
0,300
0,200
0,100
0,000
y = 0,2681x - 0,05
R² = 0,3229
0,000
0,500
1,000
1,500
2,000
2,500
Concentration [mg/L]

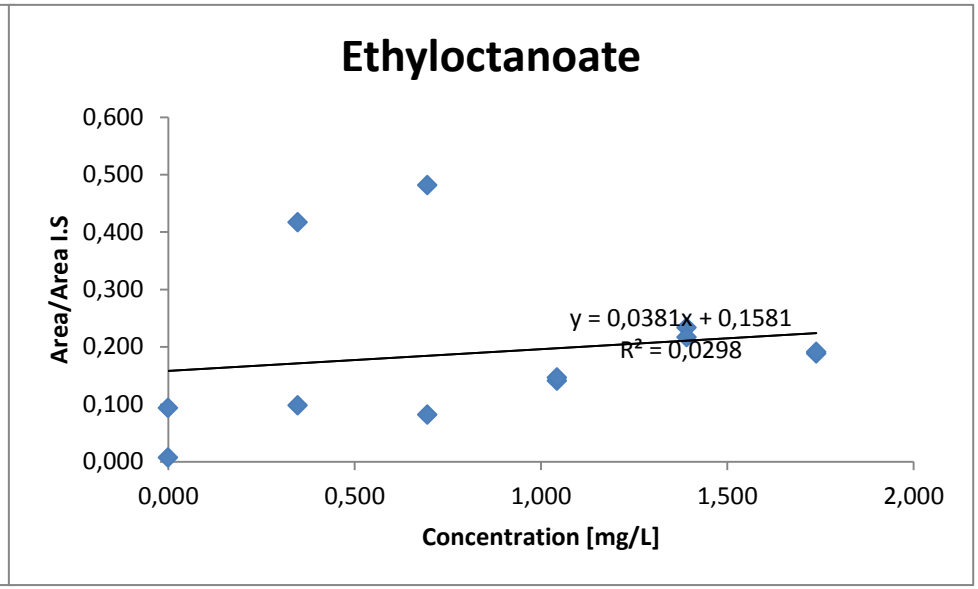

N-Propanol
Area/Area I.S
Concentration [mg/L]
y = 0,0272x + 0,2772
R² = 0,0553
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
2,000
4,000
6,000
8,000
10,000
12,000
14,000
16,000


Isobutanol
Area/Area I.S
Concentration [mg/L]
y = 0,0319x + 0,2776
R² = 0,1452
1,800
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
5,000
10,000
15,000
20,000


Isoamylacetate
Area/Area I.S
Concentration [mg/L]
y = 0,2726x - 0,1621
R² = 0,8755
5,000
4,500
4,000
3,500
3,000
2,500
2,000
1,500
1,000
0,500
0,000
0,000
5,000
10,000
15,000
20,000


Ethylhexanoate
Area/Area I.S
Concentration [mg/L]
y = 0,0105x + 0,143
R² = 0,1231
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
5,000
10,000
15,000
20,000
25,000
30,000
35,000
40,000


N-Amylalcohol
Area/Area I.S
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
y = 0,0015x + 0,2129
R² = 0,0073
0,000
20,000
40,000
60,000
80,000
100,000
Concentration [mg/L]


Ethyloctanoate
Area/Area I.S
0,600
0,500
0,400
0,300
0,200
0,100
0,000
y = 0,0381x + 0,1581
R² = 0,0298
0,000
0,500
1,000
1,500
2,000
Concentration [mg/L]

4- CALIBRATION CURVES BEER 2.3

		Sample 1.1			Sample 1.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,759				
IS	**12,000**	65171	11,962		444529	12,703	
Acetaldehyde	**3,498**	3062	3,659	0,04698409	20000	3,606	0,04499144
DMS	**3,673**	362	4,081	0,00555462	201	4,208	0,00045216
Ethyacetate	**4,848**	431674	4,866	6,623713	136897	4,916	0,30795966
Ethylbutyrate	**8,698**	5404	8,597	0,08292032	1188	8,728	0,00267249
N-Propanol	**8,812**	57325	8,744	0,87960903	306595	8,932	0,68970753
Isobutanol	**10,920**	129133	10,877	1,9814488	419027	11,404	0,94263141
Isoamylacetate	**11,973**	70556	11,886	1,08262878	23100	11,818	0,05196511
Ethylhexanoate	**17,413**	13174	17,255	0,20214513	595146	16,416	1,33882379
N-Amylalcohol	**18,226**	427494	18,169	6,55957404	2927492	18,488	6,58560409
Ethyloctanoate	**23,623**	26479	23,541	0,40630035	19365	23,526	0,04356296

