INVESTIGATION OF NON-PROCESS COMPOUNDS DETECTED IN THE RESIDUAL SOLVENT ANALYSIS OF PHARMACEUTICAL SUBSTANCES USING HS-GC-FID/MS

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ABSTRACT

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Investigation of Non-Process Compounds Detected in the Residual Solvent Analysis of Pharmaceutical Substances Using HS-GC-FID/MS

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Ensuring the safety of active pharmaceutical ingredients requires analytical methods capable of detecting production-related contaminants at low concentrations. The aim of this thesis is to identify volatile impurities that may arise from non-process materials used in drug substance production, using the HS-GC-MS/FID device.

In the literature review the working principle of HS-GC-MS/FID was outlined. Multi detector systems in gas chromatography were also described as well as the different approaches used in GC data analysis and analytical method development.

The applied section investigates the potential contaminants that could arise from non-process materials such as detergents, packaging products lubricants HS vial septa used in API production and analytical quality control. Diluted detergent solutions, and detergent solutions simulated under process conditions, were analyzed. Packaging products and the HS vial septa were investigated by exposing them to different solvents under different conditions. In addition, information on the additives used in the packaging materials was collected from literature. Finally, samples of mineral oil were analyzed for volatile impurities. Volatile impurities that arise from the synthetic process such as residual solvents are effectively controlled in the QC testing of drug substances using HS-GC-FID. Thus, the analysis of these substances is outside the scope of this study.

The experimental data indicated that certain detergents include volatile compounds that can be detected with HS-GC-MS/FID. The polyethylene packaging products did not produce volatile impurities; however, they did dissolve into certain solvents at higher temperatures. The mineral oil and headspace septum samples did not produce any volatile impurities either.

TIIVISTELMÄ

Lappeenrannan–Lahden teknillinen yliopisto LUT

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Kemiantekniikka

Magnus Kaurinkoski

Lääkeaineiden jäämäliuotinanalyysissä havaittujen ei-prosessiperäisten yhdisteiden tutkimus hyödyntäen HS-GC-FID/MS laitetta

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Lääkeaineiden laadunvalvonta edellyttää analyysimenetelmien käyttöä, jotka havaitsevat prosessiepäpuhtaudet jo alhaisissa pitoisuuksissa. Tämän työn tavoitteena on tunnistaa ei-prosessi peräisiä haihtuvia epäpuhtauksia, joita voi muodostua lääkeaineiden tuotanto materiaaleista, hyödyntäen HS-GC-MS/FID laitetta.

Kirjallisessa osassa esiteltiin HS-GC-MS/FID laitteen toimintaperiaatteet. Lisäksi esiteltiin monidetektoriyhdistelmiä sekä GC datan käsittelytapoja ja analyysimenetelmien kehitystä.

Kokeellisessa osassa tutkittiin HS-GC-MS/FID laitteella mahdollisia epäpuhtauksia, jotka voisivat olla peräisin tuotannon ja laadunvalvonnan materiaaleista, jotka eivät osallistu lääkesynteesiin. Näitä materiaaleja ovat pesuaineet, pakkausmateriaalit, voiteluaineet sekä headspace-näytepullojen tiivistemateriaalit. Analysoitiin laimennettuja pesuaineliuoksia sekä simuloiduissa prosessiolosuhteissa valmistettuja pesuaineliuoksia. Pakkausmateriaaleja ja tiivisteitä tutkittiin uuttamalla materiaaleja eri liuottimissa eri olosuhteissa. Lisäksi kirjallisuudesta haettiin tietoa pakkausmateriaalien lisäaineista. Lopuksi analysoitiin myös mineraaliöljy näyteitä. Synteesiprosessista peräisin olevat haihtuvat epäpuhtaudet, kuten jäämäliuottimet, ovat tehokkaasti hallinnassa lääkeaineiden laadunvalvonnassa. Joten näiden yhdisteiden analyysi ei kuulu tämän tutkimuksen piiriin.

Tulokset osoittivat, että tietyt pesuaineet sisältävät haihtuvia yhdisteitä, jotka havaittiin HS-GC-MS/FID-laitteistolla. Pakkausmateriaaleista ei muodostunut haihtuvia yhdisteitä, mutta liukenivat tietyissä liuottimissa korkeammissa lämpötiloissa. Mineraaliöljy ja headspace tiiviste näytteet eivät tuottaneet haihtuvia epäpuhtauksia.

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Espoo 25.05.2026

Magnus Kaurinkoski

SYMBOLS AND ABBREVIATIONS

Symbols

A = Area found by detector

$\propto$ = Proportional to

C_G = Gaseous analyte concentration

C_s = Analyte concentration liquid sample

C_0 =Concentration of the added sample

K = partition coefficient

k = Capacity factor

β = Phase ratio

V_G = Gaseous volume

V_S = Volume of liquid sample

k_a = Molar response factor for an analyte

k_b = Molar response factor for an internal standard

C_a = Concentration for analyte

C_b = Concentration for internal standard

S_a = Signal response for analyte

S_b = Signal response for internal standard

Abbreviations

API = Active Pharmaceutical Ingredients

GMP = Good manufacturing practice

FDA = Food and Drug administration

EMA = European Medicines Agency

LDPE = Low density polyethylene

HDPE = high density polyethylene

FCC = Fluid Catalytic Cracking

LPG = Liquified Petroleum Gas

HS = Headspace

GC = Gas chromatography

MS = Mass spectrometry

FID = Flame ionization detection

QC = Quality control

RT = Retention time

SNR = Signal-to-Noise ratio

t_r = Retention time

t_0 = Retention time of an unretained compound

DECLARATIONS

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Responsibility

The author, Magnus Kaurinkoski, takes full responsibility for the content of this thesis and has reviewed and edited the content generated by the possible use of AI tools

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1. Introduction

Innovations in medicine require rigorous quality control measures to ensure the safety and purity of medicines. This is very important because the pharmaceutical supply chain is very complicated, and in recent years certain events such as the covid-19 pandemic demonstrated the vulnerability of this system *(Coates, 2026)*. Therefore, scientifically sound, specific and accurate quality control measures have become increasingly crucial for ensuring patient safety and public health. Drug substance quality control relies heavily on different analytical equipment and methods to ensure the purity and safety of the products. The purpose of the analytical equipment is to detect, identify and quantify the impurities that are present in the products. This thesis utilizes headspace gas chromatography combined with mass spectrometry and flame ionization detection to investigate and identify potential process related contaminants. Certain samples were prepared by simulating the production conditions whilst other samples were prepared by modifying certain variables, such as temperature and extraction time.

Research on the interaction of polyethylene with certain solvents has been conducted. This research suggests that certain solvents are not compatible with polyethylene which raises concerns about the migration of contaminants when these solvents are exposed to polyethylene. *(Simoes, 2019).*

However, limited research has been conducted on volatile impurities that could arise from different detergents, polyethylene packaging and lubricants. The aim of this thesis is to identify volatile impurities that may arise from these materials used in drug substance production and to determine the sources of non-process impurities that have been detected in the residual solvent analysis using HS-GC-FID. The HS-GC-MS/FID system was used to detect and identify volatile impurities originating from essential production and laboratory materials such as detergents, packaging, lubricants and headspace vial septa. The structure of this thesis is divided into 2 sections. This includes a literature review as well as an applied section. The literature review outlines the working principle of HS-GC-MS/FID system and discusses both qualitative and quantitative data analysis. It also explains how analytical methods can be developed and describes the different impurities that appear in drug substance production. In addition, the structure and properties of low-density polyethylene

were outlined along with the design and operating principles of different production equipment used in drug substance manufacturing. Volatile impurities that arise from the synthetic process such as residual solvents are effectively controlled in the QC testing of drug substances using HS-GC-FID. Thus, the analysis of these substances is not in the scope of this study.

The applied part outlines the different experiments that were conducted on the production materials to investigate the impurities that may arise from them. After this the data was analyzed thoroughly. Visual, chromatographic and mass spectrometric data were recorded and analyzed.

Research questions:

What are the sources of non-process compounds detected in residual solvent analysis?

What volatile impurities can arise from the utilization of different detergents in drug substance production?

How are packaging materials made of polyethylene affected when exposed to different process solvents under different conditions and what, if any, volatile impurities do they produce under these conditions?

What volatile impurities can arise from the utilization of different lubricants in drug substance production?

What volatile impurities can arise from headspace sample preparation materials?

LITERATURE REVIEW:

2. Principles of HS-GC-MS/FID

This chapter discusses the basic working principles of sample introduction methods for GC-MS mostly focusing on headspace sampling. The working principle and data analysis methods of gas chromatography, mass spectrometry and flame ionization detectors are also

discussed as well as the principles of multidetector systems. In addition, chromatographic data analysis techniques such as qualitative and quantitative analysis are covered.

2.1. Gas chromatography (GC)

The main principle of gas chromatography is to separate volatile components for analysis. In chromatography there is always a stationary phase and a mobile phase. The mobile phase is often a liquid or gas that contains the substance that is being analyzed and carries it through the stationary phase. The components in the analyte travel across the stationary phase depending on their chemical and physical properties. *(East Texas A&M University, 2021)* Gas chromatography is best for analyzing thermally stable and volatile organic analytes *(Frolova, 2025)*.

In gas chromatography the mobile phase is called a carrier gas. Selecting a suitable carrier gas is important for optimizing efficiency and accuracy of the analysis. Helium, hydrogen and nitrogen are common carrier gases. It is essential that the carrier gas does not react with the analyte. Therefore, the selection of the appropriate carrier gas for each analysis is based on the chemical inertness and the separation capabilities of the gas. Helium separates components well and is also very inert, therefore it is an ideal choice. However, the availability of helium is limited, increasing the price significantly. Hydrogen and nitrogen are also good options as they are less expensive; they also have certain limitations such as the reactivity of hydrogen, and nitrogen requires lower flow rates leading to slower analysis. *(Phenomenex, 2025)*

The carrier gas transports the gaseous analyte across the stationary phase which is a thin layer that is inside of a glass or metal column. As the analyte travels through the column, the different components will interact with the stationary phase. Different components will travel at different rates causing different retention times. Once the compounds elute through the column, they exit the GC and are detected by detectors such as flame ionization detectors (FID) or mass spectrometers (MS). This whole process is illustrated in figure 1 below. *(East Texas A&M University, 2021)*

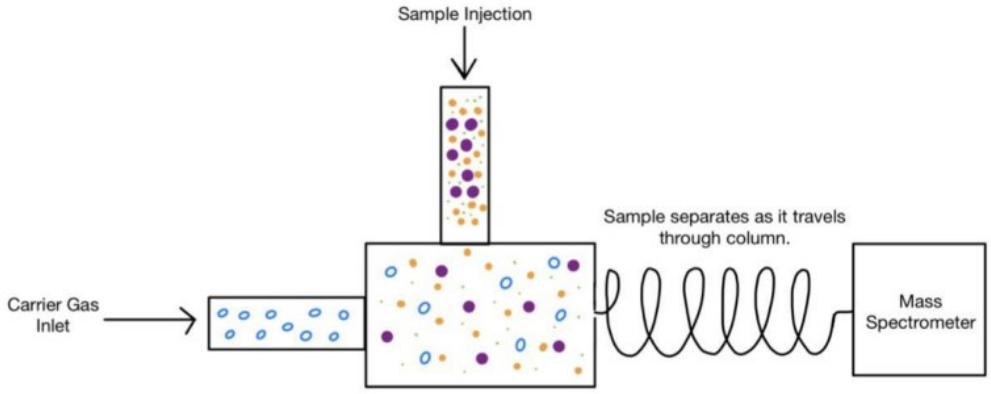

Figure 1: Illustration of the GC process *(East Texas A&M University, 2021)*

2.2. Flame Ionization Detector (FID)

The flame ionization detector is a common detector used in GC analysis. It is used to detect organic compounds and works by creating a continuous flame made up of a mixture of hydrogen and air that burns the samples as they leave the GC column. This ionizes the carbon atoms in the compounds. After the ions have been formed, they create a current at the detector's electrodes. This current is formed from the accumulation of these ions. The current is then transformed into an electrical signal. The different parts of the FID detector are displayed in figure 2. *(Peak Scientific, 2019)*

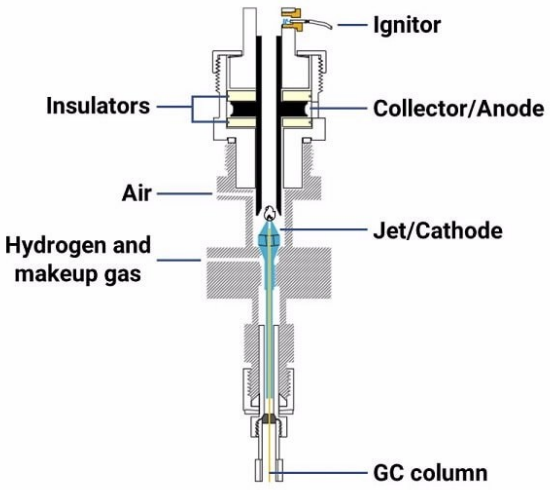

Figure 2: Illustration of the different parts of the FID detector *(Agilent, n.d.)*

2.3. Mass spectrometry (MS)

The mass spectrometer is one of the detectors used in GC analysis that identifies and quantifies chemical components that have been separated in the GC. The retention time is delivered by the GC however mass spectrometry gives information about the molecular weight, fragmentation patterns and isotopic ratios of the different components in the analyte. This information can be used to identify and quantify the different molecules in the analyte as well as aid in determining their chemical properties and structure. *(Khalifea & Ali, 2025)*

When the analyte chemicals elute to the mass spectrometer from the GC, they first encounter the ion source. This is where the neutral molecules are ionized. An electron ionization source is commonly used as the ion source. They often contain a metal filament that receives an electrical current which emits electrons at the molecules in the analyte. These electrons break the compounds into smaller fragments (or ions) giving many of them a positive charge. The ions produced can be used for the identification of a molecule. This process is illustrated in figure 3. (*Van Bramer, 2022)*

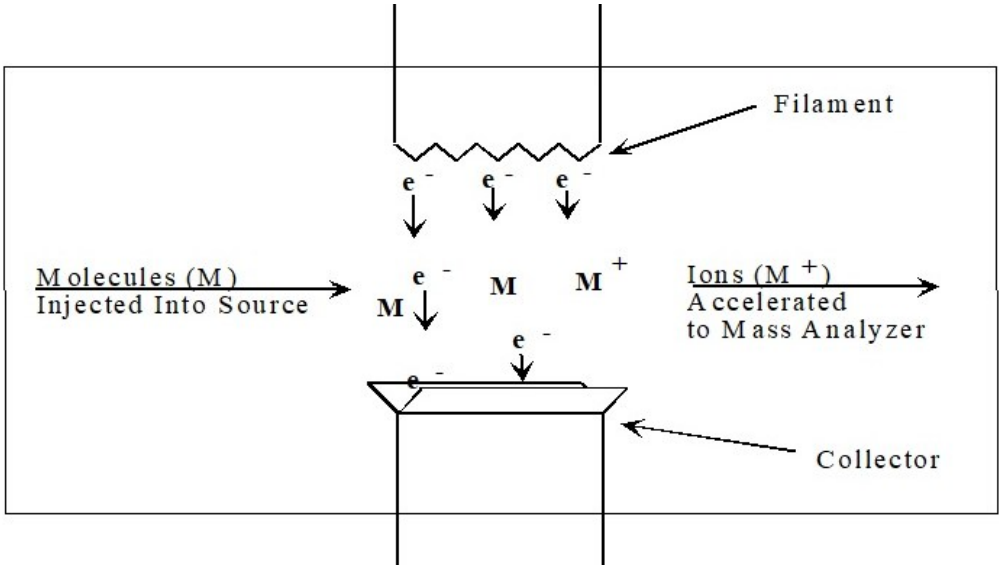

Figure 3: Ionization process (*Van Bramer, 2022)*

The ion source consists of several electrodes that are called lenses which lead the ions towards the detector. Before the ions enter the detection, they go through mass analyzer which is also known as a mass filter (*Busch, 2009)*. An example of a common mass analyzer is the quadrupole mass analyzer. This filter is made up of four rods that receive a direct current voltage and radio frequency. Different combinations of these forces are applied to

the rods which ensure that only ions of a specific mass-to-charge (m/z) ratio get transferred through the mass filter to the detector at a given time. *(Smith, 2013)*

The mass spectrometer can operate in full scan mode or then selected ion monitoring can be used. In full scan mode the mass spectrometer scans the different mass ranges from the smallest mass of ions to the largest, allowing these ions of different m/z ratios to pass through at different times *(Bhanot, 2016)*. After the ions have passed through the mass filter, they are measured with a detector. This data can then be used to identify the compounds present in each sample *(Smith, 2013).*

Full scan mode is useful for qualitative analysis whereas selected ion monitoring is useful for the quantitative analysis of specific ions in the analyte of interest. When this mode is used, only ions of selected masses are allowed to pass through the mass analyzer, increasing specificity.

2.4. Multidetector systems in gas chromatography

Within gas chromatography the basic principle includes a sampler, a column and a detector. When conducting a GC analysis confirmation of the reliability of the results is often required. This confirmation is required when analyzing complex samples as different errors can occur. These errors are usually caused by co-elution of compounds and matrix interferences. The traditional confirmation method is to perform an additional analysis with another column. However, analysis confirmation can also be conducted by implementing additional columns or detectors to the system. These multidetector systems were invented in the early years of GC development and they enable the user to analyze the same analyte using 2 different columns and/or detectors simultaneously. *(Dąbrowski. Ł, 2018).*

There are many different configurations of multidetector systems as can be seen in figure 4. When only considering the detectors, they can either have a parallel set up or they can be connected in series. When detectors are connected in series, they should have the same operating parameters, and the first detector cannot be destructive as this will affect the analysis of the second detector. The serial connection of detectors is not often used however they do provide certain advantages such as their simplicity. Serial connection is simpler

because the sample is not split into fractions, therefore the whole sample is analyzed, and a splitter is not required. *(Dąbrowski. Ł, 2018).*

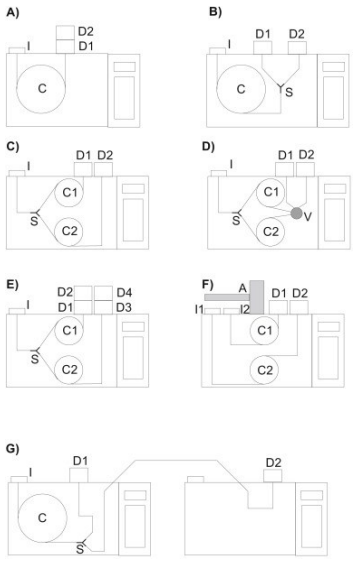

Figure 4: Illustration of the different multidetector system configurations *(Dąbrowski. Ł, 2018).*

When using a parallel set up a clear advantage includes the possibility of altering operating conditions between the detectors as they operate separately. The most common parallel set-up that is used includes the splitting of either the injection effluent or the column effluent. They can be split into two or more streams. These streams lead to separate detectors. The detectors can either be the same or different type. The effluent is split into separate streams using a splitter which is a press-fit glass Y-shaped device. The different multidetector combinations are illustrated in figure 4.

