# Analysis of Test Mixture and Water Contaminants Using Advanced Chromatographic Techniques

Perizat Abdigali

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Robert E. Synovec
Dan Fu

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**Abstract**

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Perizat Abdigali

Chair of the Supervisory Committee:

Robert E. Synovec

Department of Chemistry

This thesis work highlights Solid Phase Extraction (SPE) coupled with Intuvo 9000 Gas Chromatography (GC), comprehensive two-dimensional (2D) gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS), and one-dimensional gas chromatography-mass spectrometry (GC-qMS) to study the chemical components of a Test Mixture and contaminated by a diesel water sample. Diesel compounds were found during the first step of the analysis on one-dimensional GC-qMS. However, due to significant peak overlap, the compound resolution and identification were limited. GC×GC-TOFMS was used to analyze samples and its SPE extracts to overcome those limitations.

During the analysis of the Test Mixture, a consistent temperature program and other conditions were adjusted on GC-qMS developed method. Nonadecane peak intensities were used as an

example compound to find the limit of detection (LOD), which produces detection ability at low concentrations, and gave an instrumental practical value of 4 ppm.

To analyze changes and determine which compounds were most reduced or eluted during the extraction, chemometric techniques such as Principal Analysis (PCA) and tile-based F statistic ratio (F-ratio) analysis were used. A significant change in analytes was shown by the separation of the extracts and original neat sample by PCA. To reduce noise and correct retention time shifts, the chromatograms with more than 2500 differential areas were reduced to around 1216 significant features using F-ratio analysis.

A detailed analysis of a typical peak at mass-to-charge ratio 120 discovered that the Extracts had a weaker signal intensity than the Original Neat Diesel Sample, which shows partial compound loss after extraction. When comparing the real laboratory data with the NIST library mass spectrum, it has strong match across significant fragment ions, confirming the identification of 1-ethyl-2methylbenzene.

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## ACKNOWLEDGEMENTS

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Last but not least, I want to thank my family. My parents, You gave me everything so I could pursue my dream and grow as a strong person. My brother, you were always ready to listen and support me. My husband, you are my best friend and were not afraid to move overseas, leaving behind everything you had, just because you believed in me. My daughter, you are the reason why I am on this way. I want you to always follow your dreams like your mom.

## DEDICATION

*To my dear parents, Kanat Begassilov and Lyazzat Toishybayeva, my brother Yerkebulan,*

*my husband Kanat and daughter Ayala.*

*Without YOU by my side and YOUR endless support, I could not be who I am today.*

# Chapter 1. Overview of Gas Chromatography

## 1.1 Introduction to Gas Chromatography

Gas Chromatography (GC) is an analytical technique to separate and analyze volatile, semi-volatile compounds due to differences in how analytes distribute through the stationary phase by carrier gas [1]. GC is one of the widely accepted analytical tools in chemical research because of the combination of analytical precision, ease of use, and effectiveness in separating volatile compounds. According to chemical and physical characteristics, the components of a test mixture pass through the stationary phase at various speed leading to their separation [2].

There are different forms of chromatography. These types are classified according to the mobile and physical states of stationary phases: Liquid Chromatography (LC), Gas Chromatography (GC), and Thin-layer Chromatography (TLC). GC is best for the analysis of volatile and semi-volatile substances because it offers high efficiency, short analysis times, and a high level of sensitivity. These benefits allow the use of this technique in such fields as pharmaceutical, environmental, forensic, and petrochemical [3]. Gas Chromatography originated by Martin and James in 1952 and is based on the use of a long, narrow packed column with a solid adsorbent like alumina or silica gel [4].

This study is about the application of gas chromatography for the analysis of a test mixture consisting of about 76 compounds, using the Agilent Intuvo 9000 GC system. The main components of a gas chromatographic process are carrier gas – most commonly helium, nitrogen, or hydrogen gases – an injection system, a separation column, and a detector. After being vaporized in the injection slot, the analytes are being transported by carrier gas through the column, where their molecular interactions with the stationary phase cause them to separate. Mass Spectrometry

(MS) or Flame Ionization Detection (FID), depending on the analytical requirements, are used for identification[5]. Additionally, the effectiveness of sample preparation techniques for complex ones is tested using solid-phase extraction (SPE) using alumina and silica cartridges.

## 1.2 Fundamentals of Gas Chromatography Separation of Complex Samples

The gas chromatography separation method divides analytes between stationary liquid or solid phases coated within a capillary column and a mobile gas phase, as shown in Figure 1. Driven by changes in boiling temperatures, numerous interactions with the stationary phase and polarity, some separate components migrate through the column at different speeds as the sample is added into the system [3].

Many important parameters affect GC separation efficiency, such as stationary phase composition, column size, and column temperature. Providing a suitable resolution between overlapping peaks in complex test mixes with dozens of analytes, such as a test mix with about 76 components combination used in this work, depends on optimizing the parameters. One of the often-used techniques to increase the analyte separation over a range of instability or overlapping is temperature programming. So, early eluting compounds can be separated at lower temperatures. The later-eluting compounds with higher boiling points can be resolved more clearly as the temperature progressively rises in the column oven, instead of keeping a constant temperature during the run. This leads to shortening and more efficient analysis time and improves peak form [6].

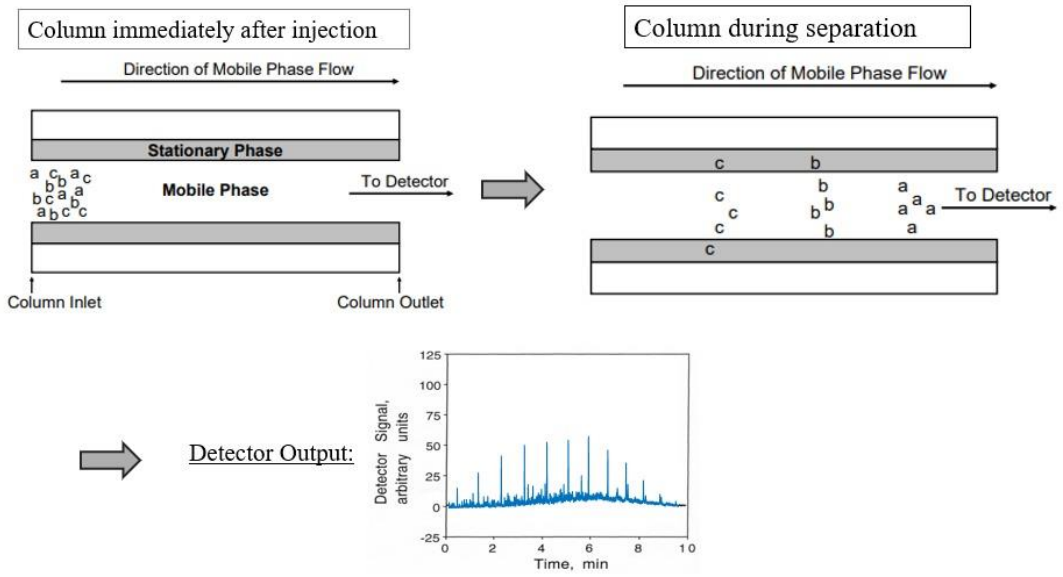



**Figure 1.** Fundamentals of Gas Chromatography: Separation Process of Complex Samples.

Chromatographic successful performance is mostly dependent on the choice of carrier gas, and because of its inertness and efficiency, helium has always been the most popular among other carrier gases. In other cases, with a different column dimension or desired resolution, hydrogen or nitrogen gases are also being used as carrier gases. The gas should have a steady and ideal linear velocity if the aim is to maximize effective mass transfer while minimizing band broadening.

Alternatively, obtaining baseline separation from complex mixtures fully depends on column selectivity – the type of the analytes gives us an idea to choose polar or semi-polar stationary phases. When single-column separation is not enough, more specifically for samples with many structurally related compounds, multidimensional techniques such as complete two-dimensional gas chromatography (GC×GC) are used [7].