		Szmple 2.1			Sample 2.1		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,792				
IS	**12,000**	75869	12,193		246497	12,457	
Acetaldehyde	**3,498**	5928	3,49	0,07813468	17973	3,491	0,07291367
DMS	**3,673**	384	4,906	0,00506136	1036	4,103	0,00420289
Ethyacetate	**4,848**	58397	4,887	0,76970831	343061	4,902	1,39174513
Ethylbutyrate	**8,698**				2746	8,682	0,0111401
N-Propanol	**8,812**	49178	8,801	0,64819623	14911	8,852	0,06049161
Isobutanol	**10,920**	1958	10,849	0,02580764	5461	10,89	0,02215443
Isoamylacetate	**11,973**	73656	11,2	0,9708313	206173	11,332	0,8364118
Ethylhexanoate	**17,413**	1264	17,23	0,0166603	4520	17,193	0,01833694
N-Amylalcohol	**18,226**	380549	18,249	5,01586946	1426516	18,36	5,7871536
Ethyloctanoate	**23,623**	5798	23,553	0,0764212	9957	23,555	0,040394

		Sample 3.1			Sample 3.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,981			6,09	
IS	**12,000**	273159	12,065		380228	12,585	
Acetaldehyde	**3,498**	28023	3,466	0,1025886	53216	3,497	0,13995813
DMS	**3,673**	3922	3,852	0,01435794	73379	3,678	0,19298684
Ethyacetate	**4,848**	822103	4,769	3,00961345	1290905	4,921	3,39508137
Ethylbutyrate	**8,698**	8508	8,338	0,03114669	9449	8,72	0,02485088

N-Propanol	**8,812**	177934	8,536	0,65139351	250462	8,898	0,6587153
Isobutanol	**10,920**	242756	10,492	0,88869852	15027	10,927	0,03952102
Isoamylacetate	**11,973**	103306	11,433	0,37818999	114296	11,817	0,30059859
Ethylhexanoate	**17,413**	14098	16,632	0,05161097	14972	17,174	0,03937637
N-Amylalcohol	**18,226**	1701566	17,98	6,22921449	2422467	18,432	6,3710905
Ethyloctanoate	**23,623**	28287	23,29	0,43404275	20256	23,555	0,0532733

		Sample 4.1			Sample 4.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,892				
IS	**12,000**	179670	12,365		213908	11,876	
Acetaldehyde	**3,498**	46335	3,492	0,25788946	43097	3,483	0,20147447
DMS	**3,673**	8418	3,673	0,04685256	11587	3,661	0,05416815
Ethyacetate	**4,848**	1238796	4,904	6,89484054	1205732	4,868	5,63668493
Ethylbutyrate	**8,698**	12478	8,682	0,06944955	13356	8,591	0,06243806
N-Propanol	**8,812**	144309		0,80318918	43923	8,739	0,20533594
Isobutanol	**10,920**	11161	10,888	0,06211944	96136	10,86	0,44942686
Isoamylacetate	**11,973**	136795	11,826	0,76136806			
Ethylhexanoate	**17,413**	18484	17,237	0,1028775	21125	17,235	0,09875741
N-Amylalcohol	**18,226**	1168102	18,333	6,50137474	273451	18,149	1,27835799
Ethyloctanoate	**23,623**	28349	223,563	0,15778371	26065	23,528	0,12185145