The addition of the splitter may cause certain problems during analysis such as the possible leakage at the connection areas of the splitter. Another disadvantage includes the possible loss of sensitivity as the sample is split into 2 or more streams. However, this can often be fixed by injecting a larger volume of analyte. Another important factor to consider is the split ratio between the detectors. This refers to the percentage of the analyte that is sent to each detector and can be controlled by altering the length of the transfer line that connects the splitter to each detector. *(Dąbrowski. Ł, 2018).*

2.5. GC data analysis

Depending on the detector, gas chromatography can be used for qualitative and/or quantitative analysis. There are also different factors that can affect the analysis which should be considered. When using GC-MS the basic idea includes a chromatogram and a mass spectrum. The x-axis on the chromatogram is the retention time, and the y-axis displays the measured response of the analyte. Each time an analyte passes through the column a response will be displayed on the chromatogram in the form of a peak as can be seen in figure 5. The retention time is measured at the apex of each analyte peak. However, when there is no analyte eluting through the column or the concentration of the analyte is below the detection limit the baseline response will be displayed. The baseline response is a representation of the combination of electrical and chemical noise which can include impurities in the carrier gas as well as other contamination, degradation or disturbances in the system. Therefore, if the baseline response is relatively high this could be an indication that the system may require maintenance. *(Turner, 2021)*

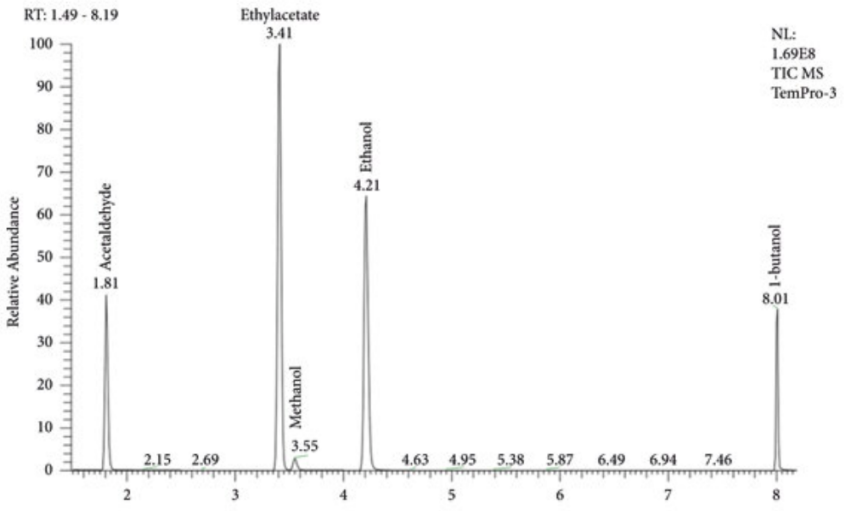

Figure 5: Shows the chromatograms of different chemicals. *(Nguyen & Le, 2023)*

Each analyte peak can be analyzed in more detail by measuring the height, area or baseline width of the peak. The area of the peak is proportional to the concentration of the analyte present and therefore can be used for quantitative analysis. When the analyte peaks are narrower, they provide better sensitivity, meaning a better signal-to-noise ratio. Narrower

peaks also provide better resolution, indicating better separation of the analyte compounds. *(Turner, 2021)*

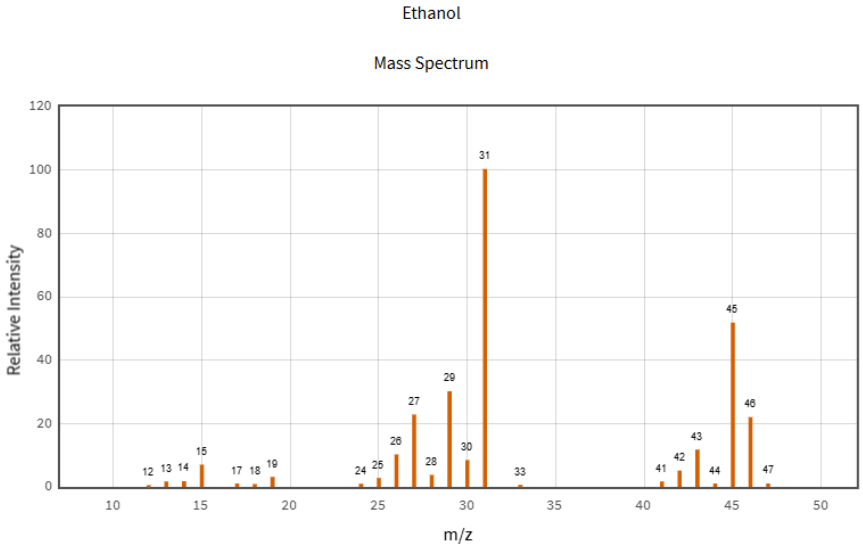

Figure 6: Mass spectrum of ethanol *(NIST, n.d.)*

When using mass spectrometry, the mass spectra provide information about the intensity of each specific ion present in the compound. The data is displayed in the form of an intensity signal. All the signals produced at a given time are presented on a graph called a mass spectrum. The mass spectrum can then be used to identify analytes as they are unique to each compound. Figure 6 displays ethanol's mass spectrum. Each signal in the mass spectrum represents a specific ion. *(Nugraha & Nandiyanto, 2021)*

2.5.1. GC Qualitative analysis

Qualitative analysis refers to the identification of the analytes in the mixture. The simplicity of the analysis depends on the available detectors. When using an MS detector, the qualitative analysis is simple as the mass ion detected is a representation of the molecular weight of the analyte. The structure of the molecule can also be determined from the fragmentation patterns of the ions that appear in the spectra. *(Haas, 2022)*

When using mass spectrometry for identification a large challenge is background noise. Identification of the spectra can be improved by subtracting the background and therefore isolating the analyte peaks. This produces clearer and better spectra, enhancing the efficiency

of the qualitative analysis. Another challenge with qualitative analysis occurs when compounds co-elute i.e., they have a similar retention time. When the compounds co-elute their chromatograms appear on top of each other, preventing the extraction of the mass spectra of the pure compound, which is needed for identification. When analyzing many different samples in the same sequence, residues from the earlier samples can contaminate the subsequent samples causing the carryover effect. This means that the analytes from the previous samples also appear in chromatograms of the subsequent samples as traces of the previous samples remain in the column. This poses a significant challenge for the qualitative analysis. All of these issues also complicate quantitative analysis.

When an MS detector is not available qualitative analysis can be conducted by comparing the retention time of known standards to the retention time of unknown compounds. This technique can also be used with a MS detector to further justify qualitative data. Another qualitative analysis method for determining a member of a homologous series includes comparing the capacity factor of a standard series to the capacity factor of the unknown. *(Haas, 2022)*

The capacity factor is a measure of the extent of the analyte retained by the stationary phase relative to the pure mobile phase or an unretained compound. It provides insight about the interactions between the mobile phase and the stationary phase. *(Ravisankar1 et al, 2017)* In gas chromatography this factor is influenced by column temperature, the properties of the stationary phase as well as the nature of the analytes *(Lee, 2025)*.

$$k = \frac{t_R - t_0}{t_0} \qquad (1)$$

The mathematical expression for the capacity factor is displayed in equation 1. In this expression, k is the capacity factor, t_r is the retention time of the analyte and t_o is equal to the retention time of an unretained compound. The unretained compound does not interact with the stationary phase at all and therefore is not slowed down in the column. A high-capacity factor value indicates that the analyte is highly retained in the column and that it has interacted with the stationary phase for a long time. The k value can be a more insightful parameter within qualitative analysis as it is not sensitive to fluctuations in certain chromatographic conditions such as flow rate. *(Ravisankar1 et al, 2017)*

2.5.1. GC Quantitative analysis

Quantitative analysis is used to determine the amount of analyte present in the mixture. In an ideal situation the amount of analyte injected onto the column is proportional to the area/height of the peak in the chromatogram. The peak area is calculated by integration. When two compounds partially or fully co-elute their peaks in the chromatogram overlap. This makes the quantification of specific compounds more complicated, however there are various methods that can be used to determine the area of each peak separately. *(Haas, 2022)*

To complete precise and accurate quantitative analysis a calibration curve needs to be established. This curve compares the area response to the concentration of the analyte present. This can be done by using external or internal standardization. Standards are solutions that contain different concentrations of the analyte being measured. *(Bates et al, 2023)*

In the external standard method, the standards are analyzed and the results are displayed on a graph. The x-axis is the concentration, and the y-axis is the area response displayed on the chromatogram. After this the calibration curve can be calculated from the data points.

$$Area\ response = mC + b \qquad (2)$$

After the unknown sample is analyzed, it can be quantified using the line equation of the calibration curve which is displayed in equation 2. The slope (m) and the y-intercept (b) is calculated from the calibration curve. After the area response of the analyte is recorded the concentration can be determined from the equation.

External standardization in GC can be problematic because it requires the volume of each injected standard to be identical. Achieving accurate GC injection volume repeatability is challenging even under ideal conditions. Therefore, when accurate and precise quantitative analysis is required, it is best to use internal standardization. *(Haas, 2022)*

The internal standard method includes a compound called the internal standard with a known concentration that is added directly into the analyte sample as well as each standard. The internal standard is a compound that is completely different from the analyte's compound, and it is used as a reference to compare to the analyte's signal. If there is a variation in the injected volume, then the signal of the internal standard will change proportionally to the

change in the analyte's signal as they are both under the same conditions. Therefore, the ratio between these two signals is unaffected by the changes in injection volume. *(Haas, 2022)*

An important factor to consider when conducting this method is that the sensitivity of the response can differ drastically between substances which can lead to unreliable qualitative data. An approach to combat this issue is to establish a molar response factor for the substance being analyzed. *(Haas, 2022)*

$$k_a = \frac{peak\ area}{moles\ of\ substance\ injected} \quad (3)$$

The molar response factor can be determined from the calibration curve using equation 3. When the molar response factor (k) for an analyte (a) or an internal standard (b) is calculated the signal response (S_a) of a known concentration of the same substance and the internal standard (S_b) can be expressed using equations 4 and 5. *(Haas, 2022)*

$$S_a = k_a * C_a \quad (4)$$

$$S_b = k_b * C_b \quad (5)$$

Since the ratio between these two signals is unaffected by external factors such as injection volume, this can be used to conduct precise qualitative analysis. When the molar response factors of both the analyte and internal standard are known equation 6 can be used to calculate an unknown concentration of the analyte. *(Haas, 2022)*

$$\frac{S_a}{S_b} = \frac{k_a * C_a}{k_b * C_b} \quad (6)$$

The mass spectrometer can also be used in quantitative analysis when selected ion monitoring is selected.

2.6. Headspace sampling (HS)

Headspace sampling is a technique that is used to introduce samples for gas chromatography. The headspace refers to the gas layer in the vial above the sample as can be seen in figure 7. Since this gas layer is analyzed within the GC-MS, the target compounds need to have high

volatility while the rest of the sample needs to be relatively less volatile. *(Kolb, B. and Ettre, L.S., 2006)*

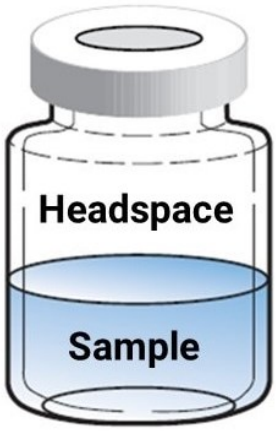

Figure 7: Illustration of a headspace vial indicating the 2 different layers. *(Agilent, n.d)*

Headspace vials are also typically larger than the regular 2ml because headspace analysis requires larger sample volume and more headspace above the sample. 10- and 20-ml vials are considered typical for headspace. *(Kolb, B. and Ettre, L.S., 2006)*

2.6.1. Benefits of headspace sampling

The main benefit of headspace sampling is its wide compatibility with almost any sample. This is because only the target compounds in the sample need to be volatile, the sample itself doesn't have to be. The solvent peak that appears in the chromatogram is significantly smaller when compared to liquid injection. This is because in headspace the number of solvents introduced into the chromatograph is much smaller. Therefore, since the solvent peak is much smaller it is less likely to interfere with the target compounds that can be seen in the chromatogram. Headspace also offers cleaner samples which reduces the maintenance required and provides higher sensitivity with good linearity and precision. *(Kolb, B. and Ettre, L.S., 2006)*

2.6.2. Headspace sampler key parts and process

The main parts of a headspace sampler are listed in table 1 along with their functions.

Table 1: Parts of the headspace sampler and their functions. *(Kolb, B. and Ettre, L.S., 2006)*

Part of sampler	Function
Temperature-controlled oven	Produces a constant temperature that incubates the samples before GC analysis
Sampling probe	Punctures the surface of the vials, injects gas into the vial to increase the internal pressure and transfers the sample into the headspace loop.
Heated sampling loop	Holds a constant volume of a sample that can be used for reproducible injections.
Heated sampling valve	Uses two flow channels which reduces carryover and ensures a constant flow of the carrier gas to the GC.
Heated transfer line	Transfers the headspace sample to the GC in a thermally controlled environment.

The headspace sampling process begins with the sample being transferred to the headspace vial. Since the target compounds are volatile, the vial needs to be capped immediately to decrease the loss of these components. After the vial has been capped the volatile target components begin to shift between the sample and the headspace layer until they reach the liquid-gas equilibrium. This phenomenon is displayed in figure 8. The time to reach the equilibrium is sample dependent and is defined experimentally. *(Kolb, B. and Ettre, L.S., 2006)*

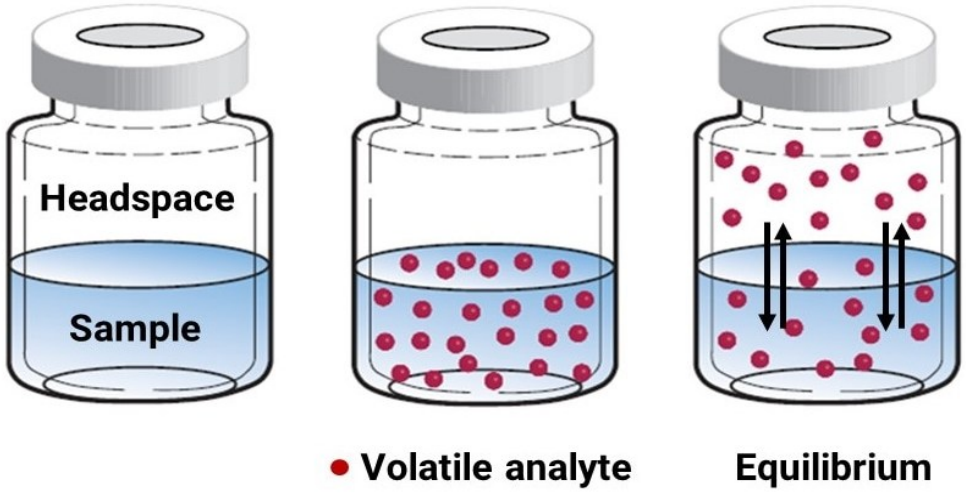

Figure 8: Illustration of the volatile target analyte moving between the sample and headspace in the vial until equilibrium is reached. *(Kolb, B. and Ettre, L.S., 2006)*

Once the sample has reached equilibrium the sampling can begin. The process is outlined in figure 9. At the start of the sampling process the sampling probe pressurizes the vial by feeding additional gas into the vial. After this the pressure in the vial is vented which then fills the sample loop with the headspace sample in the valve. Finally, the sampling valve rotates, injecting the sample into the GC inlet through the transfer line. *(Kolb, B. and Ettre, L.S., 2006)*

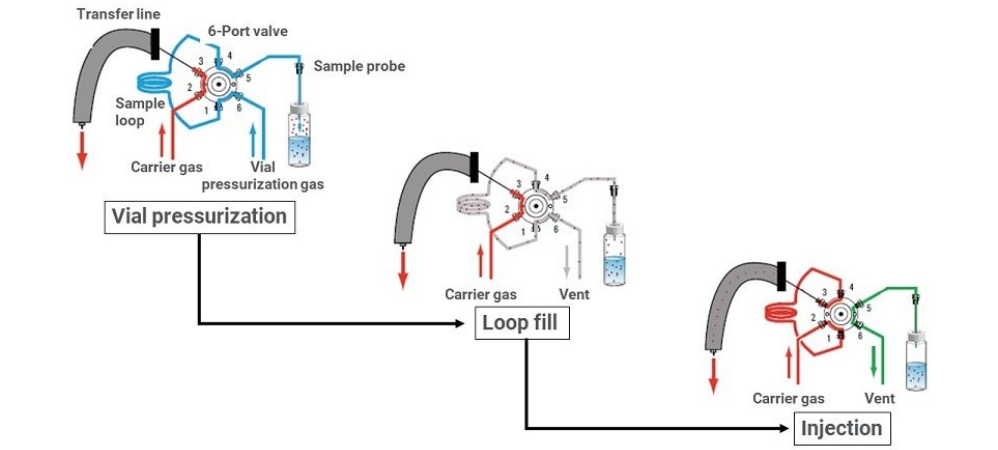

Figure 9: Three step diagrams of the headspace sampling process *(Agilent, n.d.; Kolb, B. and Ettre, L.S., 2006)*

2.6.3. Mathematical expression for headspace sampling

Equation 7 describes the relationship between headspace concentration in the vial to the GC detector response. *(Kolb, B. and Ettre, L.S., 2006)*

$$\mathbf{A \propto C_G} \qquad (7)$$

The mathematical expression shows that area (A) detected in the GC chromatogram is proportional to the gaseous analyte concentration (C_G) in the headspace of the vial. This concentration is defined in equation 8. *(Kolb, B. and Ettre, L.S., 2006)*

$$C_G = C_0/(K + \beta) \quad (8)$$

Here the original analyte concentration (C_0) that was added to the sample is divided by the sum of the partition coefficient (K) and the phase ratio (β). These parameters are defined by the equations 9 and 10. *(Kolb, B. and Ettre, L.S., 2006)*

$$\beta = \frac{V_G}{V_S} \quad (9)$$

$$K = \frac{C_s}{C_G} \quad (10)$$

The phase ratio (β) is essentially the relative volume of gas (V_g) to sample liquid (V_s) in the vial. This ratio is determined by the size of the vial and the volume of the liquid sample in the vial. The partition coefficient (K) is a temperature dependent variable which is also a relative measure of the analyte concentration (C_s) in the liquid sample to analyte concentration in the gas phase (C_G) also known as the headspace. *(Kolb, B. and Ettre, L.S., 2006)*

Since the goal in HS-GC-MS analysis is to maximize the detector response it is favorable to create conditions that minimize the sum of the partition coefficient (K) and the phase ratio (β) in equation 8. This will raise the gaseous analyte concentration and proportionally increase the area response (A) in the GC detector as shown in equation 7. The area response refers to the integrated area of a single spike. Examples of these spikes can be seen in figure 10. *(Kolb, B. and Ettre, L.S., 2006)*

To get an optimal phase ratio a general guideline is to leave at least 50% of the headspace in the vial. Therefore, using a larger vial allows for the analysis of larger sample volumes. According to equation 9, increasing sample volume decreases the value of the phase ratio (β) which in turn will increase the area response (A). Figure 10 shows this is in practice. *(Kolb, B. and Ettre, L.S., 2006)*

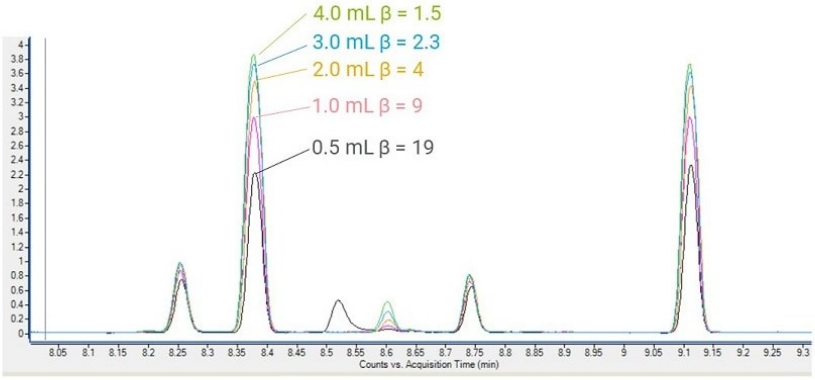

Figure 10: Chromatographic profile comparison showing how changing the sample volume in a 10ml vial impacts the area response. *(Agilent, n.d.; Kolb, B. and Ettre, L.S., 2006)*

Figure 10 displays five different chromatograms each with different sample volumes. As the sample volume increases the phase ratio (β) decreases. When this ratio is smaller the peaks are much larger, creating a larger area response. *(Kolb, B. and Ettre, L.S., 2006)*

Since the partition coefficient is a temperature dependent variable adjusting the temperature will help achieve optimal conditions. Figure 11 displays a chromatographic profile comparison where five samples were incubated for 20 minutes at different temperatures. *(Kolb, B. and Ettre, L.S., 2006)*

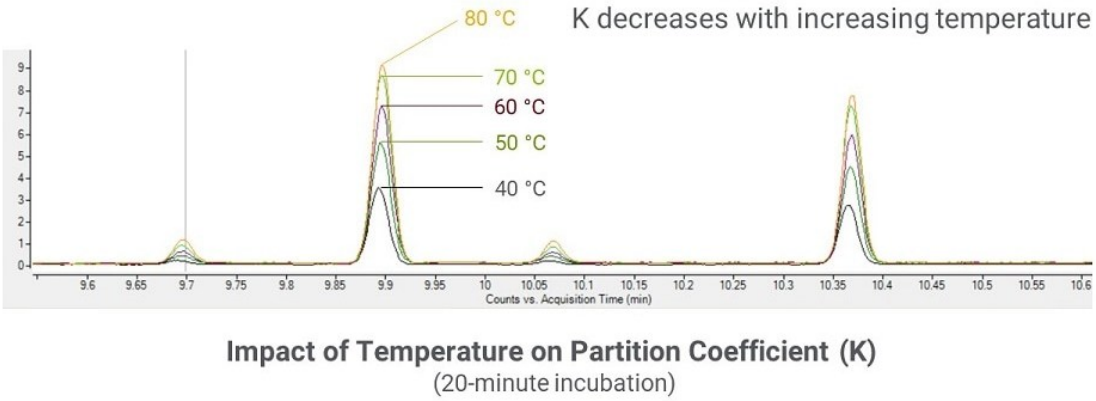

Figure 11: Chromatographic profile comparison showing how changing the temperature impacts the area response. *(Agilent, n.d.; Kolb, B. and Ettre, L.S., 2006)*

The chromatograms that had higher incubation temperatures produced higher peaks leading to a larger area response in the detectors. This also means that there is a higher concentration

of analyte in the gas phase as according to equation 10 the K value decreases when gas phase concentration increases. *(Kolb, B. and Ettre, L.S., 2006)*

There are also other parameters that can affect the area response which include equilibration time, vial shaking, sample loop volume and sample loop pressure.*(Kolb, B. and Ettre, L.S., 2006)*

2.6.4. Multiple headspace extraction (MHE)

To achieve higher accuracy with headspace sampling a technique called multiple headspace extraction can be used. The MHE technique is often used when there are interfering substances present in headspace or then a calibration standard cannot be prepared to match the sample composition, making qualitative analysis challenging. This technique involves using the same vial for a series of sampling cycles, rather than relying on a single cycle. The sample is pressurized, and a fraction of the gaseous headspace sample is taken and inserted into the GC inlet. This process is then repeated several times which improves the accuracy of the result. *(Kolb, B. and Ettre, L.S., 2006)*

2.6.5. Salting out effect

The salting out effect refers to a process in which the addition of an ionic salt such as NaCl to an aqueous solution increases the ionic strength of the aqueous layer. This causes certain non-ionic compounds to be repelled out of the water which enhances the concentration of organic compounds in the solution. This concept can be applied to headspace sample preparation by adding ionic salts directly to the samples. This can increase the concentration of the organic analytes in the headspace, leading to a better signal response in the chromatogram. *(Badawy et al, 2022)*

2.6.6. Applications of headspace

In the pharmaceutical industry a common headspace application is residual solvents. The United States pharmacopeia method (USP) 467 is an official procedure that utilizes headspace sampling to detect and measure solvents that arise from pharmaceutical manufacturing. This is done to ensure that the manufactured drugs are safe for patients and meet the required regulations. Another common application of headspace is determining the ethanol content in blood. Since this information can often be used in criminal cases the accuracy of this data is critical. Headspace is also used in environmental samples where soil and water samples are analyzed to detect volatile compounds that could pose a risk to the environment or animal and human health. *(Kolb, B. and Ettre, L.S., 2006)*

2.6.7. Liquid sampling and injection modes

Liquid injection is another common form of introducing the sample into a GC-column. The sample is introduced into the GC port by directly pipetting the liquid into it. Before the sample is injected it is heated to the initial column temperature. This then enables the evaporation of the solvents and the dissolved analytes. Before entering the column, the sample passes through a glass liner which provides a stable surface where the liquid can be converted into a vapor and transferred to the GC column without changing the sample properties *(Deepak, 2016)*. The sampling process is often automated with a device called an automated liquid sampler. *(Harvey, 2021).*

Both liquid and headspace sampling can be performed with either split or splitless injection. Split injection means that only a fraction of the carrier gas with the analyte compounds enter the column whilst the remainder exits the system through a split vent. This can be useful when wanting to control the amount of analyte compounds entering the column. In splitless injection the split vent is closed meaning that all of the injected carrier gas enters the column. *(Harvey, 2021).*

3. Development of analytical methods

This chapter discusses the development and optimization process of analytical methods. The process includes considering the physicochemical properties of the analyte sample as well as the chromatographic conditions which are discussed in detail.