Generally, the quantitative measurements of separation quality include theoretical plate count (N), selectivity (α), and resolution (Rs), which provide an understanding of the system

performance and support the ways to improve the technique. This work shows the usage of column temperature ramping, carrier gas flow modification, and column selection that works for a broad spectrum of semi-volatile compounds to improve the separation of the Test Mixture. We are aiming to develop a GC method by getting repeatable and high-resolution separation, which we need for further quantification and to identify the detection limits.

### 1.3 Intuvo 9000 Gas Chromatography System

An Agilent Intuvo 9000 series Gas Chromatography (GC) system is a modern analytical tool of choice, which was developed mostly to improve analytical performance of samples, operating efficiency, and mainly, comfortable usability to simplify daily routine in a laboratory. Difficult analytical applications such as environmental pollution identification, finding organic compound traces, and any industrial quality monitoring, as well as high-throughput laboratories, find a perfect fit with Intuvo 9000 GC [5].

The Intuvo 9000 has a ferrule-free flow channel compared to the traditional GC system, as shown in Figure 2. The system uses Intuvo Smart Connections – leak-free sealing via metal-on-metal contact, minimizing dead volume – avoiding the use of usual conventional column nuts and ferrules. This decision guarantees significantly reduced downtime relative to column maintenance and reproducibility over column setups [5]. A low thermal mass column allows rapid heating and cooling processes, and it is resulting in more effective temperature programming and faster cycle times.

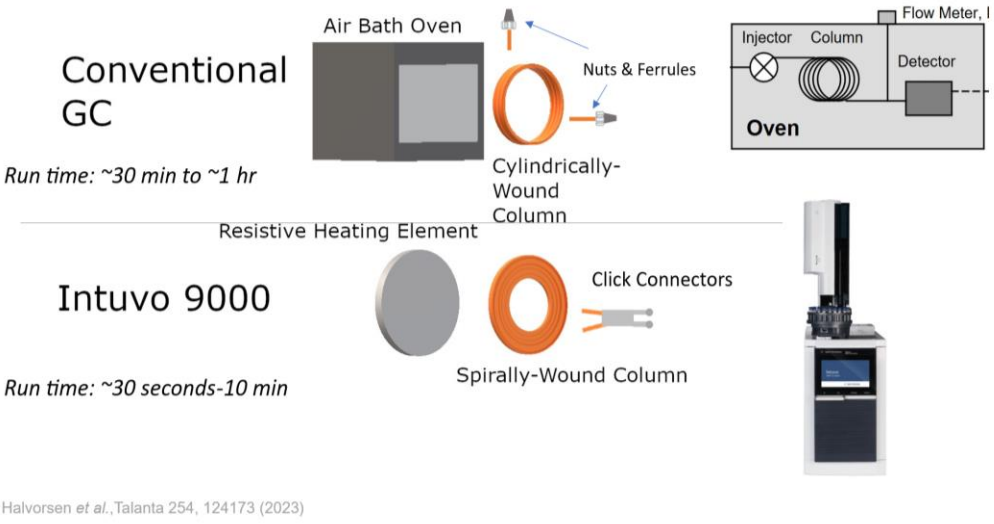



**Figure 2.** Illustration of differences between column heating in a traditional GC system and the Intuvo 9000 GC. Contrasting the air bath oven with the resistive heating element.

The Intuvo 9000 modular design is the most impressive element among all others. Faster method setup and traceability are possible because of the instant recognition of Smart ID components like columns and liners. Furthermore, the system works naturally with Agilent`s Open Lab CDS (chromatography data system), boosting data management and automation [8]. Generally, the Agilent Intuvo 9000 is analytically compatible with multiple types of detectors, including FID and MS. In this project, Intuvo 9000 is used with a FID to properly measure the Test Mixture compounds, which was chosen for its robustness and most importantly for its sensitivity in hydrocarbon analysis. All these properties make Intuvo 9000 GC a unique platform to analyze separation performance and LOD in complicated environmental mixtures.

### 1.4 One-Dimensional and Comprehensive Two-Dimensional Gas Chromatography Time-Of-Flight Mass Spectrometry Systems

Analytical chemists nowadays use more complex separation techniques rather than regular gas chromatography because industrial and environmental samples are becoming more and more complex. Gas Chromatography uses a long, narrow column packed with alumina or silica solid

adsorbent material. The Test Mixture is injected into the column, where helium or nitrogen carrier gas carries the sample through the column. As the mixture travels through, the compounds interact with the adsorbent and separate according to their polarity and boiling point chemical properties. After that, the separated products are sent to the detector. A computer analyzes the signal proportional to the concentration of each component in the mixture from the detector and converts it into the chromatogram.

For the separation of more complex compounds, using traditional one-dimensional gas chromatography with quadrupole mass spectrometry (GC-qMS), even though it has been used to analyze mixtures of volatile and semi-volatile substances, is not enough. As the number of analytes increases, the co-elution process is more likely to happen, which occurs when multiple compounds leave the column simultaneously, causing overlapping signals with resolution loss. This is mostly obvious while analyzing wastewater, fuel, and other industrial wastes, where the matrix can mask or cover low-abundance chemicals [9].

The peak capacity of one-dimensional GC is limited by being dependent on a single retention mechanism, which is dependent on column interactions and volatility. Methods are not always able to resolve molecules with the same physical and chemical properties, even with better temperature programming. The one-dimensional typical separation process can be seen in Figure 3, where overlapping peaks from unresolved components make it more difficult to distinguish as a separate peak, particularly in multicomponent mixture total ion chromatograms (TICs). It increases the need to create more advanced multidimensional techniques.

One of the multidimensional techniques is comprehensive two-dimensional gas chromatography (GC×GC). The standard schematic of the GC×GC instrument can be seen in Figure 4, and it shows how it separates alkanes, cycloalkanes, and aromatics into structured groups of compounds, in

contrast to the 1D-GC, where compounds appear unresolvable. Generally, GC×GC increases separation resolution and efficiency of the analysis, as schematically shown in Figure 5 as well. The flow path starts from the inlet part and goes to the first-dimensional ($^1$D) column, which is the same as a 1D-GC system. Two orthogonal conditioned columns: first-dimensional ($^1$D) column and second-dimensional ($^2$D) column are connected by a modulator – the "heart" of the system, which periodically traps the first column`s eluate and reinjects a large number of small fractions with lower frequency into the $^2$D column [10]. The separation happens in this column as fast as 1 to 10 seconds only, compared to the $^1$D separation, which is typically 45-120 minutes [11].

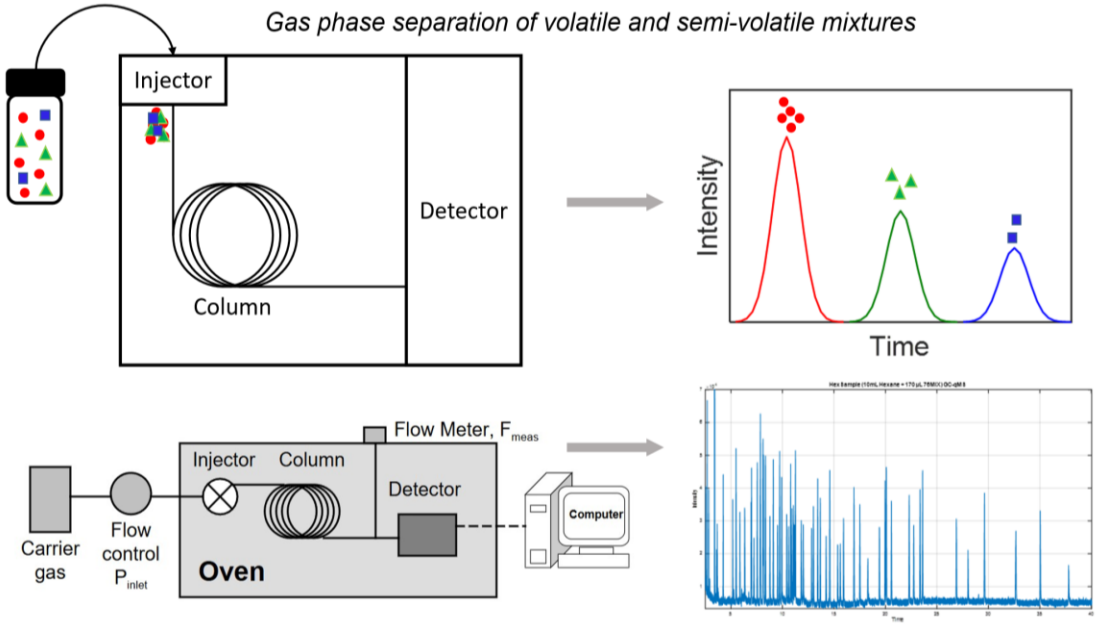



**Figure 3.** Quadrupole Mass Spectrometry (GC-qMS). The principle of complex sample separation in one-dimensional gas chromatography. Separations are mostly based on the boiling point of compounds.