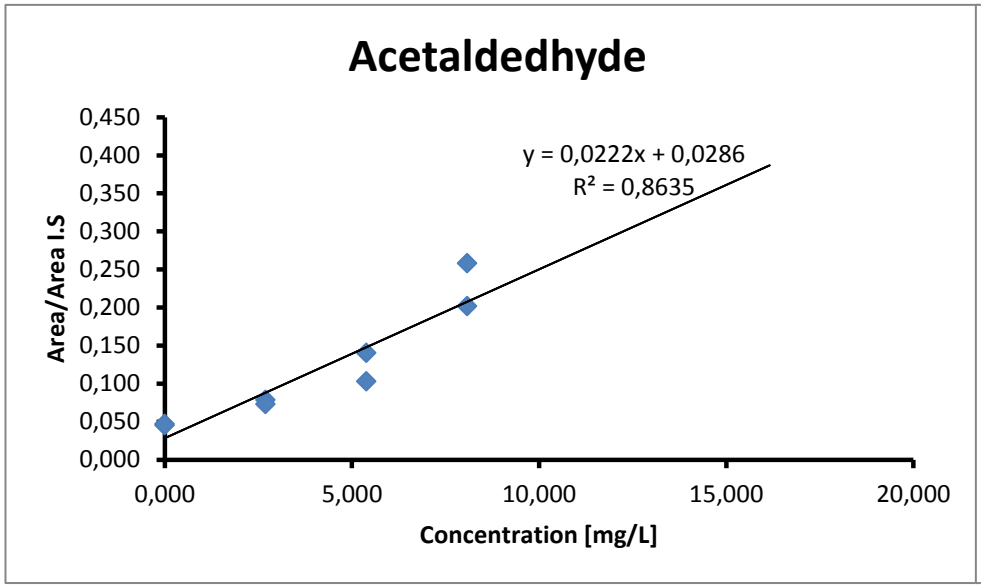
		Sample 5.1			Sample 5.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**						
IS	**12,000**	154669	11,433		86764	12,163	
Acetaldehyde	**3,498**	69194	3,421	0,44736825	66439	3,487	0,76574386
DMS	**3,673**	14748	3,567	0,09535201	14984	3,667	0,17269835
Ethyacetate	**4,848**	1641171	4,552	10,6108593	1677655	4,484	19,3358421
Ethylbutyrate	**8,698**	15171	7,749	0,09808688	17207	8,655	0,19831958
N-Propanol	**8,812**	163832	7,992	1,05924264	81303	8,805	0,93705915
Isobutanol	**10,920**	262376	10,329	1,69637096	156857	11,162	1,8078581
Isoamylacetate	**11,973**	167394	10,808	1,08227247	193494	12,163	2,23011848
Ethylhexanoate	**17,413**	21606	16,0188	0,13969186	25643	17,281	0,29554885
N-Amylalcohol	**18,226**	1384786	17,576	8,95322269	586065	18,264	6,75470241
Ethyloctanoate	**23,623**	28043	23,206	0,18130976	32604	23,574	0,37577797

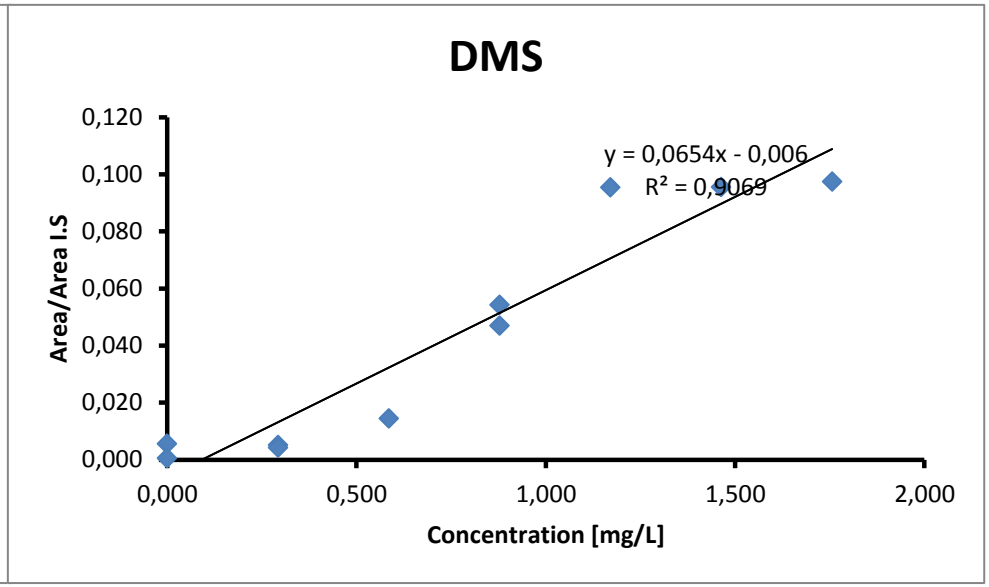
		Sample 6.1			Sample 6.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,924				
IS	**12,000**	88104	12,184		193137	11,961	
Acetaldehyde	**3,498**	62758	3,49	0,71231726	56517	3,489	0,29262648
DMS	**3,673**	8413	3,907	0,09548942	11674	3,668	0,06044414
Ethyacetate	**4,848**	1579210	4,895	17,9243848	1551885	4,89	8,03515121
Ethylbutyrate	**8,698**	14803	8,654	0,16801734	14920	8,639	0,07725086
N-Propanol	**8,812**	111911	8,808	1,27021475	31437	8,775	0,16277047
Isobutanol	**10,920**	187194	11,191	2,12469354	76788	10,798	0,39758306
Isoamylacetate	**11,973**	160785	11,856	1,82494552			
Ethylhexanoate	**17,413**	21359	17,266	0,2424294	20413	17,336	0,10569181
N-Amylalcohol	**18,226**	818968	18,27	9,29546899	165801	18,192	0,85846316
Ethyloctanoate	**23,623**	28242	23,568	0,32055298	22066	23,575	0,11425051