3.1. Physical and chemical properties of the sample

Before starting method development, it is essential to inspect the physical and chemical properties of the sample being analyzed as they will influence the selection of the methods. The properties include volatility, molecular weight, solubility, melting point and phase. The sample properties that are relevant to GC analysis such as boiling point range, polarity, functional groups, reactivity and stability should be researched as extensively as possible. The presence of water, non-volatile residues, hazardous substances or chemically and thermally unstable components such as strong acids and bases need to be checked as they may require special handling. For example, if the analyte contains non-volatile components certain pyrolysis techniques can be utilized. *(Bhardwaj et al, 2016)*

3.2. Chromatographic conditions

The selection of the chromatographic conditions plays a vital role in optimizing the separation of the components in the GC column. The conditions include selecting the appropriate headspace conditions, column, carrier gas, flow rate, oven temperature, sample size as well as selecting the optimal detector and detection temperature. *(Bhardwaj et al, 2016)*

3.2.1. Headspace conditions

Selecting the appropriate sample introduction technique and conditions is important to optimize the separation of the compounds in the analyte. There are different types of sampling techniques, and this research will primarily focus on the headspace technique. The headspace conditions that can be altered include temperature, vial volume, equilibration

time, vial shaking, sample loop volume, sample loop pressure and split ratio. Changing these conditions can affect the area response on the chromatogram. *(Kolb, B. and Ettre, L.S., 2006)*

3.2.2. Column

Selecting the correct column is an important factor when optimizing compound separation. Choosing a proper column will provide efficient separation which improves the accuracy and reliability of the data produced. Whereas selecting a column that is not appropriate for the analysis will produce improper and insufficient separation leading to inaccurate results that are challenging to interpret. Gas chromatography can analyze a very wide variety of compounds, and the amount of available GC columns is only a fraction of this number. Therefore, selecting the correct column can be challenging since there are so many different suitable applications for each column. *(Bhardwaj et al, 2016)*

The four main factors to consider when selecting the appropriate column are the stationary phase, column internal diameter, thickness of film and column length. The variation of the physicochemical properties of the analyte compounds and their interaction with the stationary phase is the fundamental principle of the separation process. Therefore, compounds with similar properties may be difficult to separate. When certain compounds interact with the stationary phase more strongly than others they are retained in the column for a longer time. This time is called the retention time that is displayed in the x-axis of the chromatogram. Therefore, changing the chemical features of the stationary phase will alter the retention time for specific components. Certain compounds can have the same retention time (i.e they co-elute and therefore do not separate) for one stationary phase and different retention times using another stationary phase. *(Bhardwaj et al, 2016)*

3.2.3. Carrier gas

There are multiple options that can be used as the carrier gas in the GC. The most common options include hydrogen nitrogen and helium. Each gas has its advantages and systems for which they are best suited for. The following factors need to be considered when selecting the appropriate carrier gas: chemical stability, dryness, absence of oxygen, safety, price and availability. *(Bhardwaj et al, 2016)*

3.2.4. Oven temperature

Compounds are separated mainly based on their boiling point thus the oven temperature is a crucial factor that is used for controlling GC analysis. Often the option that separates the compounds most effectively is not necessarily keeping the temperature constant throughout the entire GC analysis. Most of the temperature programs have a starting temperature, a slope where the temperature increases around a degree per minute and a final temperature. To improve the resolution of compounds that elute at lower temperatures the initial temperature should be lowered or kept constant for a longer period, known as the hold time. This, however, often results in longer analysis times. To improve the resolution of the compounds that elute at the middle temperature the rate of temperature change or the slope can also be adjusted. If a peak in the chromatogram is excessive the rate of temperature change can be increased to decrease the resolution of said peak and vice versa if the peak resolution is insufficient. These changes will also affect analysis time. To get better resolution of compounds that elute at higher temperatures usually the best option is to lower the rate of temperature change. However, the resolution for middle and high temperature eluting compounds can also be improved by holding the temperature constant for a period in the middle of the slope. *(Bhardwaj et al, 2016)*

3.2.5. Detector

There are many different types of detectors that can be used for detecting substances that exit the GC column. However, this research focuses on the MS and FID detectors. Since the MS and FID are both mass flow detection systems and not concentration dependent detectors the effect of lowering the flow rate lowers the intensity but not the calculated area response of the peaks. In these systems the analyte is still detected even if the flow rate is decreased. The reduction of flow rate will increase the retention time and broaden the peaks. However, the integrated area under the peak will remain approximately the same as the analyte is still fully detected. *(Bhardwaj et al, 2016)*

3.3. Development of the method and optimization

Methods are often developed for an already preexisting method, however they can also be developed from scratch. The optimal method is determined by adjusting the conditions mentioned in section 3.2 and evaluating their performance. Once a method produces appropriate separations it can be optimized. The aim of optimization is to get the most desirable separation resolution and sensitivity. The sensitivity is defined as its ability to detect small changes in the concentration of the analyte. On a general level, sensitivity can be determined numerically from the slope of the calibration curve *(Sheehan & Yost, 2015)*. During optimization one parameter is changed at a time until the optimal sensitivity and resolution have been achieved. *(Bhardwaj et al, 2016)*

4. Drug substance production impurities

This chapter discusses the different contaminants that can be found in the drug substance process. These include residual solvents as well as various other organic or inorganic contaminants.

4.1. Residual solvents

There are many different types of organic solvents that are used in the production of drug substances. These solvents are a crucial part of the synthesis, purification and preparation process. If residues of the solvents end up in the final product, they can pose a serious health risk. These residual solvents do not provide any therapeutic benefit either and therefore it is critical to make sure that they are either absent or below the acceptable levels in the final product. This is effectively monitored in drug substance quality control. *(ICH, 2021)*

The residual solvents in the pharmaceutical industry are split into 3 different categories based on their toxicity. Class 1 residual solvents have the highest toxicity level and pose significant environmental hazards. These solvents should be avoided completely unless they are necessary to produce drug substances that provide a significant therapeutic effect. A risk-benefit analysis must always be completed. Class 2 solvents have medium toxicity and can pose a significant risk to patients at higher concentrations. Class 3 solvents pose a lower risk

even at higher concentrations. However, despite the lower risk, their quantities are still controlled and should be minimized as much as possible. *(ICH, 2021)*

4.2. Inorganic and organic impurities

Other impurities can also arise during the production of drug substances. These are often classified as organic and inorganic substances. Organic impurities include substances that arise during the synthesis process. These include process related impurities such as unreacted raw materials, byproducts and unreacted intermediate compounds that have formed during the manufacturing process but are not the final product. Impurities can also arise when the compounds that have been formed start to degrade under normal storage conditions. These are called degradation products. Inorganic impurities also include contaminants that arise from the manufacturing process. These include inorganic salts that can remain in the product after synthesis, residual catalysts, ligands as well as residual heavy metals that can arise from reagents or the production equipment and be harmful in large quantities. *(ICH, 2006)*

5. LDPE-bags as packaging materials in drug substance production

LDPE bags are commonly used in drug substance production for the packaging of intermediates and products. In API packaging it is essential that the packages provide sufficient protection against contamination and degradation when the products are stored or transported. The packaging material needs to be clean and suitable with the product. It is essential that the packaging does not react with the product. *(FDA, 2016)*

5.1. Low density polyethylene structure and properties

The molecular structure of LDPE includes a main hydrocarbon polymer backbone with both short and long chain branches also made of hydrogen and carbon connected to it. The branches in these polymer chains prevent the molecules from packing closely together thus

resulting in lower intermolecular forces and lower density when compared to other forms of polyethylene such as HDPE. Figure 12 illustrates this structure. *(shobeir shimi, 2025)*

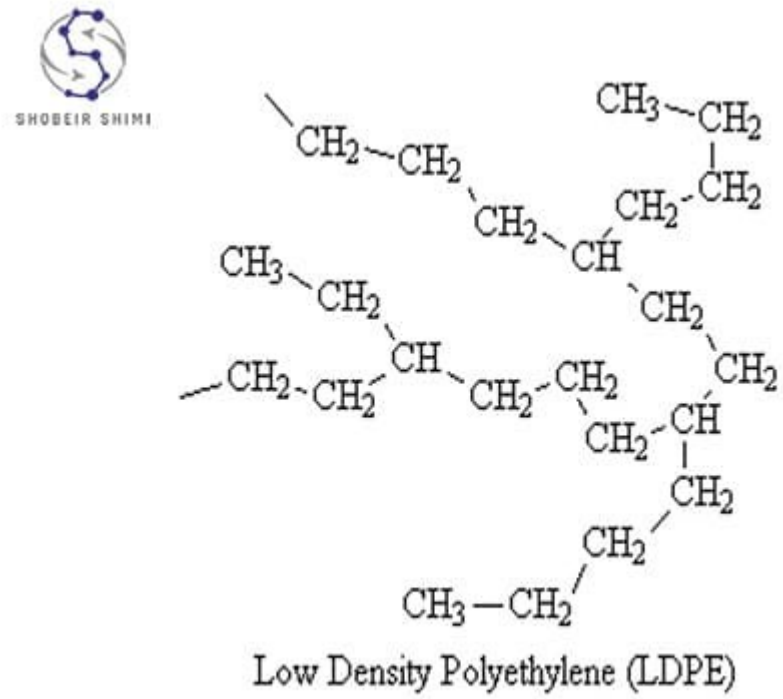

Figure 12: illustration of the LDPE structure *(shobeir shimi, 2025)*

The density of LDPE ranges from 0.910 g/cm³ - 0.940 g/cm³ whereas HDPE has a 0.941-0.965 g/cm³. The less dense and compact arrangement gives LDPE a soft and flexible composition making it easy to use in process applications. The melting point of LDPE ranges between 105 – 115 C, indicating it has a low volatility. The monomer in the polymer chain is ethylene (C2H4) therefore the chemical formula for polyethylene is C2H4n, where n is a measure of the number of repeating ethylene monomer units. *(shobeir shimi, 2025)*

5.2. Additives in LDPE

In addition to the polyethylene polymer itself, LDPE contains additives which allow for the modification or improvement of the properties and performance of the plastic product. Examples of these specific additives for different categories are listed in table 2. These

additives include plasticizers, heat and light stabilizers, lubricants, slip compounds, etc. *(Hahladakis, et al 2018)*

Table 2: Examples of additives used in LDPE *(Chapke et al, 2022)*

Category:	**Additive:**
Hydrocarbons	Undecane 1-Tridecene Tetradecane Dotriacontane
Lubricant and surfactants	Hexadecane Tetradecane Eicosane Triacontane 2-Methyl dodecane
Antioxidants	Squalene Octadecane, 3-ethyl-5-(2-ethylbutyl)- Tris(2,4-di-tert-butylphenyl) phosphate Hexanedioic acid, bis(2-ethylhexyl)
UV/thermal stabilizers	Methyl stearate Phthalic acid, hexyl oct-3-yl ester
Plasticizers	Bis(2-ethylhexyl) phthalate Tributyl acetylcitrate
Slip agents	9-Octadecenamide, (Z)- 1-Decanol, 2-hexyl
Fatty acids	Methyl tetradecanoate 16-Hentriacontanol Methyl myristoleate

Different types of LDPE have different additives based on the intended purpose of the material. If these additives end up in the human body they can have harmful health effects. These effects can include an increase in cholesterol levels, certain reproductive problems such as low infant birth weight and they can damage certain immunological organs such as the liver, kidney and thyroid. In some cases, these additives can also cause cancer. *(Chapke et al, 2022)*

During the manufacturing of plastics, additives can either be added during the polymerization stage or at the finishing stage. When additives are added during polymerization, they are typically initiators, inhibitors, lubricants, catalysts and other substances that aid with polymerization reaction. The additives that are added during the finishing stage provide the unique properties for the plastic and are adjusted based on the material's intended use. These are the additives that are outlined in table 2. *(Almeida et al, 2022).*

5.2.1. Literature example on the study of additive migration

A study was conducted on the extent of the migration of these additives into an Indian fermented milk product called Dahi by Chapke *(Chapke et al, 2022)*. To determine the additives, present in LDPE Chapke first conducted a solid liquid extraction investigation using various solvents. Different polar and non-polar solvents and solvent mixtures were tested. These included solvent mixtures prepared at a 1:1 ratio, such as acetonitrile: methanol, chloroform: methanol, and n-hexane: methanol as well as pure acetonitrile, chloroform and n-hexane. Five milliliters of each solution was used to extract additives from separate 0.2 g LDPE samples at various extraction times. *(Chapke et al, 2022)*

The chloroform: methanol solution extracted the highest number of additives, and the optimal extraction time was 18 hours as there was no observed increase in the number of additives extracted after this time. These optimal solvent and time conditions were used for further additive extraction investigations. *(Chapke et al, 2022)*

The additives of eleven samples of LDPE dahi packaging films were extracted using these optimal conditions. After which they were analyzed and quantified using a GC-MS device. Of all the samples a total of 76 chemical additives were extracted and classified into 13 different categories, similar to the ones in table 2. *(Chapke et al, 2022)*

Chapke also studied the extent of LDPE additive migration directly into dahi under normal storage conditions. This was investigated by exposing the dahi to LDPE for 160 hours at 4 °C and analyzing samples at different exposure time intervals. The samples were analyzed with GC-MS and a total of 12 different categories of LDPE additives had migrated into the dahi, including plasticizers, antioxidants, fatty acids, etc. The additives that are listed in table

2 include some of additives that were extracted in this investigation. *(Chapke et al, 2022)* All of the samples were analyzed using the GC conditions outlined in table 3.

Table 3: GC parameters for the Dahi-LDPE investigation *(Chapke et al, 2022)*

Carrier gas	Helium		
Flow	1ml/min		
Mode	Splitless		
Column	SLB-5MS 30mm x 0.25mm x 0.25 µm		
Injection volume	1 ml		
Injector temperature	280 °C		
Oven program	Rate/°C/min	Value/°C	Hold time/min
	-	70°C	-
	10 °C	300°C	20

Before the analysis each sample was filtered through a 0.22 µm syringe filter. After each sample was analysed the GC-column was washed with hexane to prevent contamination from the previous sample.

5.2.2. European pharmacopoeia 3.1.5 polyethylene with additives

The European pharmacopoeia establishes the general quality standards for APIs and medicines for human and veterinary purposes in Europe. These standards provide a legal and scientific foundation for quality control throughout the different stages in the pharmaceutical industry. These standards also apply to the additives found in polyethylene packaging. Certain polyethylene packages used in the pharmaceutical industry contain different additives that enhance their processing or their chemical, physical and mechanical properties. Table 4 outlines some of the different additives that can be used in the pharmaceutical industry as well as their maximum usage levels according to the European pharmacopoeia. *(EUROPEAN PHARMACOPOEIA 3.1.5, 2025)*

Table 4: Different permitted additives used in the packaging of products in the pharmaceutical industry *(EUROPEAN PHARMACOPOEIA 3.1.5, 2025)*

Additive Type	Additive compound	Maximum usage level (%)
Antioxidant (Total maximum level = 0.3%)	Butylhydroxytoluene	0.125
	Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	0.3
	Ethylene bis[3,3-bis[3-(1,1-dimethylethyl)-4-hydroxyphenyl]butanoate]	0.3
	Dioctadecyl disulfide	0.3
	Dioctadecyl 3,3′-thiodipropionate	0.3
	Didodecyl 3,3′-thiodipropionate	0.3
	Tris [2,4-bis(1,1-dimethylethyl)phenyl] phosphite	0.3
Lubricant and antiblocking agents	Hydrocalite	0.5
	Alanamides	0.5
	Alkennamides	0.5
	Sodium silico-aluminate	0.5
	Liquid paraffin (mixture of saturated hydrocarbons) *(RAHA Paraffin Co, 2016).*	0.5
	Magnesium oxide	0.2
Opacifying agent	Titanium dioxide	4

There are many different types of permitted additives and not all of them are listed in table 4. Additives that are not permitted according to the pharmacopoeia can also be used, however they must first be approved by regulatory authorities. *(EUROPEAN PHARMACOPOEIA 3.1.5, 2025).*

6. Production equipment used in drug substance manufacturing

Drug product manufacturing involves the use of a wide range of production equipment such as reactors, centrifuges, mixers, dryers, filters, tanks, and autoclaves. All of these have their

own specific function. This section will discuss the structure and working principle of different reactors and filter-dryers, similar to the ones used at Fermion's manufacturing sites. *(Whitmer, 2025)*.

6.1. Pharmaceutical reactor

In the drug manufacturing industry, the fundamental design of a pharmaceutical reactor is a large vessel with an agitator where essential reactions take place. It is an important part of the process because the APIs and intermediates are synthesized within these reactors where a wide range of different chemical reactions occur. It is crucial that the appropriate temperature, pressure and mixing conditions are provided. The practices in the pharmaceutical industry must always be compliant with the good manufacturing practice (GMP) regulations. These regulations are overseen by different regulatory agencies such as the FDA to ensure the purity, reproducibility and safety of the products. Therefore, it is essential that the production equipment also complies with these regulations. *(Stalwart International, 2025)*

Examples of the different reactors used in pharmaceutical production include batch reactors, continuous flow reactors, jacketed reactors and high-pressure reactors. The reactor material is a key factor as the material must be suitable with the chemical reactions and the cleaning procedures carried out in the reactor. It is crucial that the material does not react with the compounds in the reactor, therefore common reactor materials include a corrosion resistant alloy SS316L, Hastelloy or glass-lined steel. Since the chemical reactions are either endothermic or exothermic the thermal control must also be considered for the reactor design. Appropriate agitation must also be installed to avoid hotspots and to ensure the even dispersion of the reactants in the reactor. *(Stalwart International, 2025)*

6.2. Filter-Dryer

The filter dryer is another important device that is used for the separation of liquids and solids from a slurry. The fundamental design includes a vessel with a full diameter filter disc that collects solid particles. The liquid in the slurry often contains undesired chemicals, and the solid particles are usually the desired final product in the form of highly pure crystals as

this device is usually used in the final purification step. Figure 13 illustrates a simplistic diagram of a filter dryer during the filling stage of the process. *(Pope Inc, 2021).*

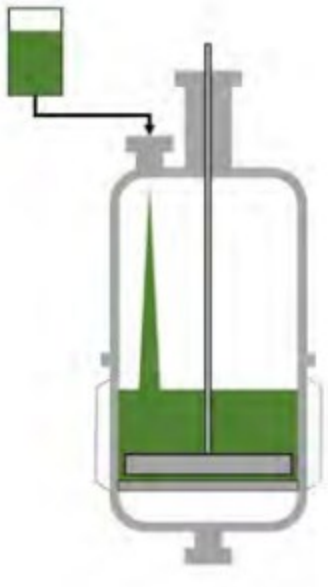

Figure 13: Illustration of the filling stage of a filter dryer *(Pope Inc, 2021).*

The working principle of the filter dryer involves transporting the slurry from the reactor after crystallization to the filter with a pump, gravity or pressurizing the reactor. After this all the ports, excluding the filter drain, are closed. Pressurized air or a nitrogen source is then installed in the vessel to help push the liquid past the solids and into the drain. As this happens, the solids start to produce a layer called the filter cake on top of the filter disc. After all the free liquid has been separated from the solids the filter cake will remain slightly wet. Therefore, the next step is to wash the filter cake as some of the chemical is still present in the filter cake. This is done by spraying fresh solvent onto the filter cake, mixing it into a slurry and filtering it again. This process can then be repeated if necessary. After the filter cake has been washed enough times, it is usually dried as in most cases the desired product is a dry crystal. In the drying process all the ports except the top port are closed again. A vacuum is installed onto the top port. Once a vacuum has been reached in the vessel, the solvent will start to evaporate off the solids. Finally, once the solids are completely dry, they can be collected into another container. *(Pope Inc, 2021).*

APPLIED SECTION

7. Potential production related contaminants at the manufacturing sites

Fermion Oy is a Finnish company which develops, manufactures and markets active pharmaceutical ingredients. Fermion Oy is a subsidiary of Orion Pharma, a Finnish pharmaceutical company with which it collaborates closely. Together they develop, manufacture and market both generic and proprietary medicines for humans and animals.

Fermion has two approved commercial API manufacturing sites which are in Oulu and Hanko. Both manufacturing sites operate similar types of processes where they utilize similar products. This chapter discusses these processes and investigates potential contaminants that can arise from the different detergents and cleaning procedures.

7.1. Detergent products used in API manufacturing

There are four different detergents that are used for cleaning the production equipment at Fermion's manufacturing sites. All of them are manufactured by the Finnish chemical company Kiilto and are called Proset V30L, Proset S26E, Proset S26H and F44 Sensol. This section discusses the composition of these detergents and the potential hazards associated with their use.