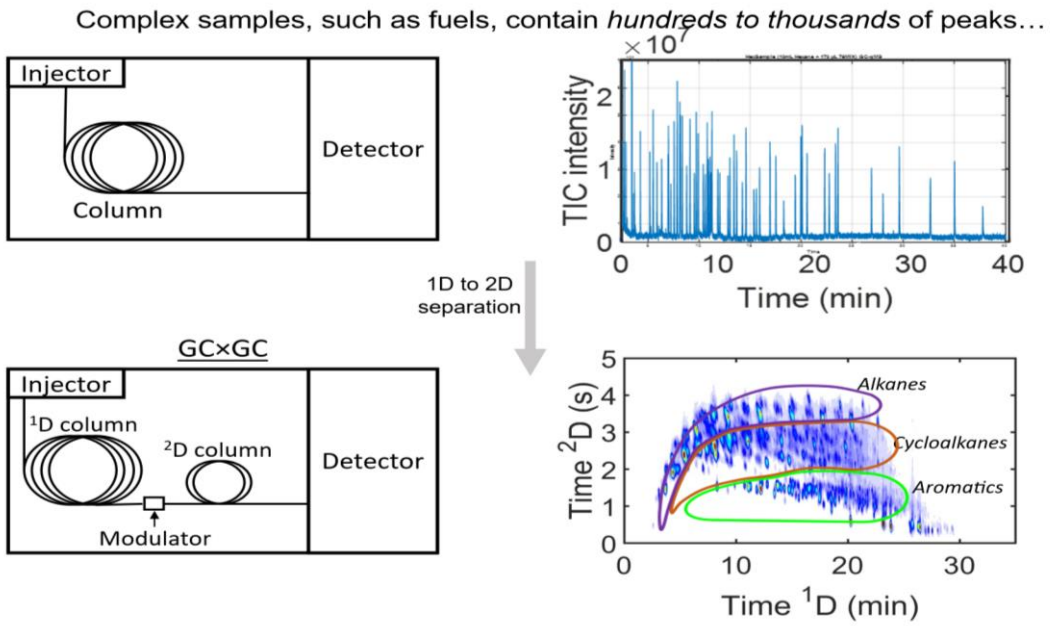



**Figure 4.** Limitations of GC-qMS and need for GC×GC-TOFMS. Complex samples contain hundreds to thousands of peaks, and most analytes are *unresolved* with 1D-GC-qMS. With the help of two-dimensional chromatography, clusters of compounds like alkanes, cycloalkanes, and aromatics can be separated.

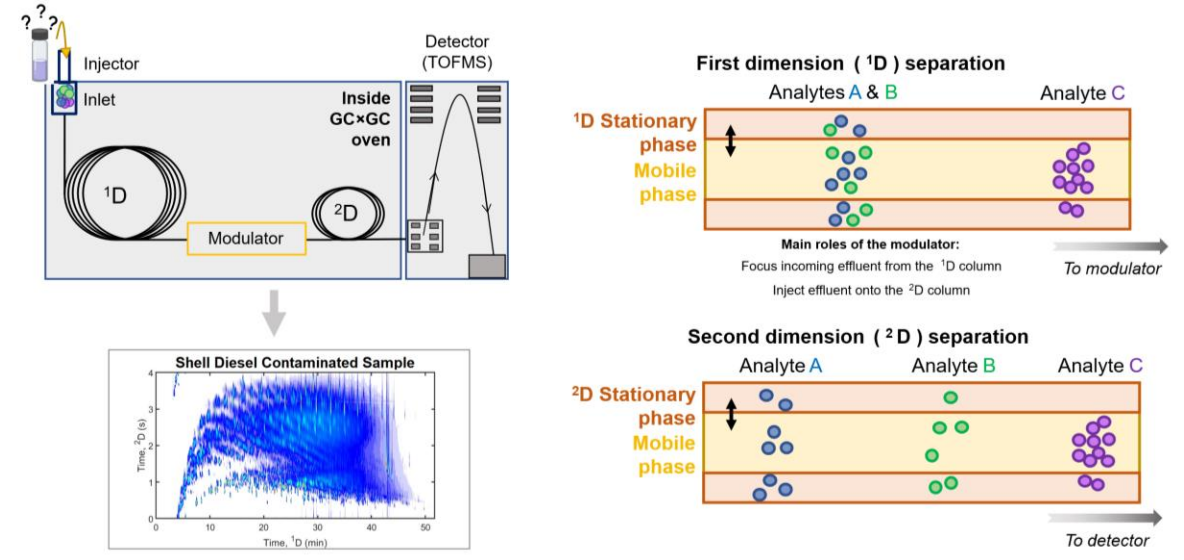



**Figure 5.** Comprehensive Two-Dimensional Gas Chromatography (GC × GC-TOFMS). The principle of complex sample separation in two-dimensional gas chromatography. An alternative approach for increasing the chromatographic resolution of volatile mixtures.

Usually, GC×GC is presented with a normal column configuration where the first-dimensional column is non-polar and the polar one in the second-dimensional column; this difference helps to achieve the orthogonality between column conditions. But for the more complex samples, such as fuel, wastewater, or in cases where other lower polarity compounds are targeted, a reverse column configuration is favorable. It allows us to see alkanes at the top of the 2D separation time with a low retention on the $^{2}$D column, aromatic compounds at the bottom of the 2D separation with a high retention on the $^{2}$D column, and cycloalkanes appear between alkanes and aromatics [11].

When GC×GC combines with time-of-flight mass spectrometry (TOFMS), it allows quick spectrum capture without losing resolution. TOFMS can identify sample compounds by stating how long every molecule flies in a drift region [12]. TOFMS can capture small peak widths in the second dimension as little as 0.5 seconds and help to provide accurate mass data for the chemical identification of the compound in a database.

### 1.5 Solid-Phase Extraction (SPE). Choosing the cartridge type.

Sample preparation is one of the most important parts of the whole analysis. The Solid Phase Extraction (SPE) helps to isolate, concentrate, and properly clean up the sample before chromatographic analysis. SPE involves the use of disposable cartridges [14] and increases the sensitivity of the technique and minimizes unwanted interferences, which is helpful for the trace level analysis of environmental contaminants. During analysis of the low-abundance chemicals in complex structures, just like wastewater, river water, or industrial pollutants, the performance of the chromatographic method is as good as the sample preparation step that leads to it. Short analysis time, ease of automation, and lowering the organic compounds concentration are counted as the main benefits of the SPE [13].