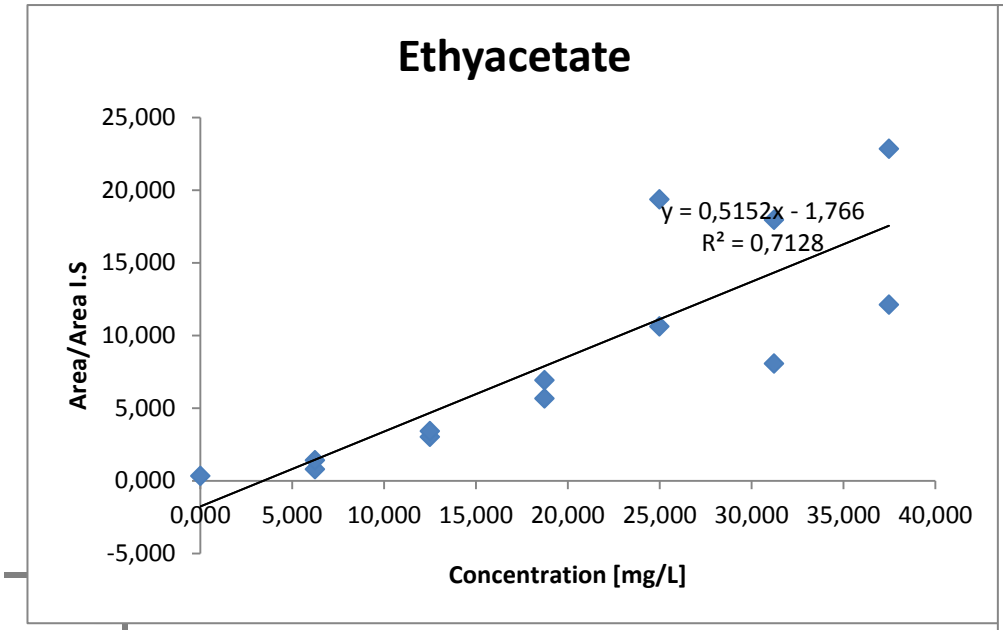
		Sample 7.1			Sample 7.2		
	Time expect	Area	Time [min]	[Area/Area IS]	Area	Time [min]	[Area/Area IS]
Et-oH	**5,802**		5,775				
IS	**12,000**	133793	11,942		233340	11,942	
Acetaldehyde	**3,498**	218245	3,485	1,63121389	148477	3,461	0,63631182
DMS	**3,673**	13034	3,662	0,09741915	8396	3,629	0,03598183
Ethyacetate	**4,848**	3052948	4,88	22,8184434	2821139	4,766	12,0902503
Ethylbutyrate	**8,698**	14763	8,618	0,1103421	18567	8,336	0,07957058
N-Propanol	**8,812**	186014	8,757	1,3903119	105772	8,504	0,45329562
Isobutanol	**10,920**	321300	10,77	2,40147093	209106	10,636	0,89614297
Isoamylacetate	**11,973**	114028		0,85227179	209106	11,575	0,89614297
Ethylhexanoate	**17,413**	6386	17,309	0,04773045	13140	17,3	0,05631268
N-Amylalcohol	**18,226**	1261720	18,172	9,43038873	428648	17,769	1,83701037
Ethyloctanoate	**23,623**	4532	23,56	0,03387322	7485	23,318	0,03207765