7.1.1. Proset V30L

Proset V30L is the most used detergent at Fermion's manufacturing sites. This product is used for cleaning dirty production equipment. It is a strong alkaline with a pH of 13.5 containing different hydrocarbon solvents and can be foamed onto surfaces. It is especially effective at removing fat-soluble organic compounds such resin and alkali-soluble inorganic residue. Another common application of this detergent is the paper industry. When handling the detergent, the safety measures include protecting eyes and skin from splashes with protective equipment. It should be stored in a cool storage room in the original package and

tightly sealed. The detergent is also prone to corrode with aluminum and zinc plated metals. The composition of the detergent is outlined in table 5. *(Kiilto, 2022)*

Table 5: Composition of Proset V30L *(Kiilto, 2022)*

Chemical:	Concentration:
Hydrocarbons, C10-C13, n-alkanes, isoalkanes, cyclical and aromatic	5 - <15%
Sodium hydroxide	5 - <15%
Sodium cumene sulfonate	1 - <5%
Potassium cumene sulfonate	1 - <5%
2-Ethylhexanol ethoxylate	1 - <5%

7.1.2. Proset S26E

The Proset S26E detergent is used less and only at the Oulu manufacturing site. It is suitable for washing production equipment and pipe systems that contain both organic and inorganic compounds. It is also a very strong alkali with a pH of 14 and does not produce foam. The detergent is more effective at removing dirt and other production residues than the conventional sodium hydroxide washing method. Also since the detergent is not pure sodium hydroxide it makes it easier to dispose of as it requires less neutralization. Table 6 describes the composition of the detergent. When the chemical is handled protective gloves, clothing and glasses should be used. *(Kiilto, 2022)*.

Table 6: Composition of Proset S26E *(Kiilto, 2022)*

Chemical:	Concentration:	Description:
Sodium hydroxide	>30%	-
Non-ionic surfactants	<5%	Non-ionic surfactants are different types of synthetic chemicals with a range of molecular structures. Examples include fatty alcohol ethoxylate and alkyl phenol ethoxylate *(Jiang, C. & Ma, J. 2000)*
Phosphonates	<5%	Phosphonates are a family of organic molecules that are made up of phosphorus, oxygen and hydrogen *(Stockreiter, 2024)*
Polycarboxylate	<5%	A linear polymer containing multiple carboxylate functional groups in the molecular structure *(The Soap and Detergent Association, 1996).*

7.1.3. Proset S26H

Proset S26H is also not as commonly used at the production sites. It is a strong acidic detergent with a pH of 1. The acid base is nitric acid, and it does not produce foam. It is effective at removing inorganic acid-soluble deposits as well as different microbes that appear in industrial pipes. When handling the detergent, it is essential to make sure that all the equipment is resistant to nitric acid and that appropriate protective gear is used. When using the detergent to remove carbonate deposits, the carbon dioxide that is released must be ventilated. The detergent should be stored and transported separately from any substances that contain chlorine. Table 7 outlines the composition of the detergent. *(Kiilto, 2025)*

Table 7: Composition of Proset S26H *(Kiilto, 2025)*

Chemical:	Concentration:	Description:
Non-ionic surfactants	<5%	Non-ionic surfactants are different types of synthetic chemicals with a range of molecular structures. Examples include fatty alcohol ethoxylate and alkyl phenol ethoxylate *(Jiang, C. & Ma, J. 2000)*
Nitric acid	30 - <50%	-
Citric acid	1 - <5%	-

7.1.4. F44 Sensol

F44 Sensol is another detergent that is commonly used at both manufacturing sites. It is a very acidic substance with a pH of 0.5 and can be foamed onto the surfaces easily. The base acid is phosphoric acid, and it is especially effective at removing limestone and residual rust deposits. It is also used in the food industry as it is effective at breaking down animal fats and protein. When applied on light metal surfaces it should be rinsed thoroughly. In some cases, an alkaline detergent should be used for neutralization to prevent the metal from corroding. It should be stored in a dry place above 5°C. When the detergent is handled protective gloves, clothing, glasses as well as respiratory protection should be used. *(Kiilto, 2025)*

Table 8: Composition of F44 sensol *(Kiilto, 2025)*

Chemical:	Concentration:
Phosphoric acid	30 - <50%
2-(2-Butoxyethoxy) ethanol	1 - <5%
Dodecylbenzenesulphonic acid	1 - <5%
Isotridecanol, ethoxylated	1 - <5%
Sodium alkyl sec. sulfonate	1 - <5%
Sulfamic acid	1 - <5%

7.2. Reactor cleaning procedures at Fermion's manufacturing sites

All the detergents mentioned in section 7.1 are used at both Fermion's manufacturing sites excluding Proset S26 E which is only used in Oulu. The detergents are mostly used to ensure that all impurities have been removed from production equipment. The detergents are either foamed onto the surfaces of the production equipment, or they are used in reflux boil washes. Each site has slightly different cleaning procedures, however the main principle is the same. There are many different solvents used throughout the cleaning procedures; however, this section describes the reactor cleaning procedures that involve detergents. The most used detergents are F44 Sensol and Proset V30L.

Reactors are typically cleaned using a foaming or boiling procedure. The selected procedure and detergent are selected based on the equipment type and the product synthesized in the

reactor, as different products have different solubilities. For the foaming procedure, the detergents Proset V30L and F44 Sensol are mainly used. It is essential that they are handled with care and that the appropriate safety gear is used as the detergents are very strongly acidic or alkaline.

The foaming procedure begins by foaming the detergents onto the surfaces using one of the foaming devices mentioned in table 9.

Table 9: Foaming devices used at Fermion's manufacturing sites

Foaming device:	Maximum operating pressure (bar)	Volume (liter)	Material
Indumatic 20 M	6	20	Stainless steel
MFU75	9	75	Stainless steel
FOAM-MATIC 5 E - VAAHDOTIN	4	5	Detergent tank: HDPE, Pump: PP, Hose and seals: EDPM
FOAM-MATIC 5 P - VAAHDOTIN	4	5	Detergent tank: HDPE, Pump: PP, Hose: PVC and Seals: Viton

When using the F44 Sensol detergent it is diluted to approximately 20 % in the foaming device. For example, this can be done by adding 2 ± 1 liters of detergent to 8 ± 2 liters of water.

After the detergent has been foamed onto the reactor surfaces it is left to dwell for around 10 minutes. If impurities are difficult to remove a brush or heat can be used if necessary. After this the reactors are rinsed with a substantial amount of water. This process must be repeated until the reactor is visually clean. After using the foaming device, the hose and detergent tank must also be rinsed with a substantial amount of water.

The reflux boil wash procedure includes diluting the detergent into water, into a small percentage solution (approximately 0.5%) and adding it to the reactor. After this it is heated close to boiling point and stirred within the production equipment for approximately one hour. As the solution boils it is refluxed back into the reactor. After one hour the solution is cooled to 50°C and emptied into a drain.

7.3. LDPE packaging at Fermion

Certain intermediate products within API production remain as solvent-wet solids because sometimes production equipment like the filter dryer does not remove the solvent from the product completely. Packing these solvent wet solids in LDPE-bags can, in some cases, be problematic. Particularly problematic solvents that are used within the production process at Fermion include Dichloromethane, Perchloroethylene and Toluene. According to *(Cole-Parmer, 2019)* the compatibility of each of these solvents with LDPE bags is either fair or severe. This is described in more detail in tables 10.

Table 10: Compatibility of different solvents with LDPE bags *(Cole-Parmer, 2019)*

Solvent:	Compatibility:
Dichloromethane	Class D: Severe Effect not recommended for any use.
Perchloroethylene	Class D: Severe Effect not recommended for any use.
Toluene	Class C Fair moderate Effect, not recommended for continuous use. Softening or loss of strength, and swelling may occur.

When these solvent-wet solids are packed into LDPE bags they can pose a safety and product quality risk. These solvents can soften these bags to the extent that they can break, creating a significant safety hazard. The product quality risk emerges from the interaction between the LDPE bag and the solvent. There is a possibility that the contaminants from the bag will end up in the product.

There are three main types of polyethylene used at Fermion's manufacturing sites. They are all low-density polyethylene; however, the material used contain slightly different additives and properties which are outlined in table 11. The different materials are manufactured by the companies DOW and Borealis.

Table 11: Properties of the different LDPE materials

Material name	Additives used	Intended applications	Key features	Density (g/cm^3)
BOR3221 *(SHANGHAI SUSHENG, n.d.).*	Antioxidant	Agricultural applications, films, food packaging and Heavy-duty bags	Recyclable material, good strength	0,922
BOR5224 *(Borealis, 2022).*	Antioxidant, Anti-blocking agent (850 ppm) and lubricant (450 ppm)	Consumer and food packaging	Good strength, high pressure heating resistance, moderate smoothness etc	0,922
DOW312E *(DOW, 2026).*	Anti-blocking agent, lubricant	Health and hygiene films, food packaging, shopping bags etc	Excellent processability and drafting performance; can be blended with LLDPE	0,924

Table 11 clearly shows that the LDPE materials are very similar and only have minor differences in the additives used and features. The LDPE material that was used in the investigation in section 8 was BOR5224.

7.4. Lubricants in drug substance production

At Fermion's pilot production site in Espoo lubricants such as mineral oil are used for sealing different production equipment such as the agitator shaft in the reactor. The purpose of mineral oil is to provide lubrication, cooling and prevent corrosion as well as many other similar purposes. Since reactors are used in API synthesis there is a small risk that contaminants can end up in the final product.

8. HS-GC-MS/FID investigation of potential contaminants produced during drug substance production

The HS-GC-MS/FID can detect impurities that arise during the production process of certain products. Certain non-process related impurities have been detected during residuals solvent analysis at Fermion's quality control laboratories using HS-GC-FID. The purpose of this investigation is to determine whether these impurities could originate from the different materials used in production. These substances include the production of detergents, packaging materials and lubricants. In addition to this, potential analytical impurities were investigated from the headspace vial septa used in the QC laboratories. To investigate this, different samples were produced and analyzed under different conditions with the HS-GC-MS/FID.

8.1. General residual solvent method parameters

Tables 12 and 13 outline HS-GC-MS/FID method that was used for the analysis of all the different samples in this investigation. This same method has been used to detect non-process related impurities in residual solvent analysis.

Table 12: Headspace conditions of the HS-GC-MS/FID method used in this investigation

Oven:	80°C
Loop:	90°C
Transfer Line:	100°C
Vial equilibration:	15 min
Injection Duration:	1 min
GC Cycle:	25 min
Vial shaking:	5
Vial Fill Mode:	Custom, Flow to Pressure
Fill Flow:	50 ml/min
Fill Pressure:	15 psi
Pressure Eq. Time	0.5 min
Loop Fill Mode	Custom
Final Loop Pressure	20 psi/min
Loop Equilibration	0,05 min

Table 13: Chromatographic parameters of the HS-GC-MS/FID method used in this investigation

Column	DB-624 UI, 30 m x 0,32 mm, 1,8 µm		
Injector	220°C		
Detector	250°C, FID		
Constant flow	1,8 ml/min		
Split	2:1		
Carrier gas	Helium		
Oven program	Rate/°C/min	Value/°C/min	Hold time/min
	-	45	0,2
	2,5	60	0
	10	240	20

8.2. Preparation of the detergent samples

To investigate detergent related contaminants some of the reflux boil wash procedures were simulated. Since reflux boil washes are used in both production plants, the effect of temperature on the chromatographic results produced was also investigated. The reflux boil wash was simulated by measuring 500mg of each detergent in a 100 ml volumetric flask and filling the rest with distilled water. This produced a 5 mg/ml solution, which is approximately the same as the 0.5% solution used at the production sites. The reactor was modelled using a round bottomed flask with a magnet and a condenser fitted above it, as can be seen in figure 14. The agitation was simulated with a magnetic stirrer. The solution was then placed in this flask and using a hot plate, boiled under reflux for about an hour. The condenser prevented any loss of the solvent through evaporation. After this the solution was cooled down.

Figure 14: Experimental setup of reactor reflux cleaning simulation

Since the detergents are also used at the manufacturing sites without heat in the foaming procedure, the same solution was analyzed without boiling the solution. 5 ml of each detergent solution was taken and placed in separate headspace vials and analyzed with HS-GC-MS/FID. Since the detergent solution was diluted in distilled water, samples of pure distilled water were also analyzed for reference in the chromatograms.

8.3. Preparation of packaging samples

To investigate the packaging related contaminants a series of experiments were conducted under different conditions. The main purpose of this investigation was to understand the interaction between the solvents and the LDPE under different conditions. In addition, the investigation evaluated the possibility of additive migration from the LDPE material and whether the impurities detected in residual solvent analysis originate from the LDPE packaging materials. The solvents that were investigated are perchloroethylene, dichloromethane and toluene. The different conditions that were investigated included temperature, exposure time and the effect of stretching the polyethylene material.

8.3.1. Temperature investigation

The effect of temperature was investigated by placing roughly 1x2cm pieces of the LDPE bags into 9 different headspace vials. The solvents were then added to the vials by pipetting 5 ml of each solvent to 3 separate vials. After this each solvent-bag extraction solution was heated to a specific temperature. These different temperatures were 40, 60 and 80 °C. The vials were heated on a hot plate for about 10 minutes. The temperature was measured by placing a thermometer in a beaker with water on the hot plate, as can be seen in figure 15. After this the vials were allowed to cool down and analyzed with HS-GC-MS/FID. Photographs of the vials were also taken before and after heating them to record the visual results.

Figure 15: Experimental setup showing vials heated to 80°C

8.3.2. Exposure time and LDPE deformation investigation

The effect of exposure time and stretching the LDPE were investigated. A total of 48 HS-vials were prepared. The same 1x2cm pieces of the bags were placed inside the vials, however half of the pieces were manually stretched before being placed in the vial. Figures 16 displays a stretched bag piece on the left and an unstretched bag piece on the right.

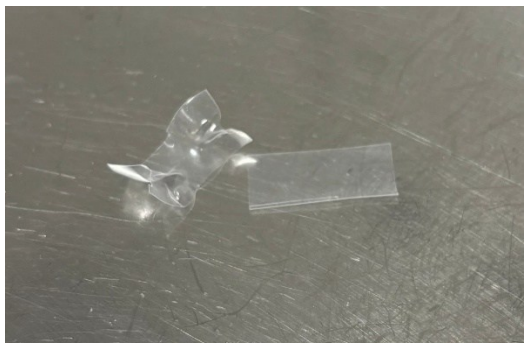

Figure 16: Piece of LDPE, stretched on left and unstretched on right

The solvents were then added to the vials by pipetting 5 ml of each solvent to 16 separate vials. So, 5 ml of each solvent was added to 8 vials with an unstretched bag and 8 vials with a stretched bag. After this the 48 vials were separated into 8 different sets. Each set contained 6 vials, with 2 vials per solvent, one with a stretched piece and one with an unstretched piece.

Each set of pieces was then extracted in the solvent for a different amount of time and analyzed with HS-GC-MS. The solvent extraction solutions were investigated under the following exposure times T = 1, 2, 3, 4, 5, 6, 7 and 14 days. The solvents were also analyzed separately without the LDPE extraction to compare the data with the extraction samples. Visual data was also recorded by photographing the vials after each day of extraction.

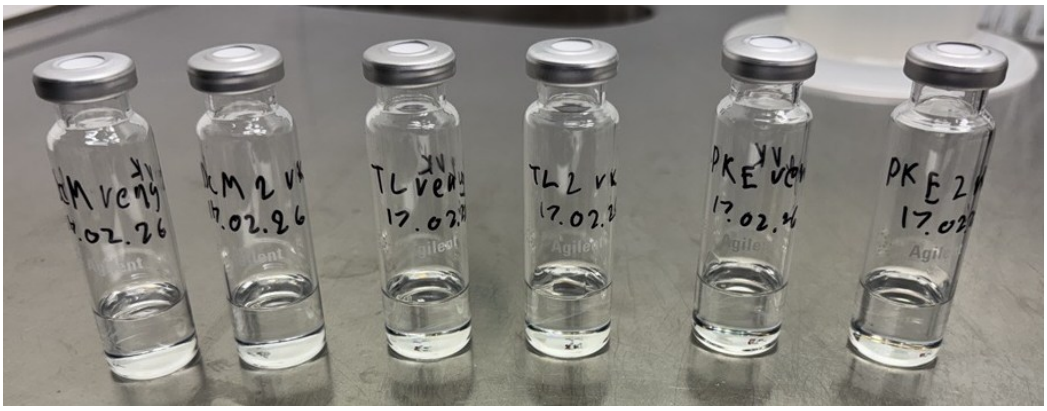

Figure 17: the LDPE-bag solvent solutions immediately after preparation

8.3.3. Salting out investigation

Adding an ionic salt such as NaCl to an aqueous solution can strengthen the chromatographic signal of certain organic compounds as this increases the ionic strength of the aqueous layer. This concept is discussed in more detail in section 2.6.5. According to *(ATSDR, 2017)* and *(IARC, 1995)* perchloroethylene and toluene can contain small amounts of water. Therefore, the effect of adding ionic salt on the chromatographic response was also tested for the LDPE packaging samples. Since the polyethylene bag only partially dissolved in the dichloromethane solvent at higher temperatures, the salting out investigation was only carried out for toluene and perchloroethylene. 20 ml of both solvents were placed into separate beakers. Four pieces of LDPE were placed into each solvent. Both solutions were heated to around 70 C or until the polyethylene had completely dissolved into the solution. After this NaCl was added to the solution. NaCl was added until the solution was fully saturated. The solutions were then kept under the heat for 20 minutes and stirred occasionally. Watch glasses were placed on top of the beakers to prevent the solvents from evaporating. Figure 18 displays the experimental set up of the salting out investigation.

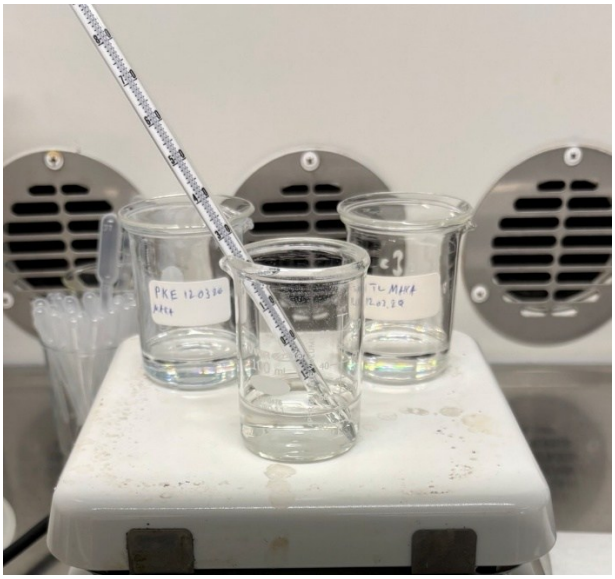

Figure 18: Experimental set up of the salting out investigation

After this 5ml of each solution was pipetted into a separate headspace vial. Around 500 mg of NaCl was also added directly to the HS-vial to ensure constant saturation. An additional piece of the LDPE was also added to each vial. After this each vial was analyzed with HS-GC-MS/FID. Figure 19 shows the samples prepared in the HS vials

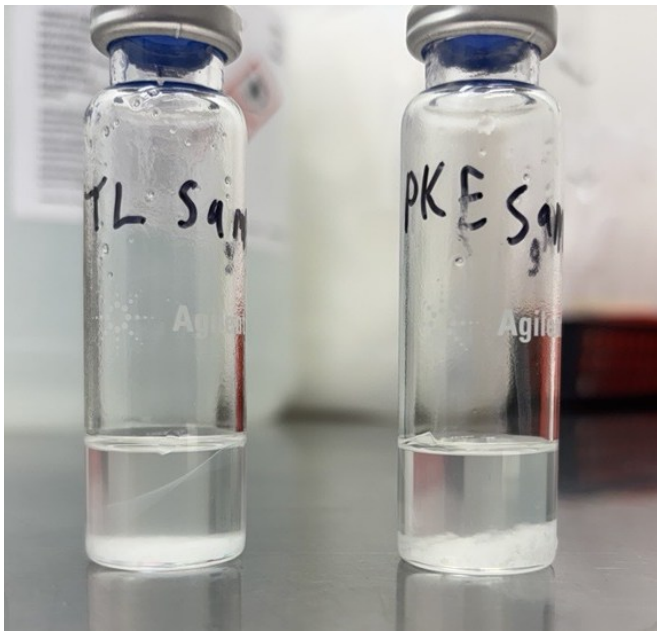

Figure 19: Prepared samples for the salting out investigation, toluene left, perchloroethylene right

In figure 19 the undissolved NaCl can be seen at the base of the vial, indicating full saturation.

8.4. Mineral oil investigation

Two different samples of mineral oil were prepared. The product name is petrocanada mineral oil, which is the same lubricant that is used at Fermion's pilot production site in Espoo. The 2 samples were prepared by placing a drop of mineral oil into 2 separate headspace vials. 5 ml of distilled water was added to one vial and the other only contained the mineral oil. The vials were then analyzed with HS-GC-MS/FID device.

8.5. Headspace vials septum investigation

The caps in the headspace analysis vials contain a septum which provides an airtight seal for each vial. The seals are made of polytetrafluoroethylene and are sealed using a device called a crimper. To investigate whether impurities could arise from the septa the following experiment was conducted.

Two septa were removed from their caps and were each cut into 4 separate pieces. The pieces were then placed into 2 separate beakers with each beaker containing 4 pieces. 25 ml of toluene was added to one beaker and 25 ml of dimethyl sulfoxide was added to the other. The pieces were then swirled in the beakers for a few minutes using a glass rod to stir. Figure 20 shows the experimental set up.