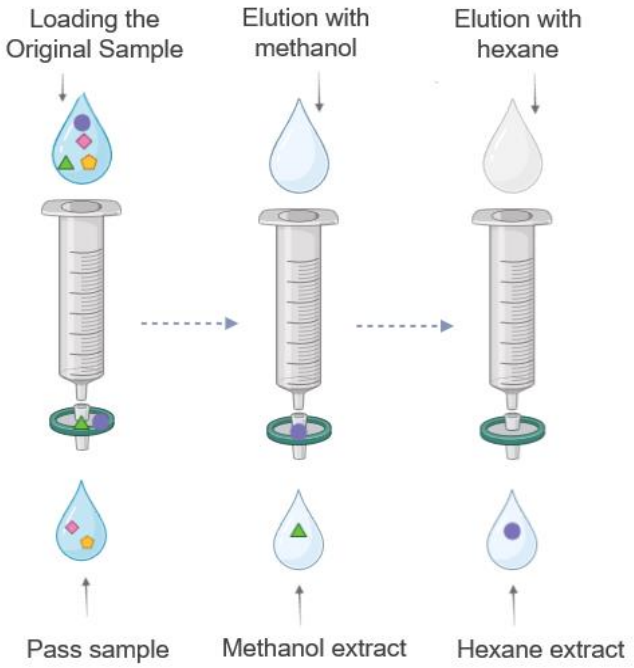



**Figure 6A.** Solid-Phase Extraction (SPE) process. Shows the contaminated sample Loading Step and the Elution Steps with methanol and hexane.

The idea of SPE, as can be seen in Figure 6A, is to analyze the partition between the sample and the sorbent. The sorbent can be performed in different ways: as a syringe, a cartridge, or discs (Figure 6B). The process usually includes the following steps: conditioning (solving the sorbent to interfere with the sample better), loading (desired species stick to the sorbent and the rest goes to the pass sample), drying (to evaporate extra water), the first elution (with a more polar solution), and the second elution (with a more non-polar solution). When the test mixture passes through the cartridge, non-targeted compounds (Pass samples) are washed away, and targeted analytes can be retained on the surface of the sorbent [13]. As for the second step, a small amount of solvent (such as methanol or water) is used to elute the targeted compounds after extra water evaporates, and the cartridge has dried. It makes the sample cleaner and more compatible for GC analysis. This step

can be repeated with different stronger non-polar solvents (as hexane, dichloromethane) to get more information from the targeted compound mixture.

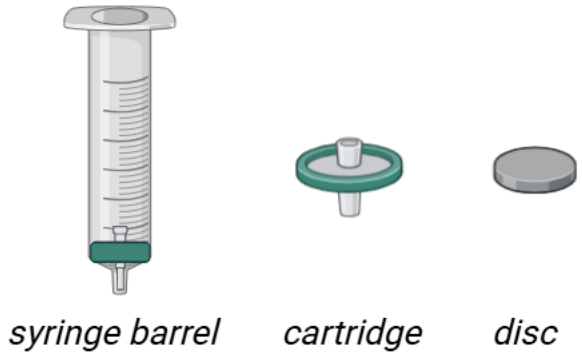



**Figure 6B.** Disposable sorbent container types.

The optimal SPE cartridge choice depends on the chemical properties of the targeted analytes and the complexity of the analyte sample. The most popular types of sorbents are reverse-phase, normal-phase, and ion-exchange [13]. Silica or alumina are examples of normal-phase sorbents and work with polar compounds. C-18 or C-8 are reversed-phase sorbents and separate well non-polar molecules. As ion-exchange sorbents come into use, it mostly deals with charged analytes like bases, acids, or ionic pollutants [14].

# Chapter 2. Analysis of Test Mixture solution and determination of the Limit of Detection using Intuvo 9000 Gas Chromatography

## 2.1 Overview of the analysis

This chapter illustrates the experimental analysis of the Test Mixture solution, which consists of volatile and semi-volatile organic pollutants with various chemical classes like alcohols, alkanes, ketones, esters, and others, can be seen in Table 1, including more than 70 components. The overview of the analysis process is shown below in Figure 7. First of all, the Standard Test Mixture preparation step, followed by serial dilution of this sample to determine the LOD for a particular sample and evaluate the instrument sensitivity over a wide concentration range; then chromatographic technique optimization process, data interpretation, and, lastly, analysis and calculations to figure out the LOD for the mixture.

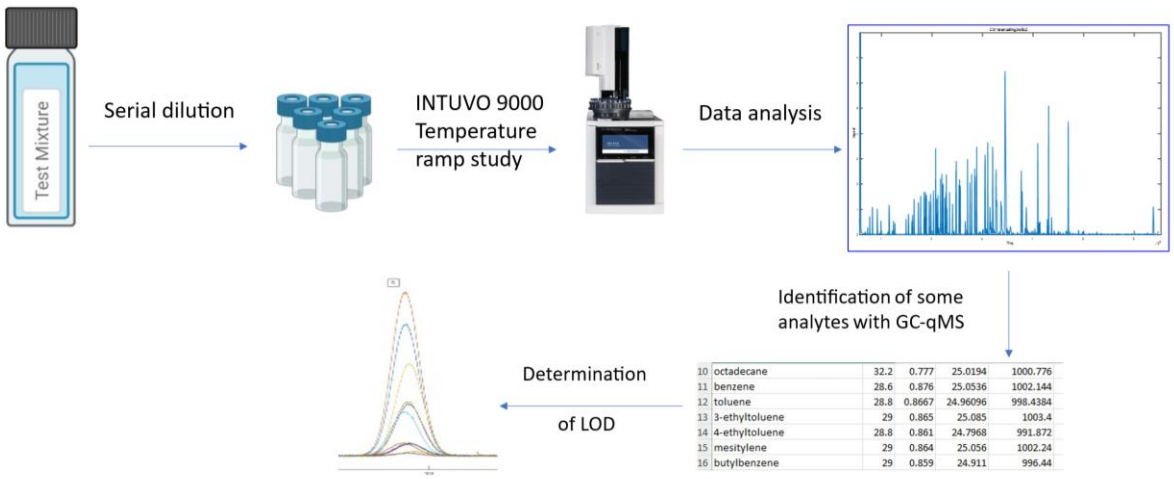



**Figure 7.** Overview for analyzing a Test mixture using Intuvo 9000 GC and GC-qMS. The procedure includes serial dilution of the mixture and followed by a temperature ramp study with the Agilent Intuvo 9000 GC. The GC-qMS was used to identify analytes, and the respective limits of detection (LOD) were determined during data analysis.

For better separation and to improve retention time distribution on Intuvo 9000 GC, the temperature optimizations were included at the beginning of the analysis. As a supporting analysis instrument and to identify several compounds, using GC-qMS helped to improve the result.

## 2.2 Experimental

### 2.2.1 Sample preparation. Serial dilution of Test Mixture solution

To start the experiment, a standard sample was prepared with the combination of over 70 analytes made in methanol, the list of chemicals shown in Table 1. The serial dilution was made from the 1000 ppm initial test solution. 0.5 mL of the current mixture was combined with 0.5 mL of HPLC quality methanol in a 1: 1 ratio to get the next solution with the lower concentration. The lower concentrations prepared from this progressive dilution were 500 ppm, 250 ppm, and 125 ppm with the same method. To avoid contamination after each dilution, the 2 mL GC vials were covered with the cap right away. Figure 8 shows the schematic process of serial dilution. To minimize the degradation, all samples were stored in a cold, dark place in amber vials.