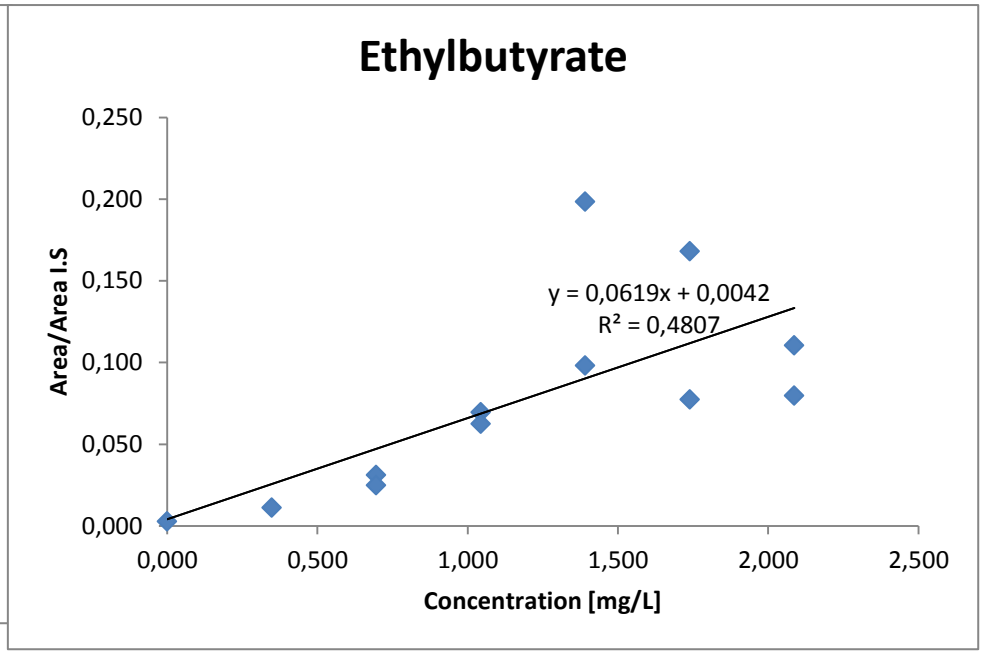
	Acetaldedhyde		DMS		Ethyacetate		Ethylbutyrate		N-Propanol	
Standard	Concentratio	Area/Area I.S	Concentratio	Area/Area I.	Concentratio	Area/Area I.	Concentratio	Area/Area I.S	Concentratio	Area/Area I.S
1.1	0,000	0,047	0,000	0,006	0,000		0,000		0,000	0,880
1.2	0,000	0,045	0,000	0,000	0,000	0,308	0,000	0,003	0,000	0,690
2.1	2,693	0,078	0,293	0,005	6,247	0,770	0,348		2,755	0,648
2.2	2,693	0,073	0,293	0,004	6,247	1,392	0,348	0,011	2,755	
3.1	5,386	0,103	0,585	0,014	12,494	3,010	0,696	0,031	5,510	0,651
3.2	5,386	0,140	0,585		12,494	3,395	0,696	0,025	5,510	0,659
4.1	8,078	0,258	0,878	0,047	18,741	6,895	1,043	0,069	8,264	0,803
4.2	8,078	0,201	0,878	0,054	18,741	5,637	1,043	0,062	8,264	
5.1	10,771		1,171	0,095	24,987	10,611	1,391	0,098	11,019	1,059
5.2	10,771		1,171		24,987	19,336	1,391		11,019	0,937
6.1	13,464		1,463	0,095	31,234	17,924	1,739	0,168	13,774	1,270
6.2	13,464		1,463		31,234	8,035	1,739		13,774	
7.1	16,157		1,756	0,097	37,481	22,818	2,087	0,110	16,529	1,390
7.2	16,157		1,756		37,481	12,090	2,087		16,529	

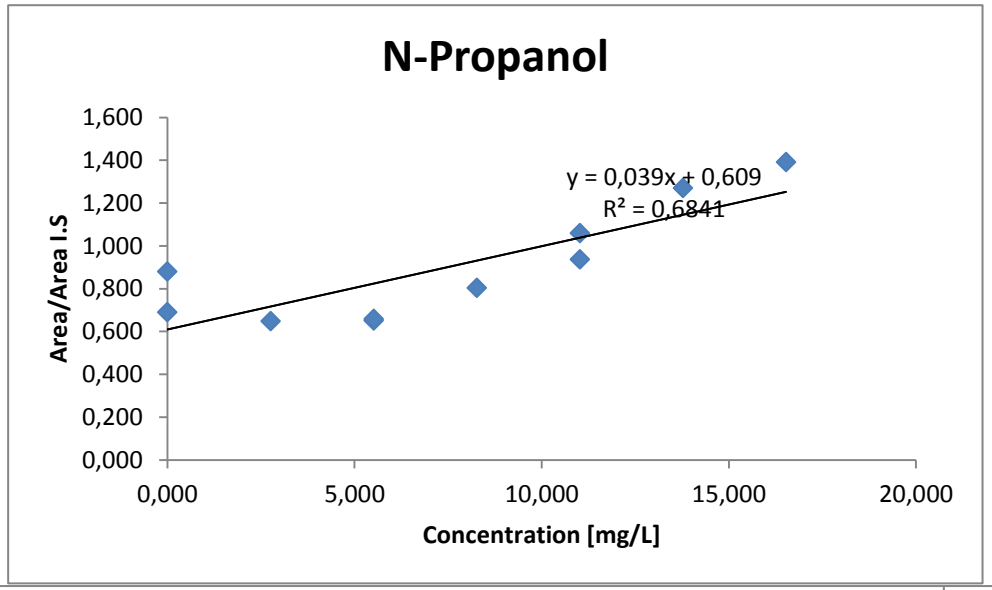
	Isobutanol		Isoamylacetate		Ethylhexanoate		N-Amylalcohol		Ethyloctanoate	
Standard	Concentratio	Area/Area I.S	Concentratio	Area/Area I.	Concentratio	Area/Area I.	Concentratio	Area/Area I.S	Concentratio	Area/Area I.S
1.1	0,000		0,000	1,083	4,000	0,202	4,000	6,560	4,000	0,406
1.2	0,000		0,000	0,052	0,000	1,339	0,000	6,586	0,000	0,044
2.1	2,763	0,026	2,996	0,971	6,247	0,017	13,946	5,016	0,348	0,076
2.2	2,763	0,022	2,996	0,836	6,247	0,018	13,946	5,787	0,348	0,040
3.1	5,527		5,992	0,378	12,494	0,052	27,892	6,229	0,696	0,434
3.2	5,527	0,040	5,992	0,301	12,494	0,039	27,892	0,025	0,696	0,053
4.1	8,290	0,062	8,987	0,761	18,741	0,103	41,838	0,069	1,043	0,158
4.2	8,290	0,449	8,987		18,741	0,099	41,838	0,062	1,043	0,122
5.1	11,054	1,696	11,983	1,082	24,987	0,140	55,784	0,098	1,391	0,181
5.2	11,054	1,808	11,983	2,230	24,987	0,296	55,784	0,198	1,391	0,376
6.1	13,817	2,125	14,979	1,825	31,234	0,242	69,730	0,168	1,739	0,321
6.2	13,817	0,398	14,979		31,234	0,106	69,730	0,077	1,739	0,114
7.1	16,580		17,975	0,852	37,481	0,048	83,677	0,110	2,087	0,034
7.2	16,580	0,896	17,975	0,896	37,481	0,056	83,677	0,080	2,087	0,032