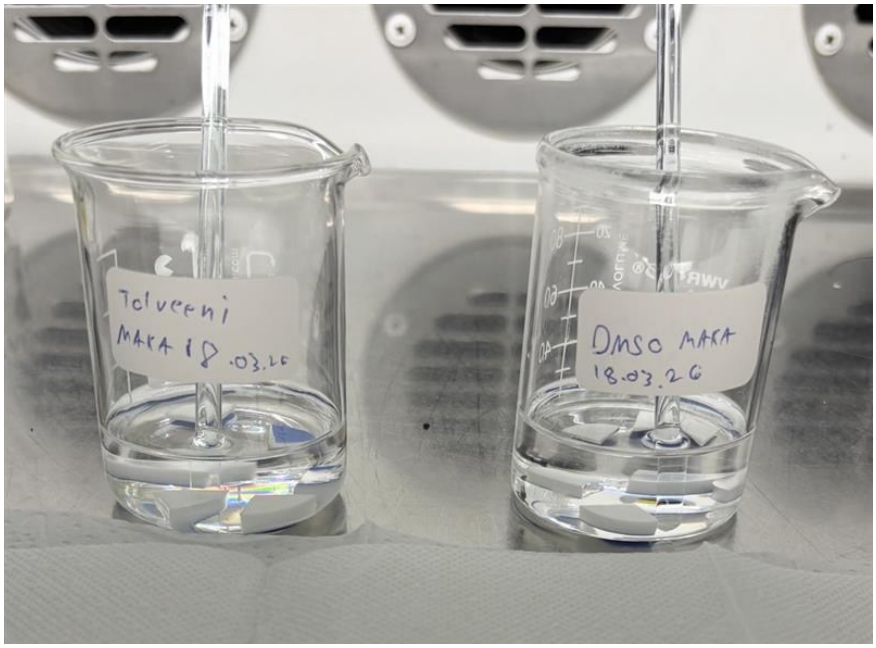

Figure 20: Experimental set up of the vial septum investigation

After this 5 ml of the toluene was pipetted directly to a vial and 5ml to a vial with a HS-septum piece. 20 ml of the dimethyl sulfoxide was added to a 100 ml volumetric flask. The rest of the flask was then filled with distilled water to the line. 5 ml of this solution was then also pipetted to two separate headspace vials with one containing a piece of the septum. The vials were then analyzed using HS-GC-MS/FID device. The pure solvent solutions were also analyzed to provide a reference for the data analysis.

Once the piece of septum was placed into the toluene it began to swell. Therefore, to further investigate an additional 5 ml of each pure solvent was added to a HS-vial containing a piece of septum and was extracted for 24 hours. The data was recorded visually with photographs.

9. Data analysis of the detergent results

The data on each of the detergents were analyzed. This included the data of both the reflux boiled solutions as well as the cold solutions. This provided valuable information about the different volatile components in each solution.

9.1. Proset V30L

The HS-GC-MS chromatograms of both the reflux boiled and cold solution of the Proset V30L can be seen in figure 21. Three main peaks are identified at RT 8.544, 9.111 and 9.673. However, the resolution of the peaks is low and there are a lot of co-eluting analytes. The reflux boiled solution shows a higher signal-to-noise ratio.

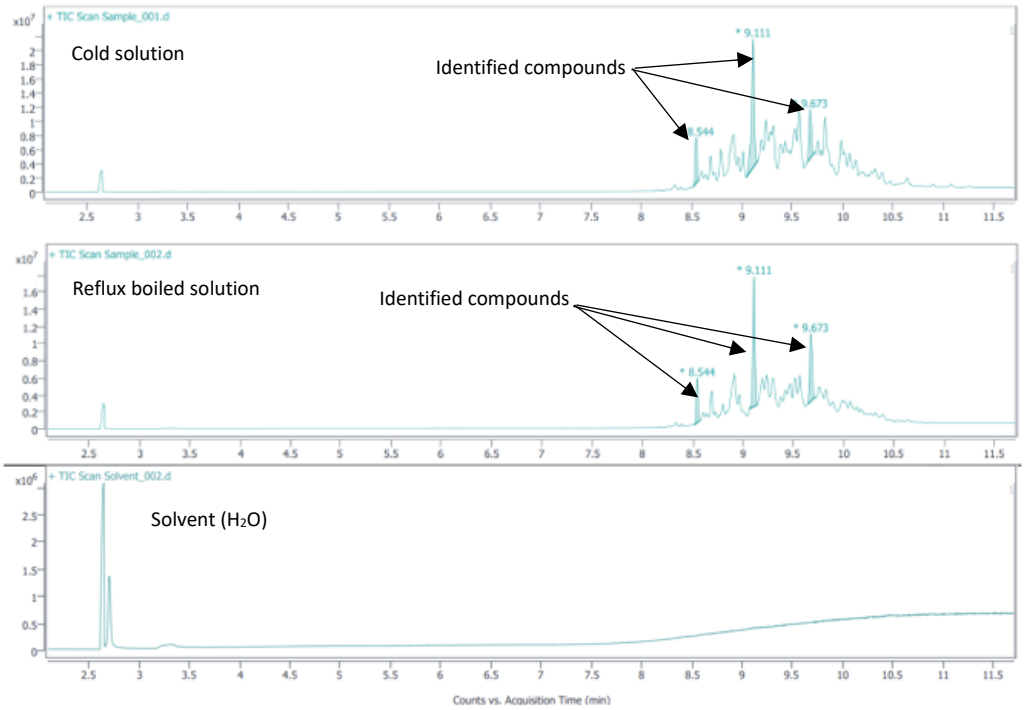

Figure 21: Proset V30L MS chromatograms of cold, reflux boiled and solvent in descending order

The numeric value of the integrated area and the signal-to-noise ratio is also presented in table 14. These values were calculated using the device's software. For the SNR value the signal was compared to the noise between RT 6-8 minutes. The signal-to-noise ratio appears to be higher for all peaks in the reflux boiled solution whereas the integrated area appears to be higher for the first two peaks in the cold solution. The reflux boiling of certain molecules could cause certain molecules to thermally degrade, leading to differences in the chromatogram response displayed. The FID chromatograms can be seen in figure 1 in

appendix A and a clear difference in the signal-to-noise ratio can also be seen in them. Since the sample and the solvent chromatograms both have a peak at around 2,7 minutes, this can be ruled out as an disturbance in the system.

Table 14: Differences in the integrated peak area and SNR values of the identified peaks between the cold and reflux-boiled Proset V30L solutions

	Integrated area		Signal-to-noise ratio	
Peak retention time (min)	Cold solution	Reflux boiled	Cold solution	Reflux boiled
8.544	8577896	6903513	64.2	68.7
9.511	34181669	22236959	182.7	199.9
9.673	11371642	13635301	69.7	103.9

The identification of the peaks using mass spectrometry was slightly challenging due to the co-eluting compounds, as the baseline response around the peaks was significant. When conducting qualitative analysis of the 3 large peaks, the background spectra was subtracted to improve the identification. All three MS spectra for both reflux boiled and cold solutions can be seen in Appendix A from figures 2 and 3.

The spectra were compared against the NIST library. This library contains a substantial number of reference spectra for different compounds which helps identify the analyte compound. When the observed spectra are compared against the library, the software suggests a compound which represents the spectra the best and provides a probability score. The NIST library results can also be seen in appendix A. The compounds that were suggested by the software were 1-Octanol, 2-butyl (RT: 8.544), 1-chlorohexadecane (RT: 9.111) and Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl (RT: 9.673) for the cold solution. For the reflux boiled solution, the NIST library suggested that the first 2 peaks were 1-Octanol, 2-butyl (RT: 8.544 and 9.111) and the last peak was phytol (RT: 9.673). These compounds can be seen in the left corner of each of the spectrum in figures 2 and 3 in appendix A. The probability scores the library gave for these compounds were between 70-90% and therefore this data is not very reliable. The low probability score derives from the low resolution of the peaks. However, all the spectra appear to resemble differently sized hydrocarbons. Therefore, it can be determined that the identified peaks derive from the volatile hydrocarbons found in the Proset V30L detergent.

9.2. F44 Sensol

The F44 Sensol detergent produced fewer and less intense peaks which made the identification of the analytes more complicated. However, there were significant differences between the FID chromatograms and MS chromatograms produced. In the MS chromatogram for the cold solution the two main identified peaks are at RT 9,982 and 10,951.

For the cold solution the FID chromatogram shows several co-eluting compounds towards the end of the chromatogram which cannot be seen in the reflux boiled FID chromatogram which further suggests that certain smaller molecules thermally degrade at certain temperature. The first 2 peaks can also be ruled out as a system disturbance as they can also be seen in the solvent chromatogram. The integrated area and signal-to-noise ratio is slightly higher with the cold solution. In these chromatograms the signals were compared to the noise between RT 3-5min. These values can be seen in table 15.

The MS spectra of the identified peaks were also compared against the NIST library. According to the library they correspond to Ethanol, 2-(2-butoxyethoxy) and Tritriacontane. The Ethanol, 2-(2-butoxyethoxy) has a probability score of 77.47, however it can be considered reliable because it is listed as an ingredient in the detergent and therefore the retention time can be localized at 9,982. The Tritriacontane is not reliable as it is not listed as an ingredient, and the resolution of the peak is poor across the different chromatograms. The MS spectra of the two identified peaks for both reflux boiled and cold solutions can be seen in Appendix A from figures 4 and 5.

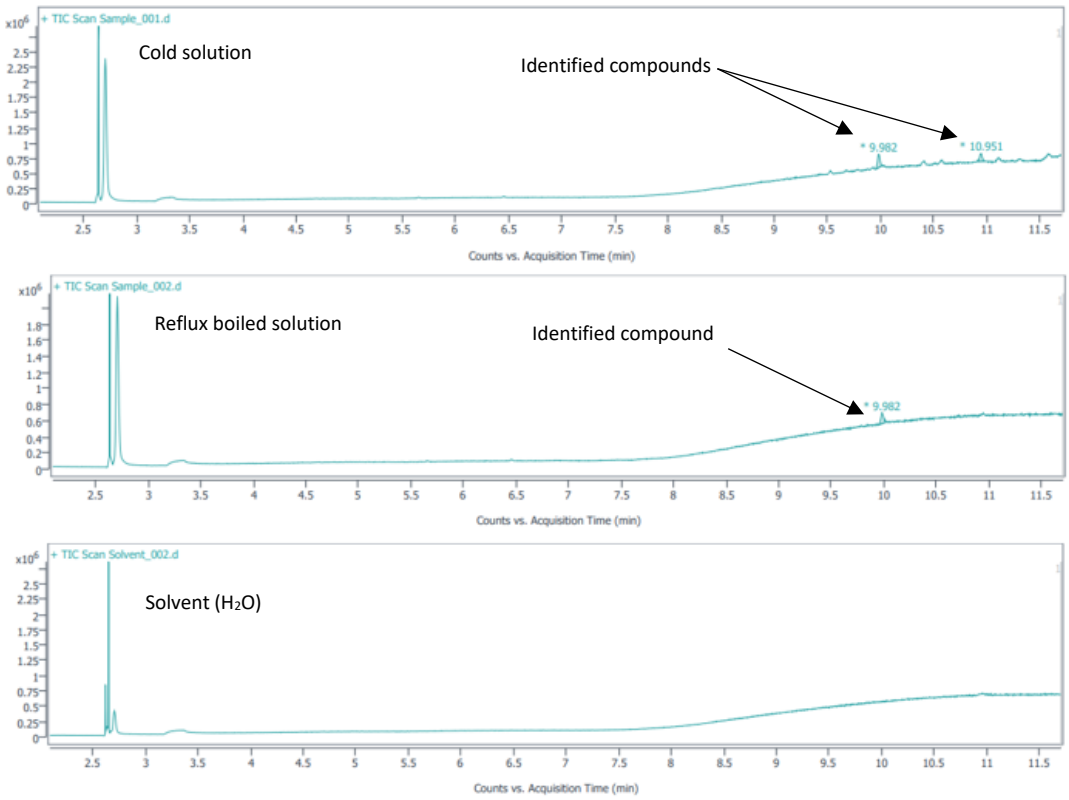

Figure 22: F44 Sensol MS chromatograms of the cold, reflux boiled and solvent in descending order

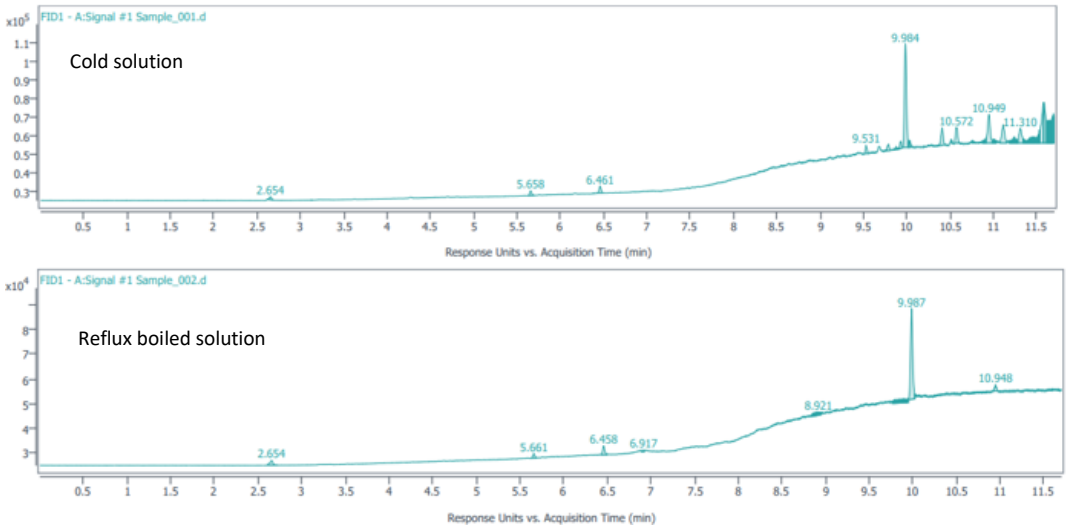

Figure 23: F44 Sensol FID chromatograms of the cold and reflux boiled solutions in descending order

Table 15: Differences in the integrated peak area and SNR values of the identified peaks between the cold and reflux-boiled F44 Sensol solutions

	Integrated area		Signal-to-noise ratio	
Peak retention time (min)	Cold solution	Reflux boiled	Cold solution	Reflux boiled
RT: 9,98 FID	371294	252106	27,6	17.3
RT: 10,95 FID	238921	-	7,6	1,2

9.3. Proset S26E

The Proset S26E detergent did not produce any peaks in the MS chromatogram for either the cold or the reflux boiled solution and therefore no mass spectra could be extracted. The FID chromatogram however did produce small peaks in both the cold and reflux boiled solutions. It is possible that these peaks derive from the organic material in the detergent, however this cannot be confirmed as the solvent solution also produced certain peaks in the FID chromatogram and therefore could be a sign of a disturbance in the system.

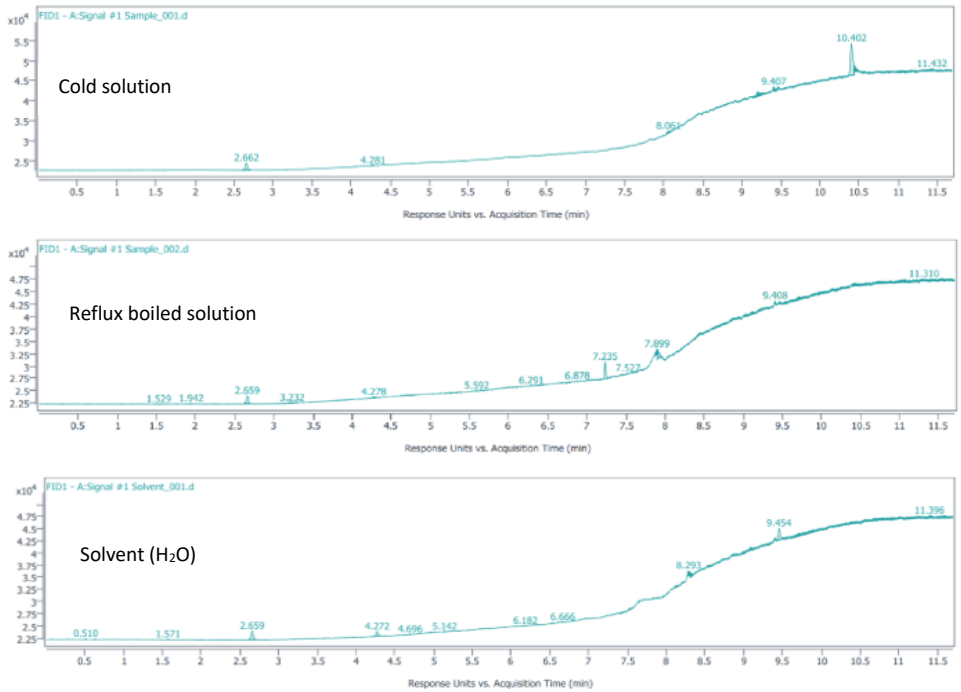

Figure 24: Proset S26E FID chromatograms of the cold, reflux boiled and solvent in descending order

9.4. Proset S26H

The Proset S26H detergent also only produced peaks at the very start of the MS chromatogram for both the reflux boiled and cold solution. These peaks were also visible in the solvent and therefore can be ruled out as a disturbance in the system. Since no peaks were produced in the chromatogram no mass spectra could be obtained. No response was detected with the FID chromatograms either as can be seen in figure 25. This data is consistent with detergent's properties as it contains little to no organic volatile material. Therefore, it can be determined that this detergent does not contain any potential contaminants that could be seen with the HS-GC-MS/FID analysis device. The chromatograms can be seen in figure 7 of appendix A.

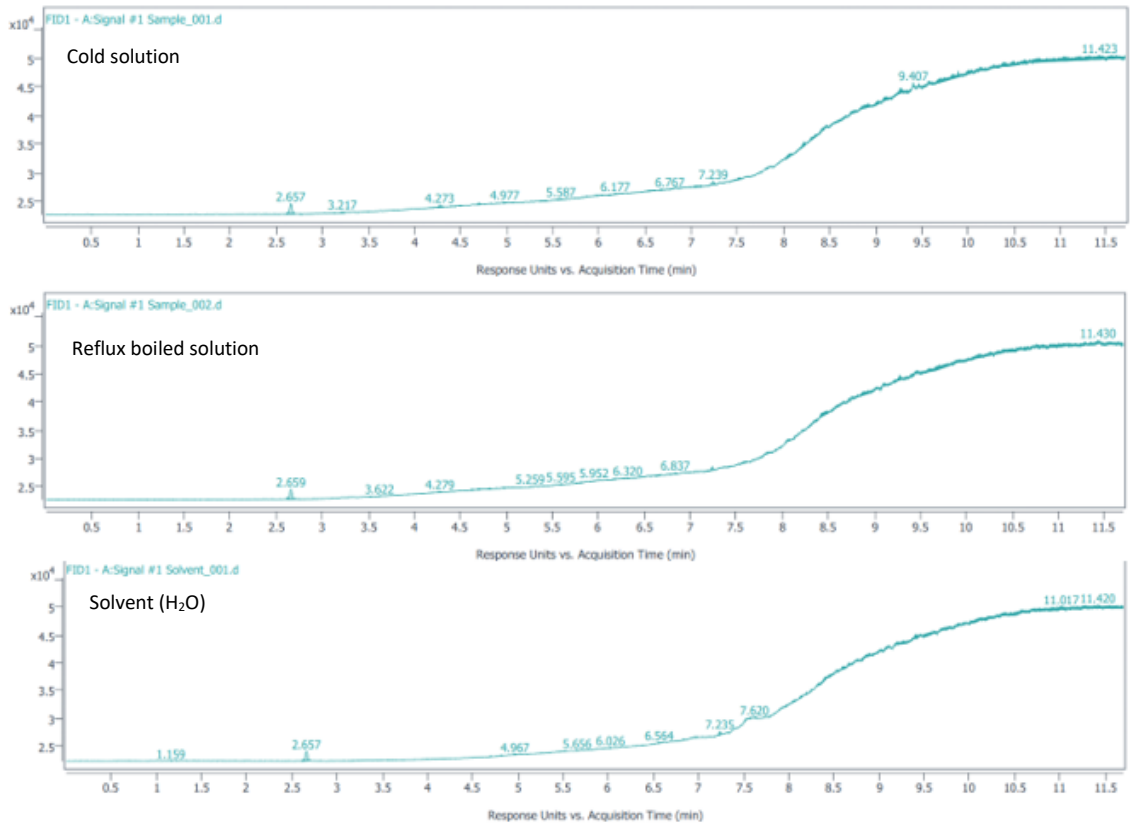

Figure 25: Proset S26E FID chromatograms of the cold, reflux boiled and solvent in descending order

9.5. Overall analysis on detergent data

Overall, the HS-GC-MS/FID analysis indicated there are differences in the integrated peak area as well as the signal-to-noise ratio between the cold and reflux boiled solutions in the Proset V30L and F44 sensol detergents. Three peaks in the Proset V30L were identified by comparing their spectra to the NIST library. The probability scores for these compounds were not very high and therefore these identifications could not be relied on. However, since the spectra mostly represents different hydrocarbons, it can be determined that the peaks arise from the hydrocarbons in the Proset V30L detergent. The F44 sensol detergent produced fewer and less intense peaks, however the Ethanol, 2-(2-butoxyethoxy) ingredient was identified. The other detergents produced little to no volatile organic compounds.

10. Data analysis of packaging results

This chapter outlines and analyzes all of the data that was gathered throughout the packaging experiments. The chromatograms of the solvents that were used in each experiment were first introduced and then all of the data from each experiment was analyzed individually.

10.1. Solvent chromatograms

The pure solvents that were used in the investigation were also analyzed so that they could be compared to the experimental data. Figure 26 below displays the different chromatograms of the pure solvent peaks. There are two peaks for toluene because both the GC and regular quality were used.