| **Alkanes** | Conc (ppm) | **Esters** | Conc (ppm) | **Cyclics** | Conc (ppm) |
|---|---|---|---|---|---|
| heptane | 592 | ethyl formate | 600 | methylcyclopentane | 536 |
| octane | 700 | methyl decanoate | 940 | cyclooctane | 764 |
| decane | 624 | methyl caprylate | 824 | butylcyclohexane | 664 |
| undecane | 660 | methyl salicylate | 1056 | bicyclohexyl | 19.2 |
| dodecane | 688 | ethyl salicylate | 1040 | **Halogenated Alkanes** | |
| tetradecane | 736 | methyl laurate | 776 | 1,5-dichloropentane | 1000 |
| hexadecane | 792 | methyl caproate | 800 | 1-chlorohexane | 928 |
| pristane | 724 | **Ketones** | | 1-bromohexane | 865 |
| octadecane | 808 | 2-hexanone | 724 | 1-bromoheptane | 1052 |
| nonadecane | 496 | 2-heptanone | 780 | 1-bromooctane | 1052 |
| eicosane | 852 | 3-heptanone | 784 | carbon tetrachloride | 800 |

| **Aromatics** | | 3-octanone | 764 | 1-iodo-2-methylpropane | 1440 |
|---|---|---|---|---|---|
| benzene | 748 | 2-decanone | 876 | **Organosulfur compounds** | |
| toluene | 832 | 2-undecanone | 728 | 1-decanethiol | 820 |
| 3-ethyltoluene | 796 | 2-dodecanone | 748 | 2-thiophenemethylamine | 1092 |
| 4-ethyltoluene | 816 | **Alkenes** | | 2-propylthiophene | 936 |
| mesitylene | 796 | 1-hexene | 540 | 2-(methylthio)-pyridine | 968 |
| butylbenzene | 876 | 1-undecene | 640 | 1,4-thioxane | 1112 |
| tert-butyl benzene | 816 | **Alkynes** | | diphenylsulfide | 1088 |
| propylbenzene | 776 | 1-hexyne | 516 | 2-metylthiophene | 820 |
| 1-ethylnapthelene | 904 | 1-heptyne | 716 | benzo[b]thiaphene | 1908 |
| cyclohexylbenzene | 868 | 1-nonyne | 772 | 1-dodecanethiol | 712 |
| diphenylmethane | 936 | 5-decyne | 780 | 2-octanethiol | 864 |
| o-xylene | 852 | **Alcohols** | | 3-methylthiophene | 884 |
| dibutyl phthalate | 1264 | 1-butanol | 740 | 2-methylthianapthene | 1480 |
| diethyl phthalate | 904 | 1-pentanol | 748 | **Organophosphorus compounds** | |
| 2,2'-dimethylbiphenyl | 964 | 2-pentanol | 784 | dimethyl methyl phosphonate | 1204 |
| sec-butylbenzene | 772 | hexyl alcohol | 860 | diethyl methyl phosphonate | 872 |
| 4-phenyltoluene | 996 | 2-heptanol | 1072 | **Other** | |
| 9-methylanthracene | 624 | 1-octanol | 940 | (-)-alpha-pinene | 872 |
| anthracene | 788 | 1-nonanol | 752 | o-toluidine | 1304 |
| naphthalene | 1128 | 1-tetradecanol | 800 | (-)-linalool | 816 |
| 1,2,3,4-tetrahydronapthalene | 1752 | 1-eicosanol | 1160 | acetophenone | 904 |
| phenanthrene | 948 | 2-ethyl-1-hexanol | 596 | dibenzofuran | 1748 |
| hexamethylbenzene | 2340 | decyl alcohol | 860 | butyrophenone | 1120 |
| | | tert-amyl alcohol | 676 | 1,5-dimethyl tetralin | 1268 |
| | | | | menthol | 540 |

**Table 1.** List of compounds in the Test mixture, with all samples diluted in methanol and concentrations in ppm.

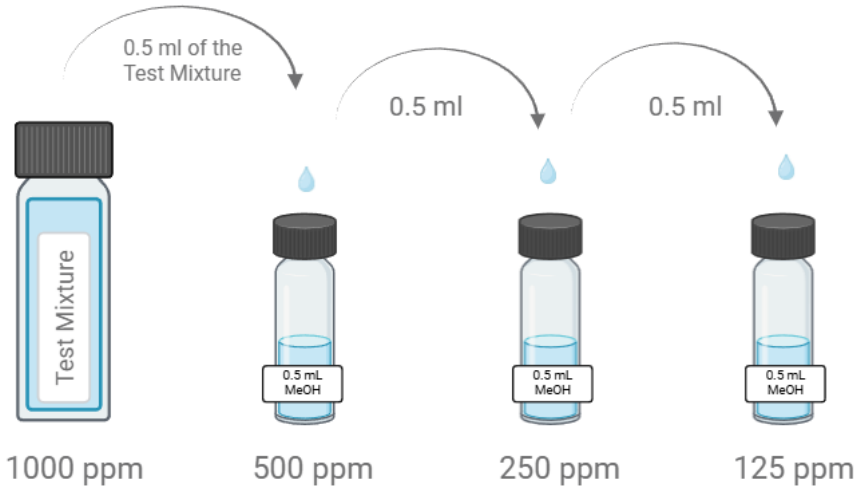



**Figure 8.** Scheme of the Serial dilution process of a Test Mixture. A solution of 1000 ppm was diluted step-by-step by mixing 0.5 mL of the previous concentration with 0.5 mL of methanol, yielding concentrations of 500 ppm, 250 ppm, and 125 ppm, respectively.

### 2.2.2 Test Mixture Solution Separation Process. Temperature ramp study optimization

In order to get a perfect chromatographic resolution for the Test Mixture components, a full temperature program was created and optimized on the Agilent Intuvo 9000 GC system. The analytes had a wide range of volatility; a multi-step oven temperature ramp was used to improve separation for both early and late eluting compounds.

The injections were done at a 1:20 split ratio and used 0.5 µL of the sample amount. The initial temperature was set at 250°C to help the vaporization happen quickly with no breakdowns. The helium was chosen as a carrier gas with a constant flow rate of 3.0 mL/min. An Agilent HP-5MS UI column (30m x 0.25mm x 0.25µm) was used for the separation process because it has low bleed, to avoid column bleeding, and generally works very well for both FID and MS detection.

In favor of early-eluting components, the oven temperature was set to 45°C to begin with, to have easier separation. It was figured out that it is best if the temperature rises in four steps, first at 3°C/min, second at 2°C/min, then at 60°C/min. and lastly at 10°C/min till the maximum 300°C/min (Table 2). This multi-step ramp was optimized to help better separate mid-volatile compounds in a controlled way, and it is easier for heavier analytes to come out separately.

All samples were run in triplicate in order to get clear and more precise results, moreover, to monitor the method reproducibility and consistency of the sample signals.

Overall, the temperature optimization was successful because it also cut down on total runtime and baseline fluctuations. These modifications were used further to identify the limit of detection.

| **Injector** | |
|---|---|
| Temperature | 250 °C |
| Split ratio | 1:20 |
| Sample size | 0.5 µL |
| **Column** | |
| Dimension | 30m x 0.25mm x 0.25µm |
| Stationary phase | HP-5MS UI |
| Flowrate | 3.0 mL/min |
| **Column Temperature Program** | |
| Initial temperature | 45 °C |
| First program rate<br>Second program rate<br>Third program rate<br>Fourth program rate | 3°C/min<br>2°C/min<br>60°C/min<br>10°C/min |
| Final temperature | 300 °C |

**Table 2.** Intuvo 9000 Operating Parameters used for the analysis of the Test mixture. The conditions were performed using a 0.5 µL split injection and a split ratio of 1:20. The HP-5MS UI capillary column with 30m x 0.25mm x 0.25µm dimensions was used with helium as a carrier

gas. Flow rate is 3.0 mL/min. The capillary column temperature program has several ramping rates with the initial temperature of 45 °C and the final temperature being 300 °C.

### 2.2.3 Data analysis

Data from Intuvo 9000 GC and GC-qMS were analyzed and processed using MATLAB R2024b (The Mathworks, Inc., Natick, MA, USA). All chromatograms were baseline corrected with the rolling ball minimum algorithm [15].

The raw data files were imported into MATLAB to analyze, create chromatograms, represent the overlay, and visualize data, including serial dilution, SPE Pass Samples, and Extracts data. The software creates zoom-in comparison within certain retention time windows and helps to understand whether samples are reproducible or not.

The limit of detection for selected analytes was established at the lowest concentration, highlighting a signal-to-noise(S/N) ratio of minimum three. The relative standard deviation(RSD) was calculated for triplicate runs to measure analytical precision under multiple conditions.