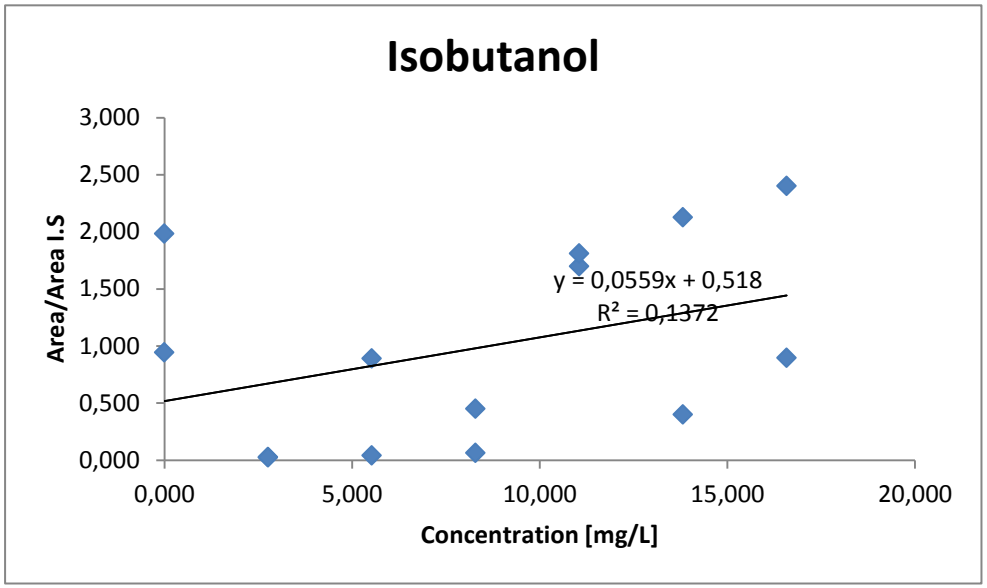

Acetaldedhyde
y = 0,0222x + 0,0286
R² = 0,8635
Area/Area I.S
Concentration [mg/L]
0,450
0,400
0,350
0,300
0,250
0,200
0,150
0,100
0,050
0,000
0,000
5,000
10,000
15,000
20,000