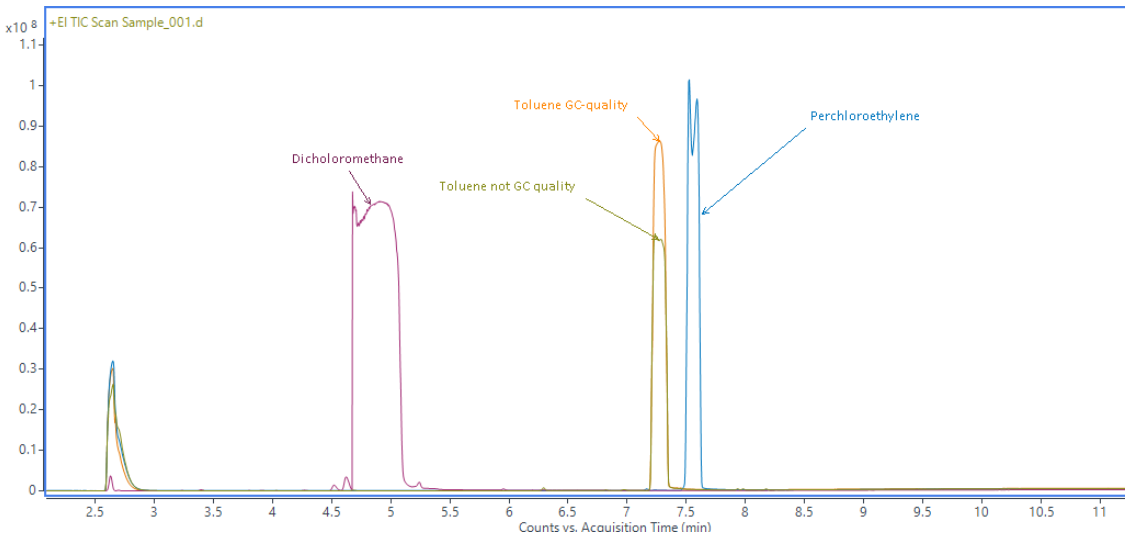

Figure 26: MS chromatograms of the solvents

10.2. Data analysis of temperature investigation

The temperature investigation provided valuable information about the dissolution of LDPE in these solvents. When the extraction solutions were heated to 40°C little to no dissolution occurred and the bags were still clearly visible within the vials as can be seen in figure 27 below. The piece of the LDPE bag can faintly be seen in each vial.

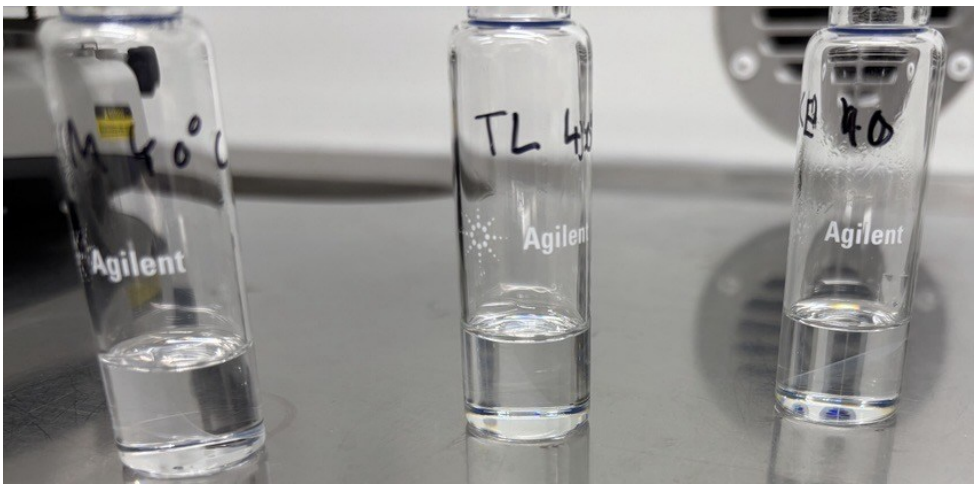

Figure 27: Appearance of the LDPE bag solvent solution following 10 minutes of heating at 40 °C. Left to right dichloromethane, toluene and perchloroethylene. Did not dissolve

When the solutions were heated to 60 °C the LDPE bag pieces began to dissolve into some of the solvents. At 60 °C the LDPE bag dissolved completely into perchloroethylene. For

toluene, the dissolution was partial but still evident. The dichloromethane solution remained undissolved at 60 °C. Figure 28 shows the visual dissolution data at 60 °C.

Figure 28: Appearance of the LDPE bag solvent solution following 10 minutes of heating at 60 °C. Left to right dichloromethane, toluene and perchloroethylene.

The LDPE piece can clearly be spotted on the surface of the solvent in the dichloromethane solution. The toluene solution is slightly cloudy, and the piece appears to be slightly smaller and swollen. There is no visible piece in the perchloroethylene solution and the solution is clear, indicating the polyethylene has completely dissolved into the solvent.

At 80 °C both toluene and perchloroethylene completely dissolved the polyethylene while partial dissolution occurred with dichloromethane. This can be seen in figure 29 below.

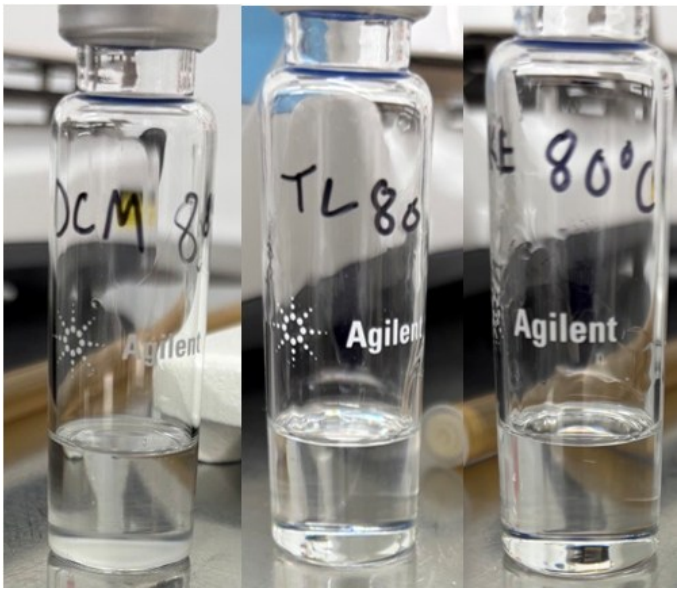

Figure 29: Appearance of the LDPE bag solvent solution following 10 minutes of heating at 80 °C. Left to right dichloromethane, toluene and perchloroethylene.

The perchloroethylene and toluene solutions appear completely clear indicating the complete dissolution of the polyethylene. A small piece of polyethylene can be spotted in the dichloromethane solution. However, it appears to be white and slightly disformed. The vial was also slightly shaken, which led to the formation of a cloudy solution that appears in figure 30 below.

Figure 30: Appearance of the LDPE bag dichloromethane solution after heating at 80 C for 10 minutes followed by the shaking of the HS-vial

Another observation was that after the solvent cooled down the polyethylene precipitated back out of the solution producing cloudy solutions that can be seen in figure 31.

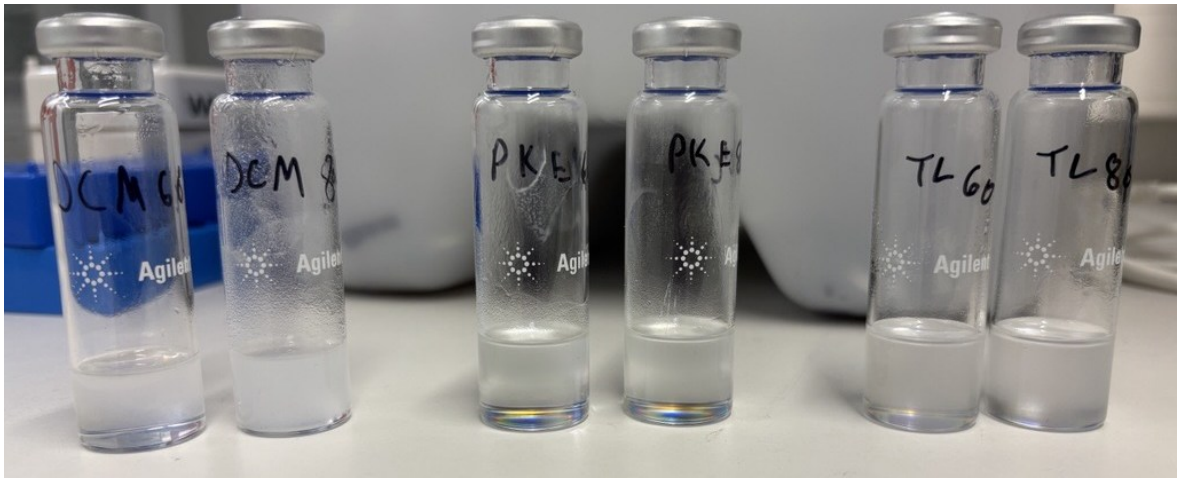

Figure 31. Appearance of the heated solvent solutions after cooling. Left to right (60 °C then 80 °C for each solvent): dichloromethane, perchloroethylene and toluene

The appearance of the solutions is clearly cloudy, and polyethylene particles can be seen floating on the top of the solvents as well as on the inner walls of the headspace vials.

After the solutions were heated, they were also analyzed with the HS-GC-MS/FID. The analyte chromatograms had no, or very little difference compared to the solvent chromatograms. Figure 32 below shows the comparison of the difference in the chromatograms between the solvents heated to 80 °C and the pure solvents without polyethylene.

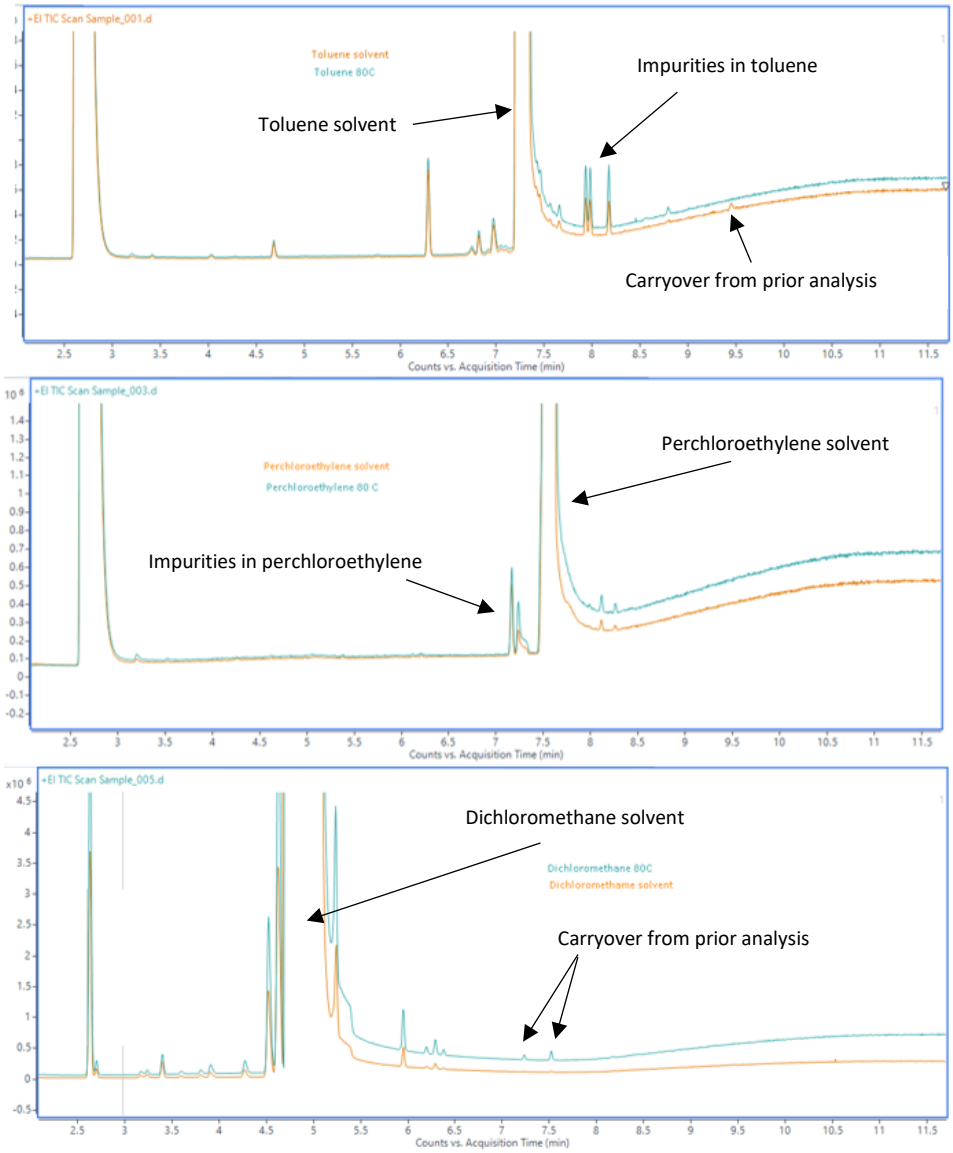

Figure 32: MS chromatograms of polyethylene-extracted samples heated to 80 °C in perchloroethylene, toluene, and dichloromethane, compared with the corresponding pure solvents.

The chromatograms of the solvents show no data that would clearly suggest that volatile impurities arise from the polyethylene. This is because most peaks that can be seen in the sample can also be seen in the solvent and therefore indicate that these compounds are impurities that originate from the solvent. In the dichloromethane sample chromatogram, a few peaks can be seen in the sample that cannot be seen in the solvent. These peaks were further inspected with the mass spectrometer and were identified as carryover from a previous analysis.

10.3. Exposure time and LDPE deformation investigation

The exposure time and LDPE deformation investigation did not provide extensive information about the interaction between the polyethylene and these solvents. Both visual and chromatographic data were recorded. Figure 33 shows extraction samples immediately after placing the polyethylene into the solvents and then after the 2-week extraction period.

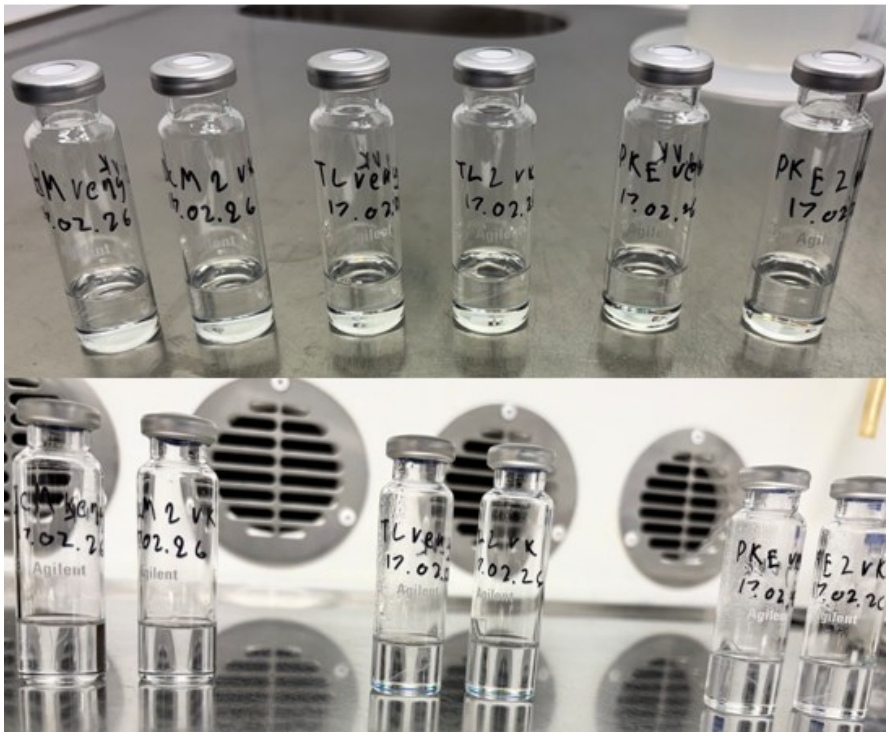

Figure 33. Visual comparison of LDPE samples at the start of extraction and after the two-week extraction period in descending order.

Figure 33 shows condensation in some of the vials in the 2-week extraction samples and the polyethylene appears to have expanded slightly. However, no noticeable visual differences were recorded after the 2-week extraction period.

Figure 34 compares the chromatograms for the solvent extraction solutions from stretched and unstretched LDPE with extraction periods of 24 hours and two weeks.

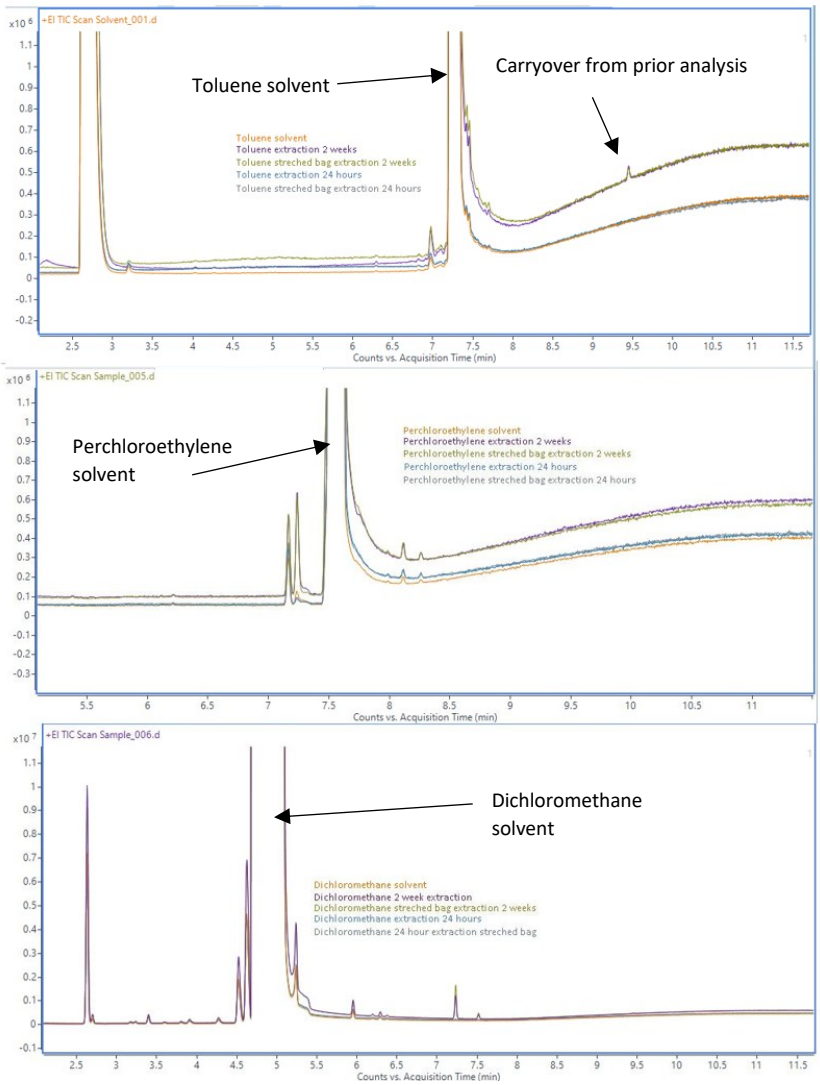

Figure 34: MS chromatograms of the LDPE extraction in toluene, Perchloroethylene and Dichloromethane

There is little to no difference between the 2 week and 24-hour extraction chromatograms and the effect of stretching the bag also appears to be limited. Most of the peaks that appear in the samples are also visible in the solvent themselves. The unique peaks that appear only in some of the sample chromatograms correspond to other solvents that are used in the same analysis according to the mass spectrometer indicating that carryover has also occurred in this analysis. There is no conclusive evidence that suggests that the polyethylene produces volatile substances that can be seen in either MS or FID chromatograms, thus HS-GC-MS/FID is not suitable for analyzing LDPE even if it is dissolved in a solvent.

10.4. Salting out investigation

The salting out investigation did not provide useful information about the polyethylene impurities either. This can be seen in the chromatograms below as all the peaks that are seen in the sample can also be seen in the solvent

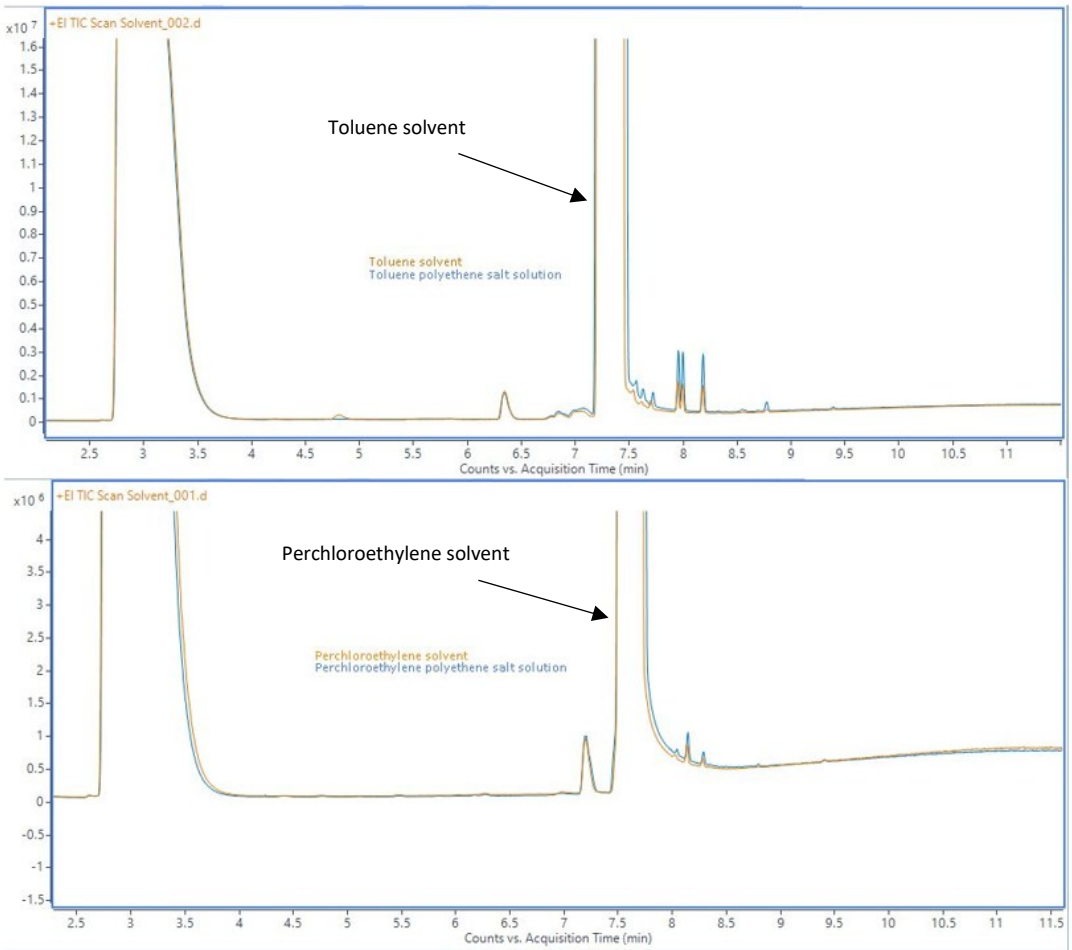

Figure 35: MS chromatograms of the salting out investigation for toluene and perchloroethylene

Some of the peaks in the salt samples appear to be slightly more intense, for example in the toluene chromatogram at around 8 min. This is most likely due to the salting out effect where the salt in the solution causes certain impurities to partition out of the aqueous phase increasing the concentration in the headspace. However, since the water concentration in these solvents is very low and NaCl is an ionic compound, it is not soluble in the organic

solvents used in this investigation *(Clark, 2013)*. Therefore, this investigation does not provide the most reliable insight for evaluating the effect of salting out.