## 2.3 Results and discussion

### 2.3.1 HexSample: Using SPE alumina and silica cartridges to identify some compounds with GC-qMS

Before starting the dilution study on the Intuvo 9000 GC, a reference sample called the HexSample was made by mixing 10 mL of hexane with 170 µL of the original Test Mixture to improve identification of compounds with GC-qMS data. The schematic of the analysis process can be seen in Figure 9.

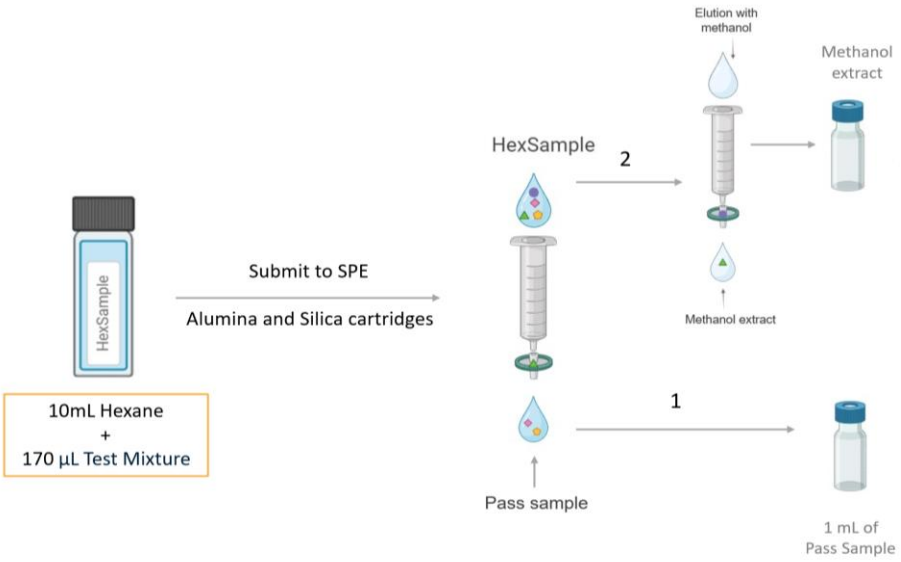


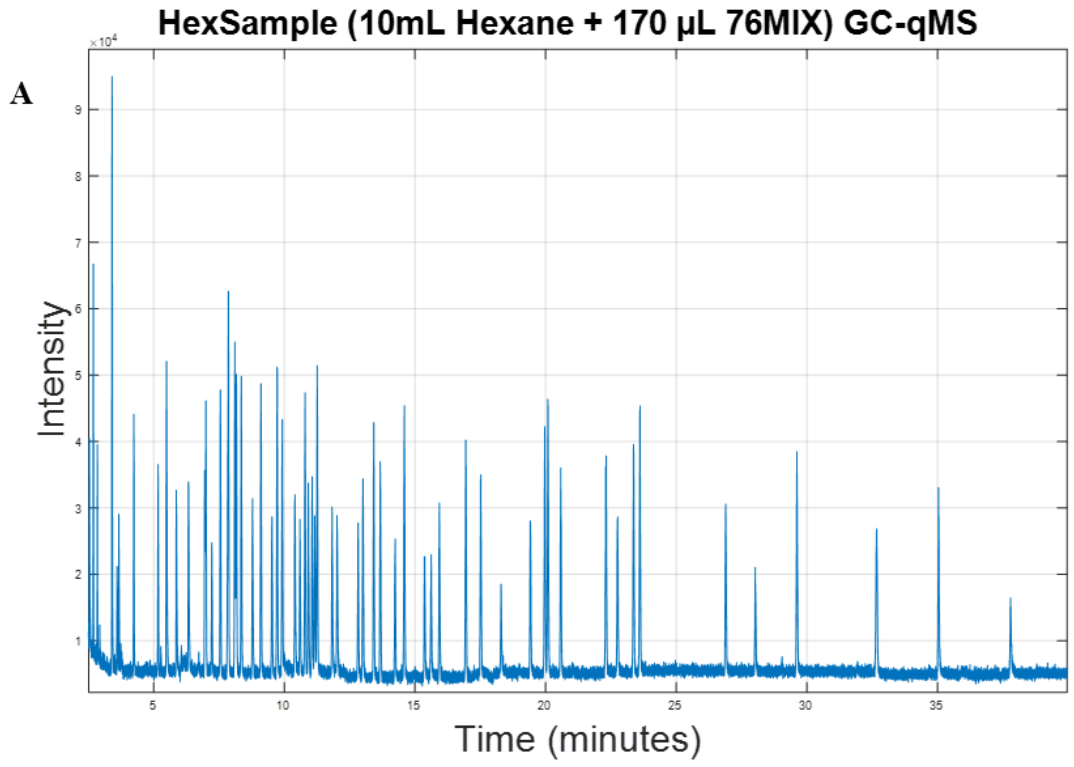

**Figure 9.** Overview for Solid-Phase extraction (SPE) of the HexSample (Hexane-based sample). The HexSample, prepared by mixing 10 mL of hexane and 170 µL of the Test Mixture, was passed through SPE cartridges, e.g., alumina and silica. The resulting Pass Sample and methanol extract were both analyzed on the Agilent Intuvo 9000 GC.

To begin with, the SPE cartridge was conditioned with 2 mL of methanol and 5 mL of HPLC-grade water, so compounds are more willing to retain on the cartridge. The next step was to load 1.0 mL of HexSample into the cartridge – the liquid that flowed directly from the cartridge was called the Pass Sample. It helps to identify cartridge selectivity and which analytes did not stick to the cartridge sorbent [13]. All components that were attracted to the sorbent by eluents are called Extracts.

Before the next step, after the sample was loaded, the cartridge was dried for 3-4 minutes with a gentle flow of air to get rid of any solvent remaining, and the sorbent content was extracted with 1 mL of methanol solution. Optimized operating conditions used to run samples in GC-qMS are shown in Table 3 below. Some components were identified, and the nonadecane peak was picked as an average representative peak through chromatogram (Figure 10).

| Injector | |
|---|---|
| Temperature | 250 °C |
| Sample size | 0.1µL |
| **Column** | |
| Dimension | 30m x 0.25mm x 0.25µm |
| Stationary phase | Rxi-1MS |
| Flowrate | 1.0 mL/min |
| **Column Temperature Program** | |
| Initial temperature | 45 °C |
| Initial hold time | 1.5 min |
| First program rate | 4 °C/min |
| Final temperature | 300 °C |

**Table 3.** GC-qMS Operating Conditions for the HexSample. The conditions were performed using a 0.1 µL sample size. The Rxi-1MS Stationary phase with 30m x 0.25mm x 0.25µm dimensions was used. The flow rate is 1.0 mL/min. The capillary column temperature program has the initial temperature of 45 °C and the final temperature being 300 °C.