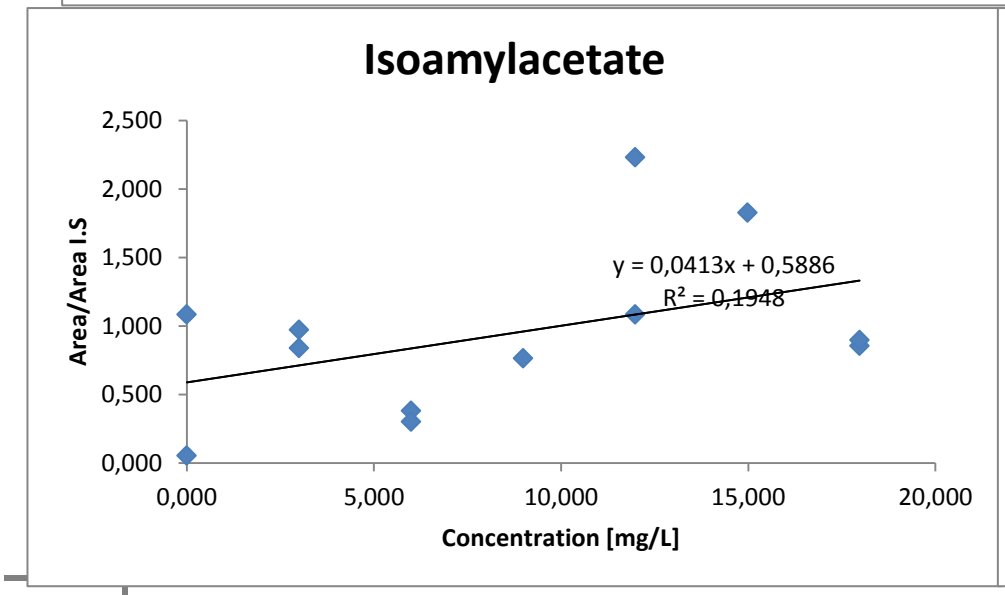

DMS
y = 0,0654x - 0,006
R² = 0,9069
Area/Area I.S
Concentration [mg/L]
0,120
0,100
0,080
0,060
0,040
0,020
0,000
0,000
0,500
1,000
1,500
2,000

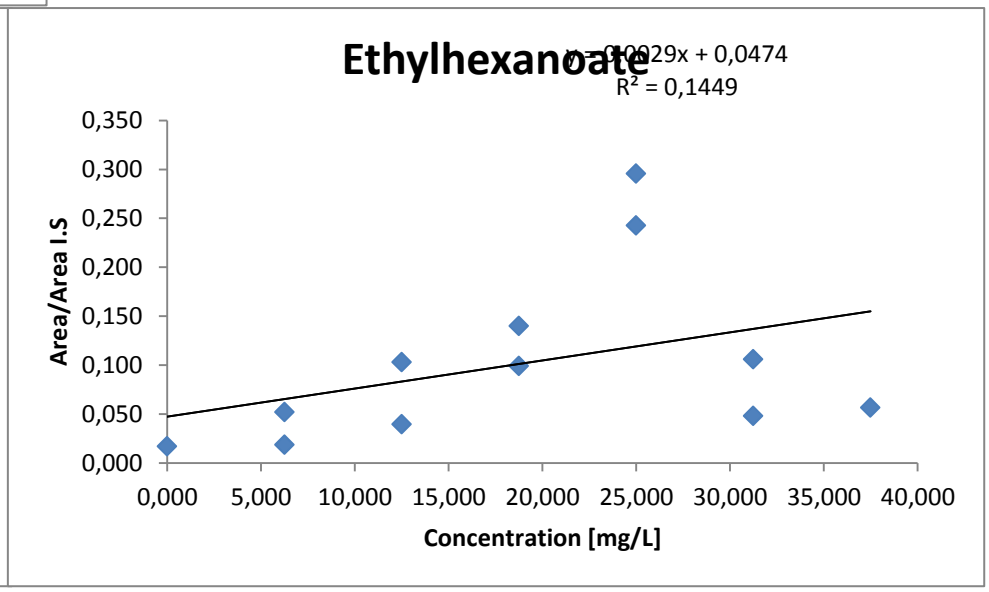

Ethyacetate
y = 0,5152x - 1,766
R² = 0,7128
Area/Area I.S
Concentration [mg/L]
25,000
20,000
15,000
10,000
5,000
0,000
-5,000
0,000
5,000
10,000
15,000
20,000
25,000
30,000
35,000
40,000