10.5. Overall analysis on packaging data

After reviewing the data, the key question that emerged was how the polyethylene could dissolve into certain solvents but could not be detected with the HS-GC-MS/FID device. Since the polyethylene is made of pure carbon and hydrogen atoms the degradation products include differently sized hydrocarbon chains.

However, according to Whajah *(Whajah et al, 2021)* and Zepp (*Zepp et al, 2023)* the depolymerization of polyethylene can only occur under specific conditions such as exposure to UV radiation or elevated temperatures making it a complex mechanism. This is a large disadvantage when it comes to LDPE because it makes the material non-biodegradable and therefore produces a large amount of plastic waste yearly.

Nevertheless Cole-Palmer *(Cole-Parmer, 2019)* states LDPE is not very compatible with certain solvents used in API production. Even though the degradation of the polymer itself is complicated, the LDPE polymer matrix can still dissolve into certain aromatic and halogenated hydrocarbon solvents at higher temperatures *(Simoes, 2019).* When this happens the intermolecular bonds between the molecules can break and the internal polymer chains are exposed. This disentangles the polymer matrix making the material incompatible with certain solvents. However, during this process the individual polymer chains do not decompose and after the dissolution the polymer is still completely recoverable. *(Chalapati, 2014)* Since the polymer matrix only disentangles into the separate polymer chains each polyethylene molecule still retains its original chemical properties. According to (*Pruitt, 2011)* the molecular weight of LDPE ranges from 30,000 to 50,000 g/mol. The large molecular weight also indicates a large boiling point. A higher boiling point also corresponds to low volatility. This means that the intact LDPE chains are non-volatile and do not evaporate into the headspace in the vial.

The additives that were present in the polyethylene packaging films were not identified either. According to the European pharmacopoeia *(EUROPEAN PHARMACOPOEIA 3.1.5, 2025)* the permitted levels of specific additives range between 0.1-0.5%. Table 11 level

outlines the concentrations of the antiblock and lubricant additives for the LDPE material used in the investigation but the concentration of the antioxidants in the material is unknown. However, the European pharmacopoeia also states that the total permitted concentration of the antioxidant level is 0.3%. This means that the concentration of the additives present in this investigation is very low, which could indicate why these substances were not detected.

The liquid paraffin additive was relevant for this research as it comprised of a mixture of hydrocarbons and it is present in almost all polyethylene products *(Ito et al, 2023)*. If these hydrocarbon chains crack it could potentially produce shorter hydrocarbon chains that would appear as volatile impurities as can be seen in the detergent data. Further research is required to investigate whether different productions conditions could cause these chains to crack.

Polyethylene dissolution has been studied for industrial purposes. This includes the conversion of waste plastic into fuel. The study by Passamonti & Sedran, *(Passamonti & Sedran, 2012)* investigated how the concentration of the LDPE added to the process affected the conversion of certain fuel products. Small amounts of low-density polyethylene are dissolved into commercial vacuum gas oil by stirring and heating them for 2 hours at 115 °C. Commercial vacuum gas oils are obtained by the vacuum distillation of crude oil and are used as feedstock in the production of lighter fuels, they consist of different hydrocarbons such as paraffins, olefins and aromatics *(Skog llc, 2026).* After this the solution was converted to a laboratory scale reactor along with FCC catalysts and were left to react at 3 different temperatures. The different temperatures were 500 °C, 525°C and 550 °C. The results showed that the LDPE underwent primary reactions of catalytic cracking that increased the yield of olefins in certain products such as LPG or gasoline. *(Passamonti & Sedran, 2012)*

11. Analysis of HS-vial septum and mineral oil data

The HS-vial septum investigation provided some useful information about the behavior of the septum with certain solvents; however, no impurities were detected in the investigation.

The main finding occurred in the 24-hour extraction investigation. Figure 36 shows the effect of 24-hour solvent extraction investigation.

Figure 36: Twenty-four-hour extraction results showing dimethyl sulphide (left) and toluene (right).

Figure 36 clearly shows that after the 24-hour extraction the toluene causes the septum piece to significantly swell whilst the dimethyl sulphide has little to no effect on the piece. The chromatographic data can be seen in figure 37.

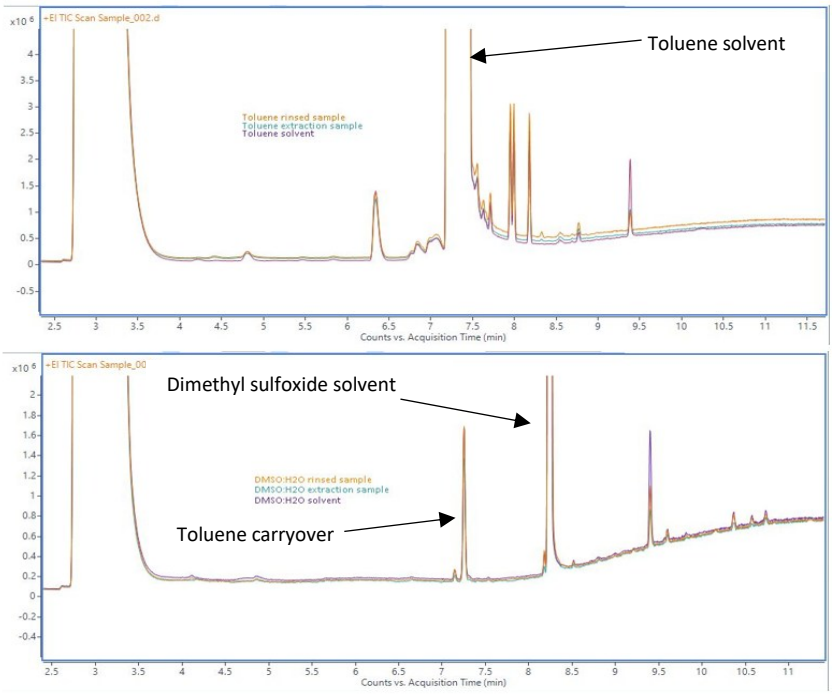

Figure 37: Chromatographic data of the vial septum investigation

Figure 37 indicates that the septum material does not produce any volatile impurities in the solvent. All the peaks that can be seen in the samples can also be seen in the solvent and therefore originate from the solvent itself.

The mineral oil samples did not produce any MS chromatograms and therefore no spectrum could be obtained. However, the FID detector produced wide large peaks for the sample with pure mineral oil. Since no peaks were produced in the MS chromatogram the FID peak is not very reliable and cannot not be identified. The resolution of the peak is also very low which could be an indication of a system disturbance as well. The chromatograms can be seen in figure 38 below.

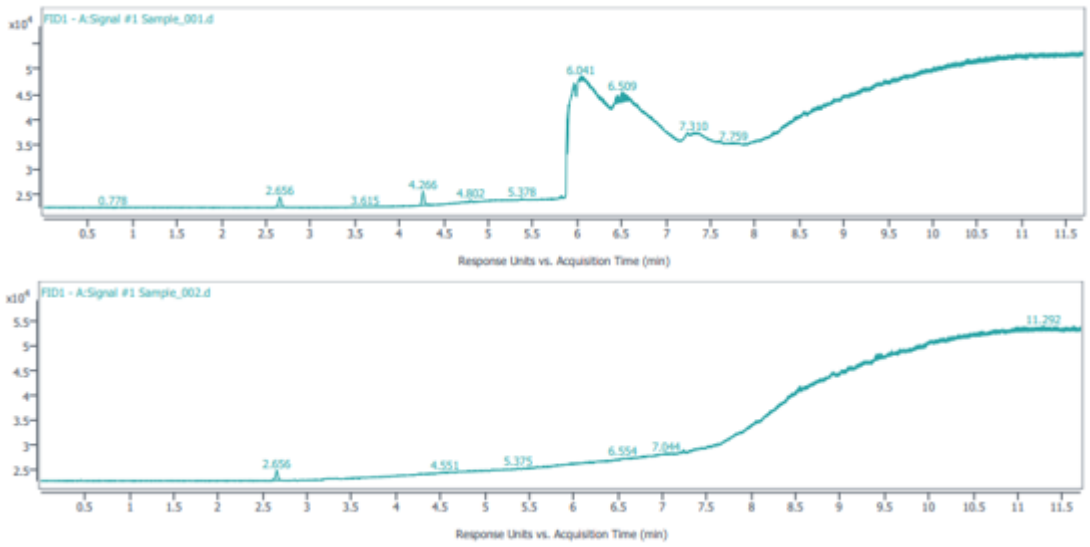

Figure 38: FID Chromatograms 1 drop petrocanada mineral oil and 1 drop petrocanada mineral oil + 5 ml H20 in descending order

12. Conclusions

The main objectives of this thesis were to identify the potential non-process related volatile impurities in different production related products and to determine the sources of non-process impurities that have been detected in the residual solvent analysis using the HS-GC-MS/FID method described in section 8.1. The device detected volatile impurities in two

different detergents. However, reliable identification of these impurities was not achieved because the identified spectra produced a low probability when compared to the NIST library. No detectable impurities were found in other detergents, polyethylene packaging or lubricants.

Although the chromatographic data did not show that the polyethylene produced any impurities in the solvent, there was one key finding in the visual data relating to the solubility of the polyethylene at different temperatures. The polyethylene started to dissolve into the solvents when heated to certain temperatures. LDPE dissolution occurred with perchloroethylene and toluene at around 60°C and dichloromethane at 80°C. This demonstrated a clear interaction between the solvents and polyethylene, indicating a significant quality and safety risk for the products that are processed with these packages and solvents. This is also consistent with previous research conducted on this matter *(Simoes ,2019)*. Therefore, when the packaging material is exposed to these solvents, it is essential to ensure proper quality control and prevent the presence of any contaminants resulting from this exposure.

Some research suggests that LDPE has certain additives that are volatile and could be detected using a GC-MS system *(Chapke, 2022)*. However, the data in this thesis does not align with this research. This discrepancy is likely due to the different sampling methods as well as the difference in the extraction solvents. A limitation to this research included focusing only on the gas chromatography as an analysis technique and therefore only volatile impurities could be detected. The european pharmacopeia states that the analysis of certain additives is completed using infrared absorption spectrophotometry and thin layer chromatography *(EUROPEAN PHARMACOPOEIA 3.1.5, 2025)*. Therefore, future research should utilize these techniques for the identification of additives in polyethylene.

The polyethylene visual data suggests that there is an interaction between the solvents and LDPE leading to the contamination of products exposed to these solvents. Therefore, future research should investigate the non-volatile contaminants using different analytical and sample preparation methods. Another limitation included the simulation of the production conditions for the packaging data. Therefore, further investigation should also be conducted into how certain process conditions could promote the production of volatile impurities through the decomposition of certain additives.

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Appendix A Detergent data:

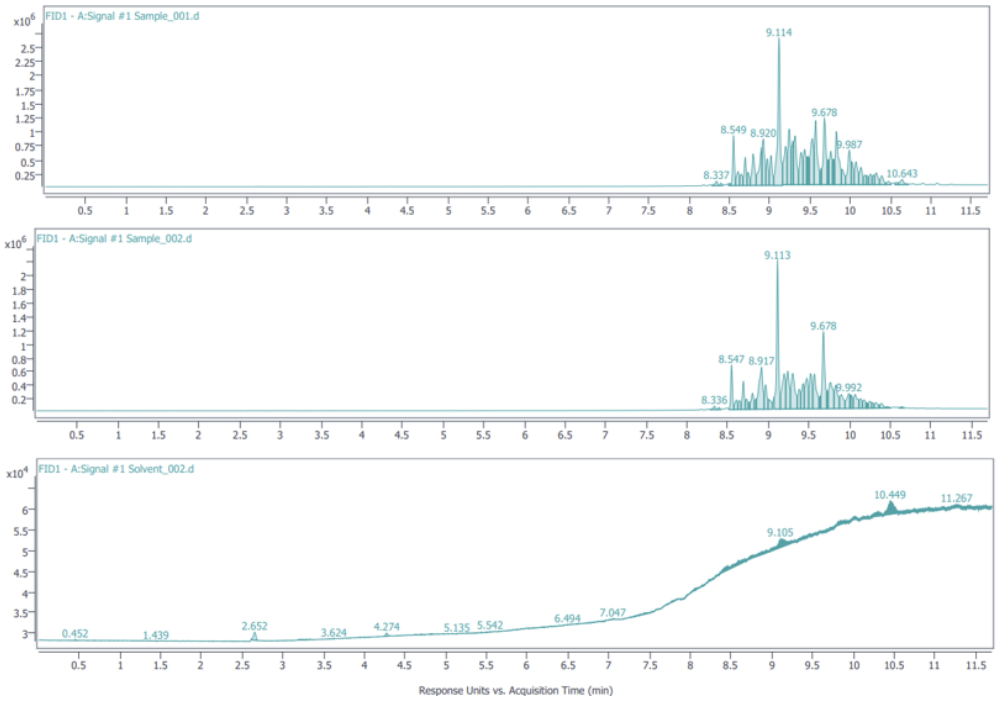


Figure 1: Proset V30L FID chromatograms of the cold, reflux boiled and solvent in descending order

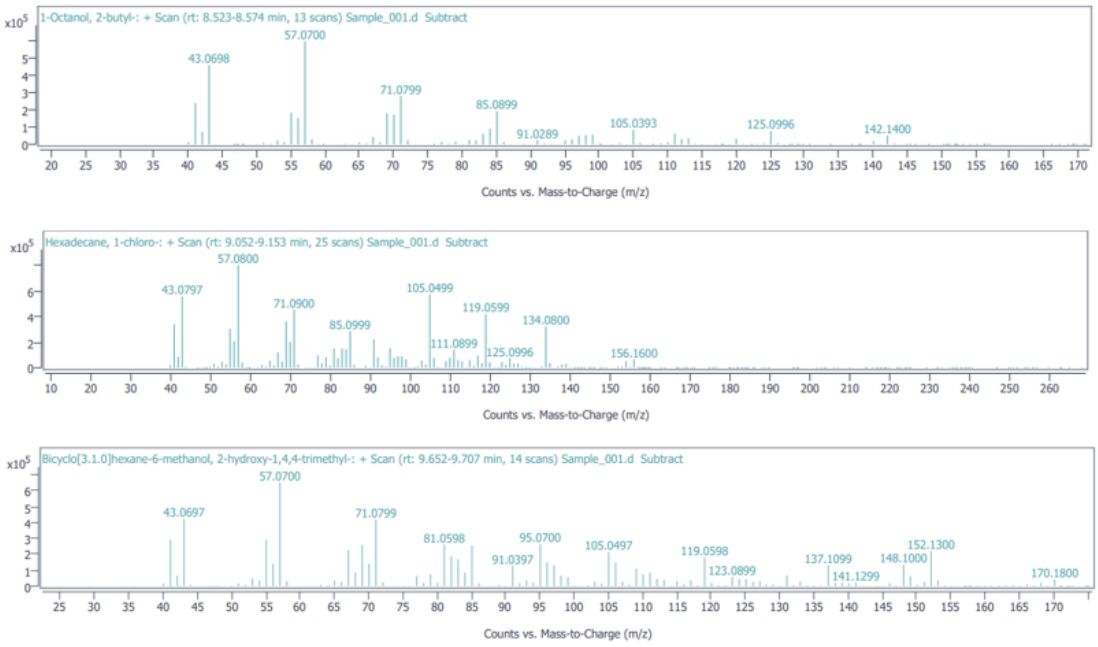


Figure 2: Proset V30L cold solution, identified spectra in descending order

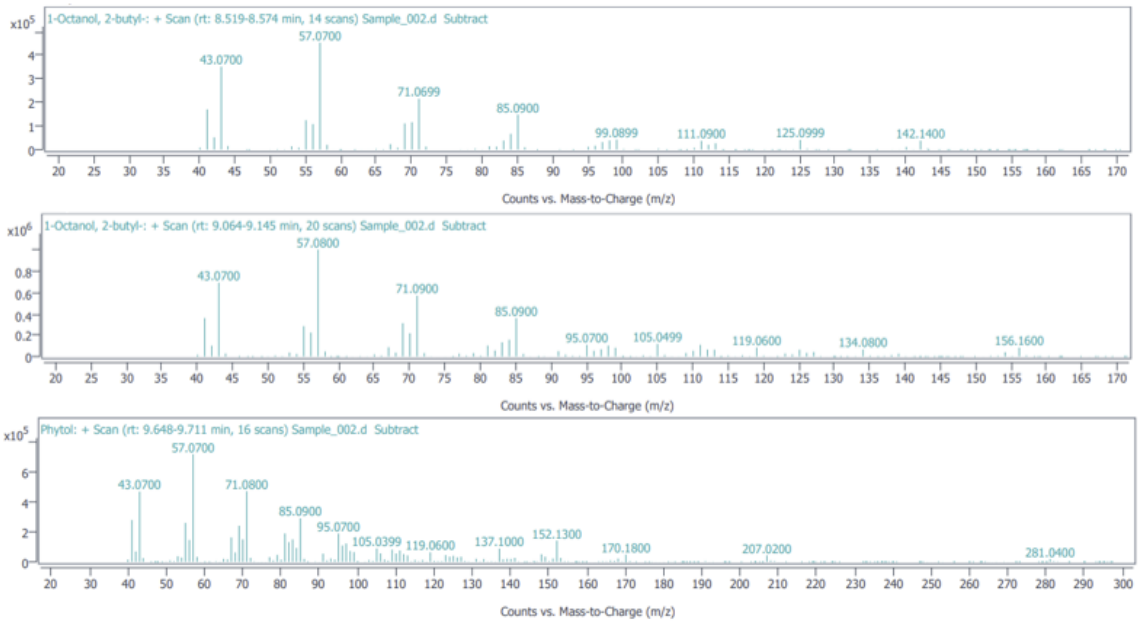


Figure 3: Proset V30L reflux boiled solution, identified spectra in descending order