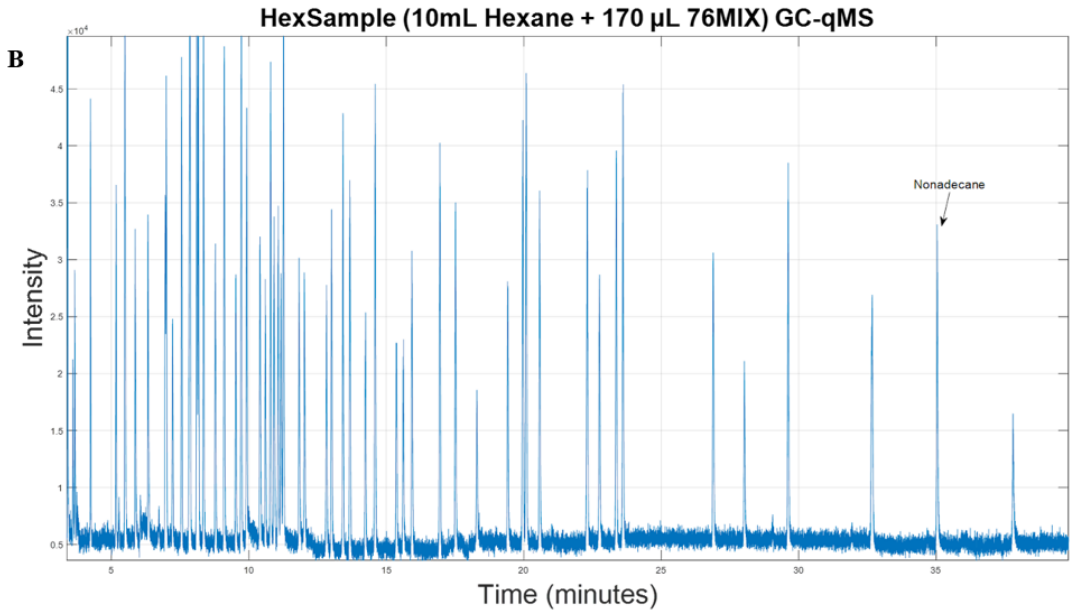



**Figure 10.** Representative HexSample chromatogram **(**GC-qMS) (A); zoomed-in peaks and indicated nonadecane peak (B).

### 2.3.2 Test Mixture: Using SPE alumina and silica cartridges

Using alumina and silica cartridges with Solid-Phase Extraction in addition to standard Test Mixture analysis helped to clean samples from the unwanted compounds and track the effect of SPE to help find specific analytes and their LODs.

The same steps described in Figure 9 were applied to Solid Phase Extraction of the Text Mixture, but the pass sample and extracts were run in Intuvo 9000 GC.

The same GC settings were used for the rest of the SPE fractions, the Pass Samples, and the Extracts. As Figure 11 illustrates chromatograms of Pass Samples, the alumina and silica cartridges held analytes in different ways. It was clear that silica sorbent held more compounds than alumina, which made the signal weaker in the Pass fraction. Zoomed-in peaks chromatogram shows that the component within the time range 2.72-2.77 minutes had much higher peak intensities in the

alumina pass sample than in silica, which means the alumina cartridge holds on to fewer compounds of the Test Mixture.

The chromatograms of both cartridge extracts showed a pattern, which supports previous results from the Pass Sample analysis. The zoomed-in peaks in Figure 12 within the time range 2.72-2.77 minutes explain that the silica extracts had higher signal intensities than alumina. This suggests that the compounds were better able to remain in the cartridge when using silica sorbent. Oppositely, alumina extracts showed lower intensities, which means fewer compounds were retained in the cartridge – supporting what was said before – most of the analytes were found in Pass Sample fractions (Figure 12).

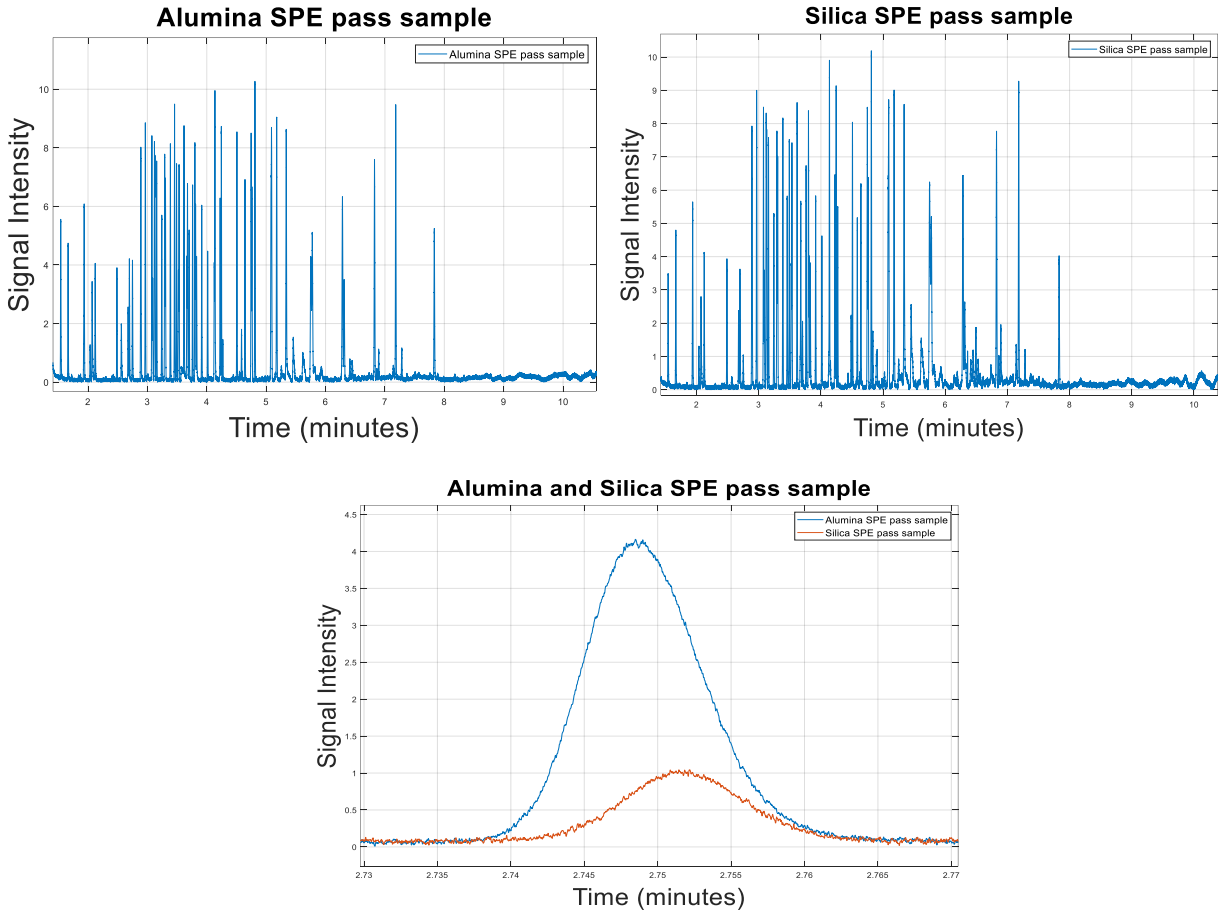



**Figure 11.** Solid-Phase Extraction (SPE) Pass samples. Alumina and Silica pass samples within the time range 2.72-2.77 minutes.