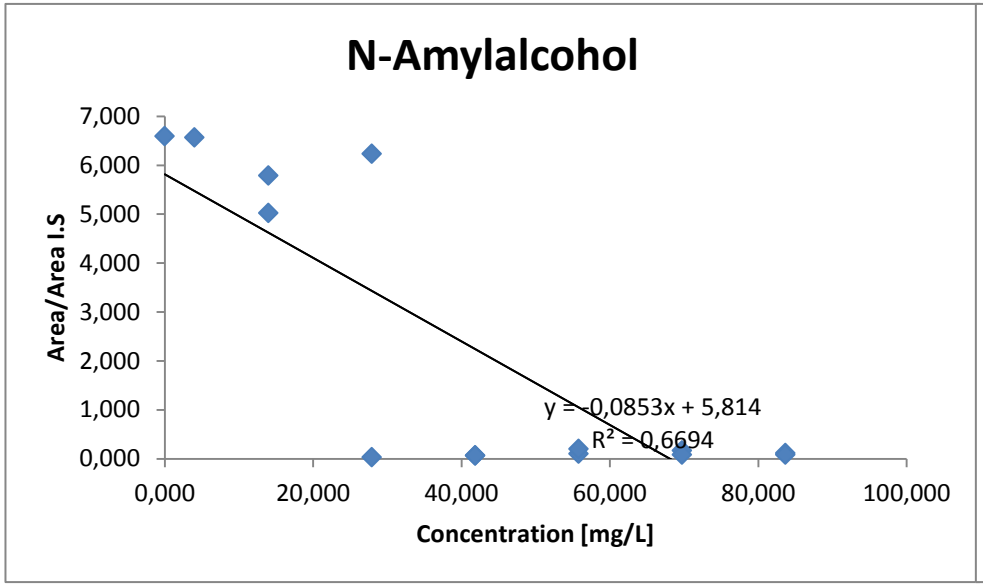

Ethylbutyrate
y = 0,0619x + 0,0042
R² = 0,4807
Area/Area I.S
Concentration [mg/L]
0,250
0,200
0,150
0,100
0,050
0,000
0,000
0,500
1,000
1,500
2,000
2,500

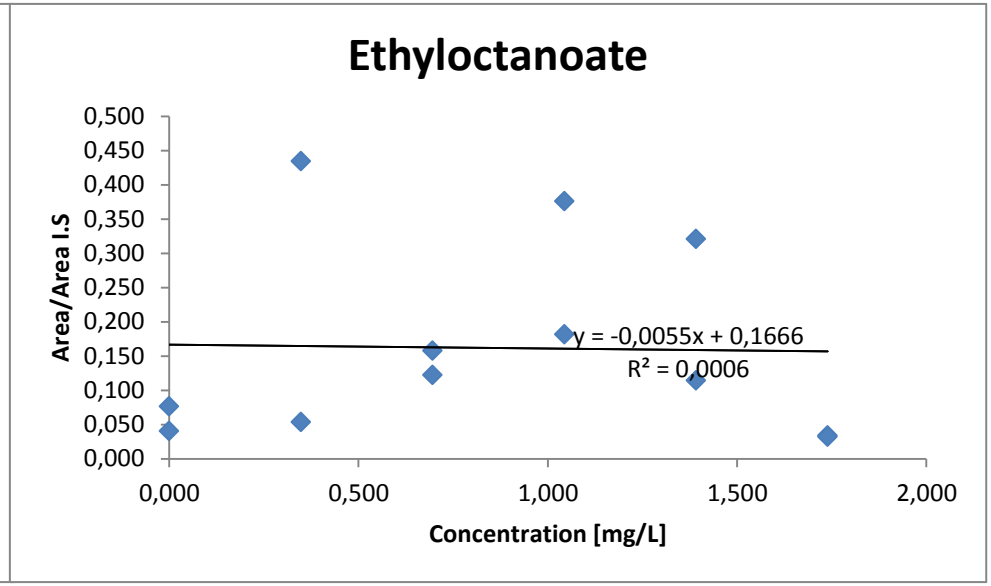

N-Propanol
y = 0,039x + 0,609
R² = 0,6841
Area/Area I.S
Concentration [mg/L]
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
0,000
5,000
10,000
15,000
20,000


Isobutanol
y = 0,0559x + 0,518
R² = 0,1372
Area/Area I.S
Concentration [mg/L]
3,000
2,500
2,000
1,500
1,000
0,500
0,000
0,000
5,000
10,000
15,000
20,000


Isoamylacetate
y = 0,0413x + 0,5886
R² = 0,1948
Area/Area I.S
Concentration [mg/L]
2,500
2,000
1,500
1,000
0,500
0,000
0,000
5,000
10,000
15,000
20,000


Ethylhexanoate
y = 0,0029x + 0,0474
R² = 0,1449
Area/Area I.S
Concentration [mg/L]
0,350
0,300
0,250
0,200
0,150
0,100
0,050
0,000
0,000
5,000
10,000
15,000
20,000
25,000
30,000
35,000
40,000


N-Amylalcohol
Area/Area I.S
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0,000
0,000
20,000
40,000
60,000
80,000
100,000
Concentration [mg/L]
y = -0,0853x + 5,814
R² = 0,6694


Ethyloctanoate
Area/Area I.S
0,500
0,450
0,400
0,350
0,300
0,250
0,200
0,150
0,100
0,050
0,000
0,000
0,500
1,000
1,500
2,000
Concentration [mg/L]
y = -0,0055x + 0,1666
R² = 0,0006