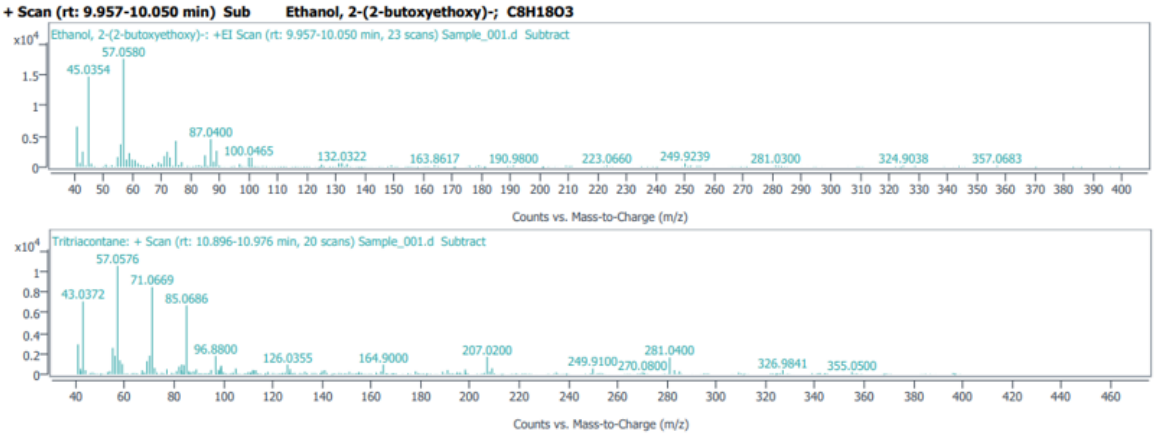


Figure 4: F44 Sensol cold solution, identified spectra in descending order

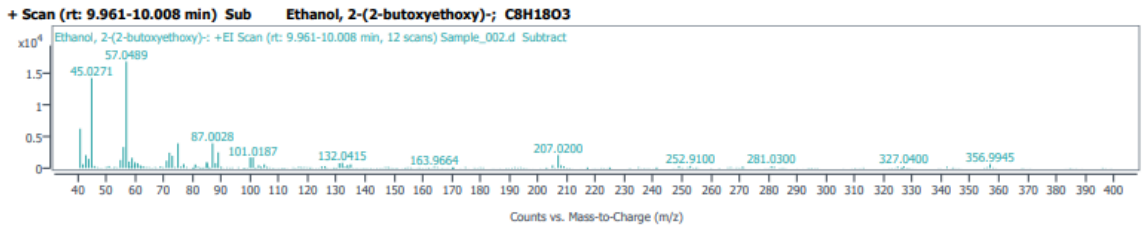


Figure 5: F44 Sensol reflux boiled identified spectrum

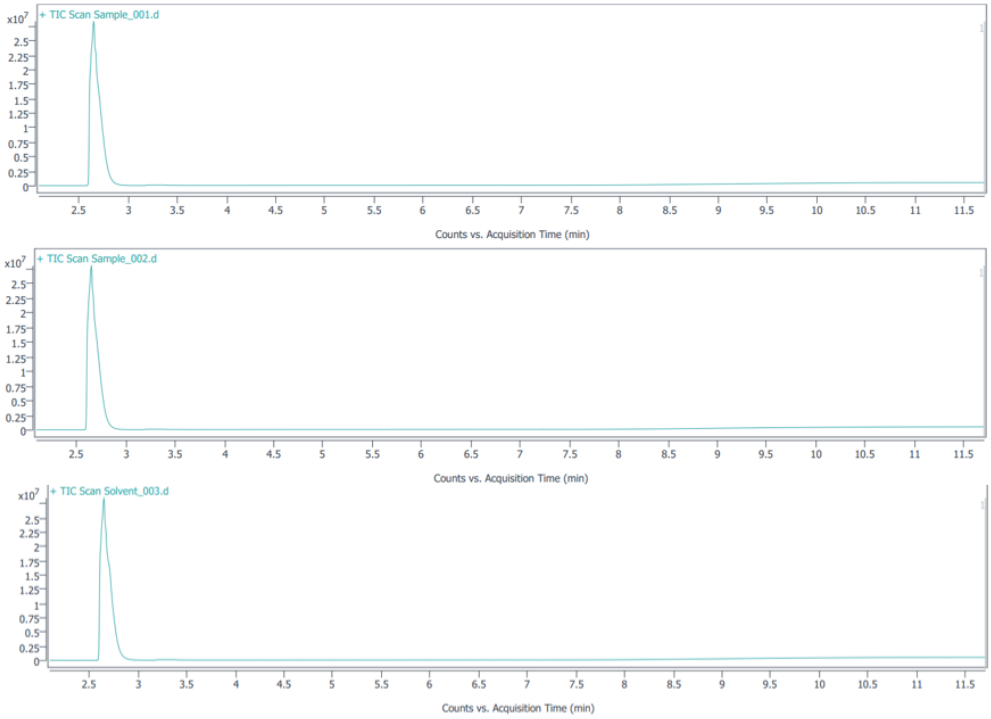


Figure 6: Proset S26E MS chromatograms of the cold, reflux boiled and solvent in descending order

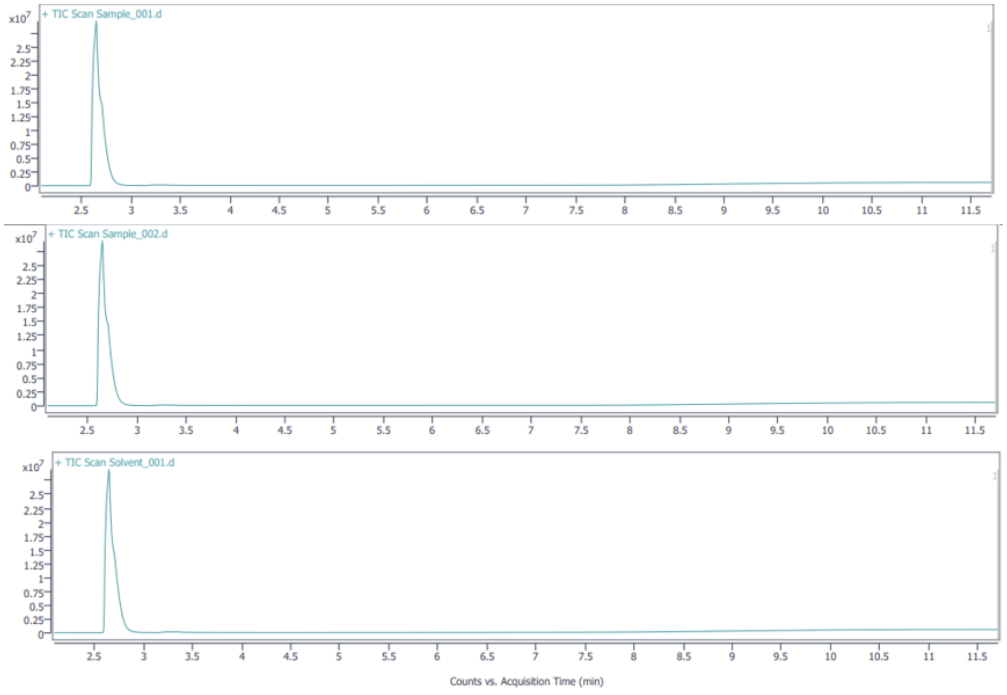
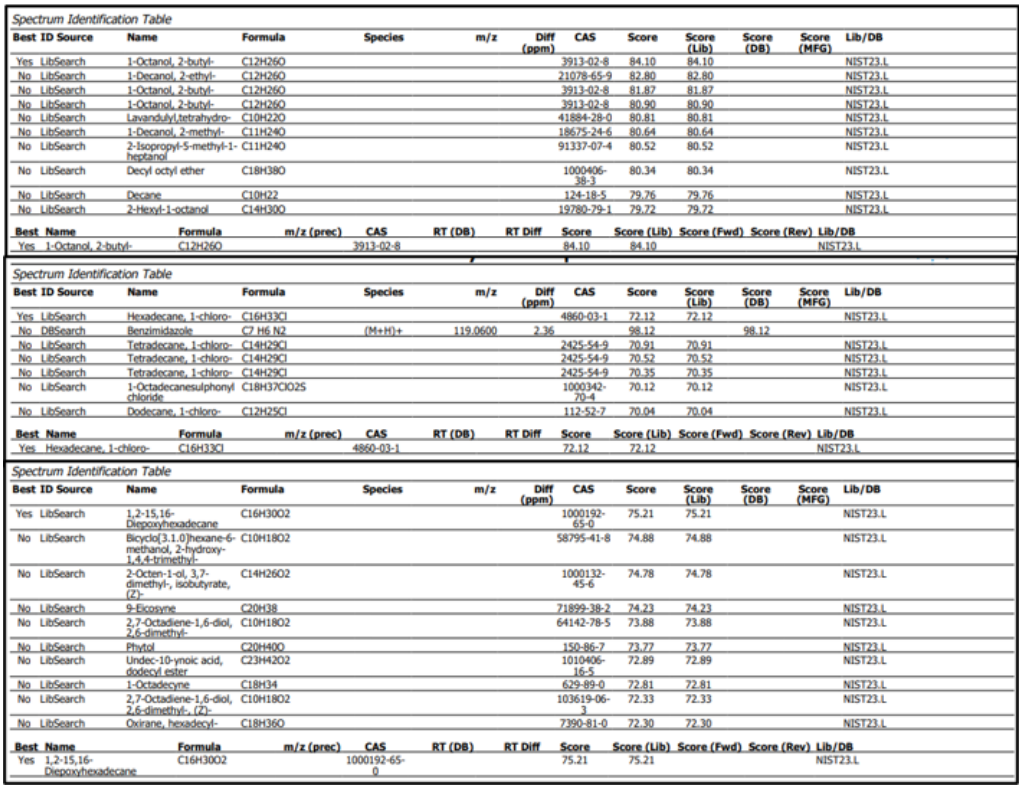


Figure 7: Proset S26H MS chromatograms of the cold, reflux boiled and solvent in descending order



Spectrum Identification Table

Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	1-Octanol, 2-butyl-	C12H26O				3913-02-8	84.10	84.10			NIST23.L
No	LibSearch	1-Decanol, 2-ethyl-	C12H26O				21078-65-9	82.80	82.80			NIST23.L
No	LibSearch	1-Octanol, 2-butyl-	C12H26O				3913-02-8	81.87	81.87			NIST23.L
No	LibSearch	1-Octanol, 2-butyl-	C12H26O				3913-02-8	80.90	80.90			NIST23.L
No	LibSearch	Lavandulyl,tetrahydro-	C10H22O				41884-28-0	80.81	80.81			NIST23.L
No	LibSearch	1-Decanol, 2-methyl-	C11H24O				18675-24-6	80.64	80.64			NIST23.L
No	LibSearch	2-Isopropyl-5-methyl-1-heptanol	C11H24O				91337-07-4	80.52	80.52			NIST23.L
No	LibSearch	Decyl octyl ether	C18H38O				1000406-38-3	80.34	80.34			NIST23.L
No	LibSearch	Decane	C10H22				124-18-5	79.76	79.76			NIST23.L
No	LibSearch	2-Hexyl-1-octanol	C14H30O				19780-79-1	79.72	79.72			NIST23.L

Best	Name	Formula	m/z (prec)	CAS	RT (DB)	RT Diff	Score	Score (Lib)	Score (Fwd)	Score (Rev)	Lib/DB
Yes	1-Octanol, 2-butyl-	C12H26O		3913-02-8			84.10	84.10			NIST23.L

Spectrum Identification Table

Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	Hexadecane, 1-chloro-	C16H33Cl				4860-03-1	72.12	72.12			NIST23.L
No	DBSearch	Benzimidazole	C7 H6 N2	(M+H)+	119.0600	2.36		98.12		98.12		
No	LibSearch	Tetradecane, 1-chloro-	C14H29Cl				2425-54-9	70.91	70.91			NIST23.L
No	LibSearch	Tetradecane, 1-chloro-	C14H29Cl				2425-54-9	70.52	70.52			NIST23.L
No	LibSearch	Tetradecane, 1-chloro-	C14H29Cl				2425-54-9	70.35	70.35			NIST23.L
No	LibSearch	1-Octadecanesulphonyl chloride	C18H37ClO2S				1000342-70-4	70.12	70.12			NIST23.L
No	LibSearch	Dodecane, 1-chloro-	C12H25Cl				112-52-7	70.04	70.04			NIST23.L

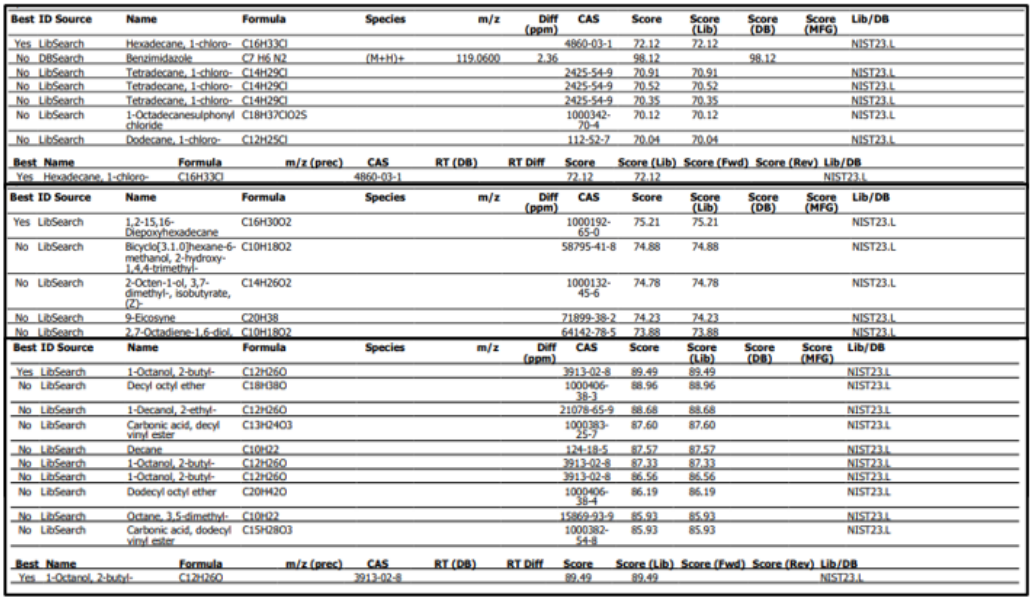
Best	Name	Formula	m/z (prec)	CAS	RT (DB)	RT Diff	Score	Score (Lib)	Score (Fwd)	Score (Rev)	Lib/DB
Yes	Hexadecane, 1-chloro-	C16H33Cl		4860-03-1			72.12	72.12			NIST23.L

Spectrum Identification Table

Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	1,2-15,16-Diepoxyhexadecane	C16H30O2				1000192-65-0	75.21	75.21			NIST23.L
No	LibSearch	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	C10H18O2				58795-41-8	74.88	74.88			NIST23.L
No	LibSearch	2-Octen-1-ol, 3,7-dimethyl-, isobutyrate, (Z)-	C14H26O2				1000132-45-6	74.78	74.78			NIST23.L
No	LibSearch	9-Eicosyne	C20H38				71899-38-2	74.23	74.23			NIST23.L
No	LibSearch	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	C10H18O2				64142-78-5	73.88	73.88			NIST23.L
No	LibSearch	Phytol	C20H40O				150-86-7	73.77	73.77			NIST23.L
No	LibSearch	Undec-10-ynoic acid, dodecyl ester	C23H42O2				1010406-16-5	72.89	72.89			NIST23.L
No	LibSearch	1-Octadecyne	C18H34				629-89-0	72.81	72.81			NIST23.L
No	LibSearch	2,7-Octadiene-1,6-diol, 2,6-dimethyl-, (Z)-	C10H18O2				103619-06-3	72.33	72.33			NIST23.L
No	LibSearch	Oxirane, hexadecyl-	C18H36O				7390-81-0	72.30	72.30			NIST23.L

Best	Name	Formula	m/z (prec)	CAS	RT (DB)	RT Diff	Score	Score (Lib)	Score (Fwd)	Score (Rev)	Lib/DB
Yes	1,2-15,16-Diepoxyhexadecane	C16H30O2		1000192-65-0			75.21	75.21			NIST23.L

Figure 8: Proset V30L cold solution NIST library results of identified peaks in descending order



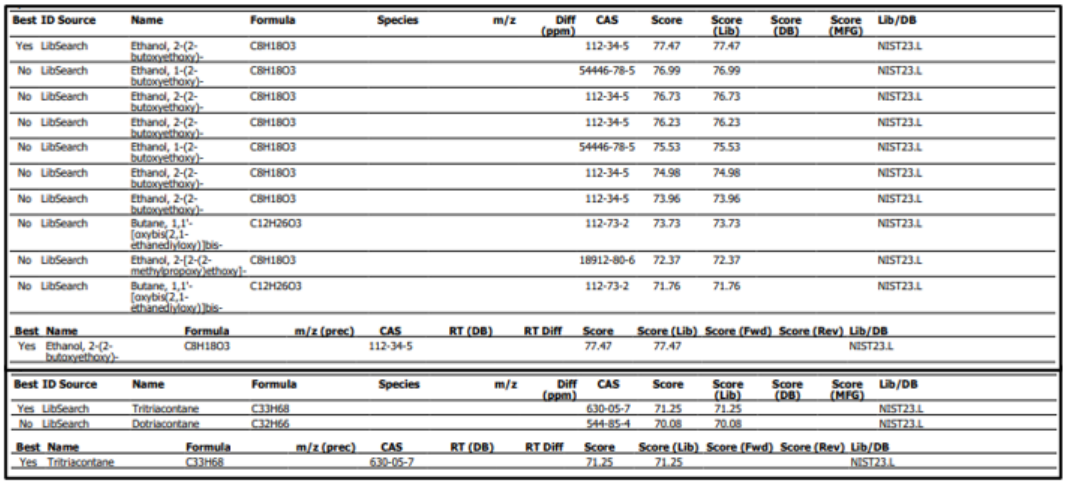
Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	Hexadecane, 1-chloro-	C16H33Cl				4860-03-1	72.12	72.12			NIST23.L
No	DBSearch	Benzimidazole	C7 H6 N2	(M+H)+	119.0600	2.36		98.12		98.12		
No	LibSearch	Tetradecane, 1-chloro-	C14H29Cl				2425-54-9	70.91	70.91			NIST23.L
No	LibSearch	Tetradecane, 1-chloro-	C14H29Cl				2425-54-9	70.52	70.52			NIST23.L
No	LibSearch	Tetradecane, 1-chloro-	C14H29Cl				2425-54-9	70.35	70.35			NIST23.L
No	LibSearch	1-Octadecanesulphonyl chloride	C18H37ClO2S				1000342-70-4	70.12	70.12			NIST23.L
No	LibSearch	Dodecane, 1-chloro-	C12H25Cl				112-52-7	70.04	70.04			NIST23.L

Best	Name	Formula	m/z (prec)	CAS	RT (DB)	RT Diff	Score	Score (Lib)	Score (Fwd)	Score (Rev)	Lib/DB
Yes	Hexadecane, 1-chloro-	C16H33Cl		4860-03-1			72.12	72.12			NIST23.L

Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	1,2-15,16-Diepoxyhexadecane	C16H30O2				1000192-65-0	75.21	75.21			NIST23.L
No	LibSearch	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	C10H18O2				58795-41-8	74.88	74.88			NIST23.L
No	LibSearch	2-Octen-1-ol, 3,7-dimethyl-, isobutyrate, (Z)-	C14H26O2				1000132-45-6	74.78	74.78			NIST23.L
No	LibSearch	9-Eicosyne	C20H38				71899-38-2	74.23	74.23			NIST23.L
No	LibSearch	2,7-Octadiene-1,6-diol,	C10H18O2				64142-78-5	73.88	73.88			NIST23.L
Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	1-Octanol, 2-butyl-	C12H26O				3913-02-8	89.49	89.49			NIST23.L
No	LibSearch	Decyl octyl ether	C18H38O				1000406-38-3	88.96	88.96			NIST23.L
No	LibSearch	1-Decanol, 2-ethyl-	C12H26O				21078-65-9	88.68	88.68			NIST23.L
No	LibSearch	Carbonic acid, decyl vinyl ester	C13H24O3				1000383-25-7	87.60	87.60			NIST23.L
No	LibSearch	Decane	C10H22				124-18-5	87.57	87.57			NIST23.L
No	LibSearch	1-Octanol, 2-butyl-	C12H26O				3913-02-8	87.33	87.33			NIST23.L
No	LibSearch	1-Octanol, 2-butyl-	C12H26O				3913-02-8	86.56	86.56			NIST23.L
No	LibSearch	Dodecyl octyl ether	C20H42O				1000406-38-4	86.19	86.19			NIST23.L
No	LibSearch	Octane, 3,5-dimethyl-	C10H22				15869-93-9	85.93	85.93			NIST23.L
No	LibSearch	Carbonic acid, dodecyl vinyl ester	C15H28O3				1000382-54-8	85.93	85.93			NIST23.L

Best	Name	Formula	m/z (prec)	CAS	RT (DB)	RT Diff	Score	Score (Lib)	Score (Fwd)	Score (Rev)	Lib/DB
Yes	1-Octanol, 2-butyl-	C12H26O		3913-02-8			89.49	89.49			NIST23.L

Figure 9: Proset V30L reflux boiled solution NIST library results of identified peaks in descending order



Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	Ethanol, 2-(2-butoxyethoxy)-	C8H18O3				112-34-5	77.47	77.47			NIST23.L
No	LibSearch	Ethanol, 1-(2-butoxyethoxy)-	C8H18O3				54446-78-5	76.99	76.99			NIST23.L
No	LibSearch	Ethanol, 2-(2-butoxyethoxy)-	C8H18O3				112-34-5	76.73	76.73			NIST23.L
No	LibSearch	Ethanol, 2-(2-butoxyethoxy)-	C8H18O3				112-34-5	76.23	76.23			NIST23.L
No	LibSearch	Ethanol, 1-(2-butoxyethoxy)-	C8H18O3				54446-78-5	75.53	75.53			NIST23.L
No	LibSearch	Ethanol, 2-(2-butoxyethoxy)-	C8H18O3				112-34-5	74.98	74.98			NIST23.L
No	LibSearch	Ethanol, 2-(2-butoxyethoxy)-	C8H18O3				112-34-5	73.96	73.96			NIST23.L
No	LibSearch	Butane, 1,1'-[oxybis(2,1-ethanediyloxy)]bis-	C12H26O3				112-73-2	73.73	73.73			NIST23.L
No	LibSearch	Ethanol, 2-[2-(2-methylpropoxy)ethoxy]-	C8H18O3				18912-80-6	72.37	72.37			NIST23.L
No	LibSearch	Butane, 1,1'-[oxybis(2,1-ethanediyloxy)]bis-	C12H26O3				112-73-2	71.76	71.76			NIST23.L

Best	Name	Formula	m/z (prec)	CAS	RT (DB)	RT Diff	Score	Score (Lib)	Score (Fwd)	Score (Rev)	Lib/DB
Yes	Ethanol, 2-(2-butoxyethoxy)-	C8H18O3		112-34-5			77.47	77.47			NIST23.L

Best	ID Source	Name	Formula	Species	m/z	Diff (ppm)	CAS	Score	Score (Lib)	Score (DB)	Score (MFG)	Lib/DB
Yes	LibSearch	Tritriacontane	C33H68				630-05-7	71.25	71.25			NIST23.L
No	LibSearch	Dotriacontane	C32H66				544-85-4	70.08	70.08			NIST23.L

Best	Name	Formula	m/z (prec)	CAS	RT (DB)	RT Diff	Score	Score (Lib)	Score (Fwd)	Score (Rev)	Lib/DB
Yes	Tritriacontane	C33H68		630-05-7			71.25	71.25			NIST23.L

Figure 10: F44 sensol cold solution NIST library results of identified peaks in descending order