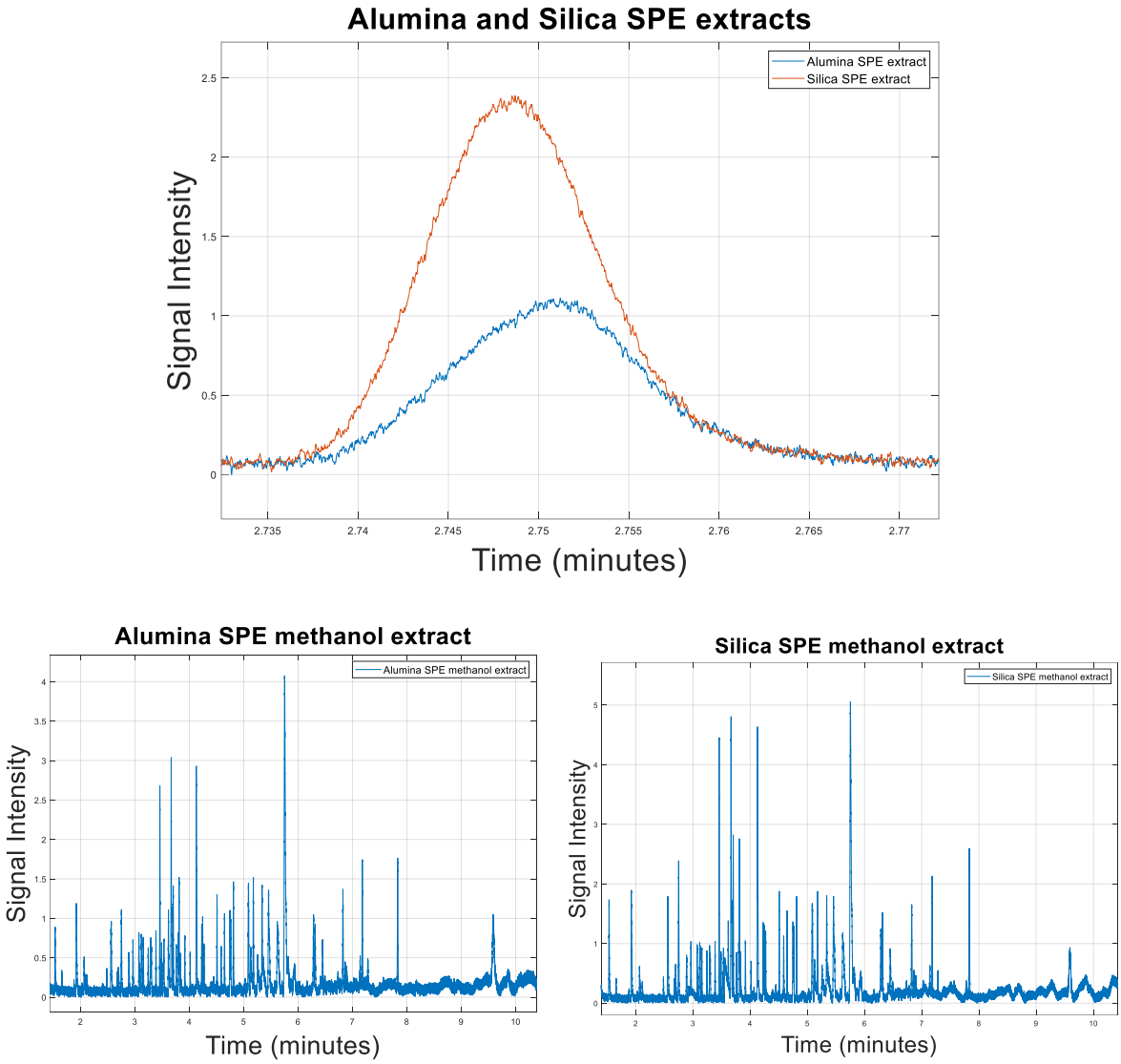



**Figure 12.** Solid-Phase Extraction (SPE) Extracts; Alumina SPE methanol extract, Silica SPE methanol extract.

The complementary results demonstrated between pass samples and extracts show how silica cartridges are much better at holding analytes, while alumina seems not as good at holding a wide range of compounds under the same conditions.

### 2.3.3 Dilution study: Exploring Limit of Detection for Intuvo 9000 GC

In order to test the sensitivity of the Agilent Intuvo 9000 GC system, a serial dilution was tested on the Test Mixture, which has more than 70 compounds. Serial dilution of the test sample with 1000 ppm concentration gave samples with lower concentrations such as 500 ppm, 250 ppm, 125 ppm, 62 ppm, 31 ppm, 15 ppm, 8 ppm, and 4 ppm. As can be seen in Figure 10, chromatograms had specific trends because the retention times were always the same, and the signal intensity decreased as the concentration decreased.

The nonadecane was chosen as an example analyte and as an average peak representative to show the limit of detection value, since it has a clear, medium volatility peak. Zoomed-in peaks of the nonadecane can be found in Figure 14A, at 1000 ppm, 500 ppm, 250 ppm, and 125 ppm concentrations, respectively, for the Test Mixture, with the retention time remaining consistent around 7.18 minutes between 7.1 – 7.2 minutes range. After getting the chromatogram of the lowest concentration, it was clear that even lower concentrations can be applied; the results can be seen in Figure 14b.

Using the International Union of Pure and Applied Chemistry (IUPAC) recommendations to check the sensitivity of the system and how low a signal can be detected, the signal-to-noise (S/N) approach was chosen with a ratio of 3:1, which means the minimum peak height (signal) must be three times higher than the noise level [16]. The nonadecane peak was visible in every concentration, and so the S/N ratio was measured to the lowest detectable signals.

Figure 14B represents zoomed-in nonadecane peaks at concentrations of C, C/2, and C/4. And C/8, where C = 1000ppm concentration. At 8ppm, the nonadecane peak shows a height of around 0.6 units with the baseline noise measured at around 0.2 units, yielding a signal-to-noise ratio of:

$$\text{signal-to-noise ratio (S/N)} = \frac{0.6}{0.2} = 3$$

This number is the same as the S/N=3 threshold to find the LOD. At 4 ppm, the signal-to-noise ratio dropped to about 2.0, which is below the acceptable level. At this point, some visible peaks can even represent and be counted as false signals and may not be interpreted correctly.

$$\text{LOD} = \frac{3\times \text{S}}{Slope}$$

S - standard deviation of the noise;

N – baseline noise;

Slope – slope of the calibration curve;

In a visual signal-to-noise ratio (S/N) method, where the baseline noise is measured, the equation above can be simplified as

LOD = S/N = 3,

so minimum signal = $3 \times S_{noise}$

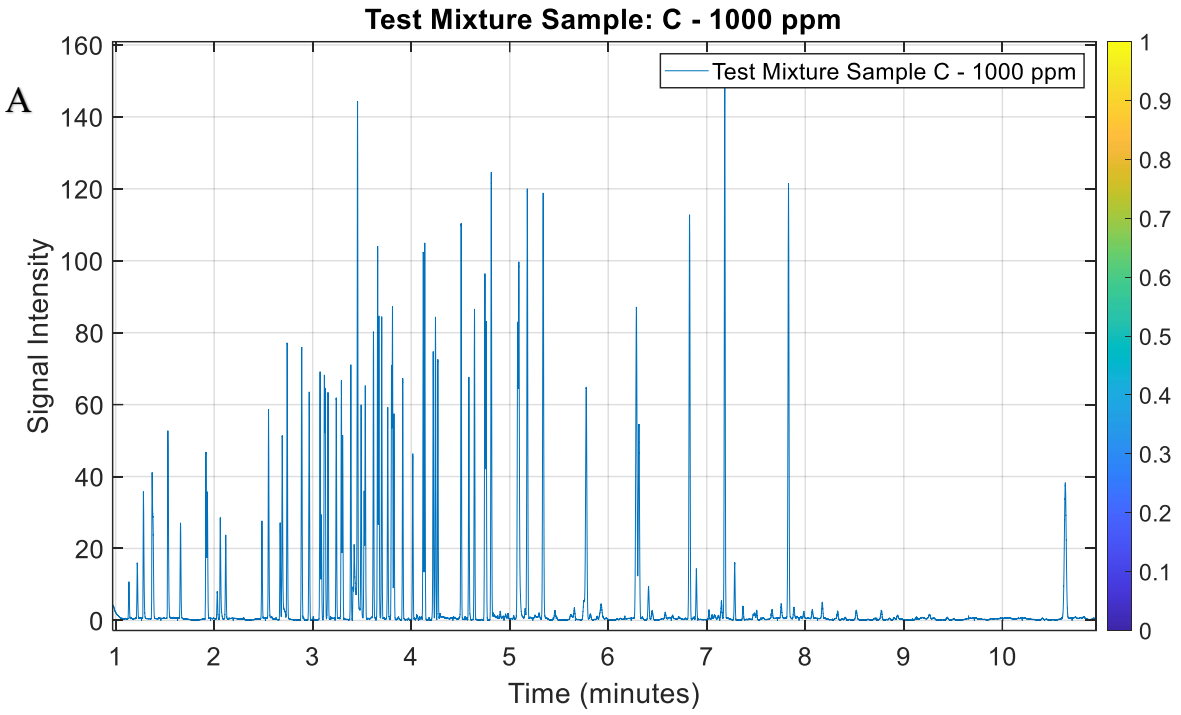
Therefore, the Limit of Detection is estimated at:

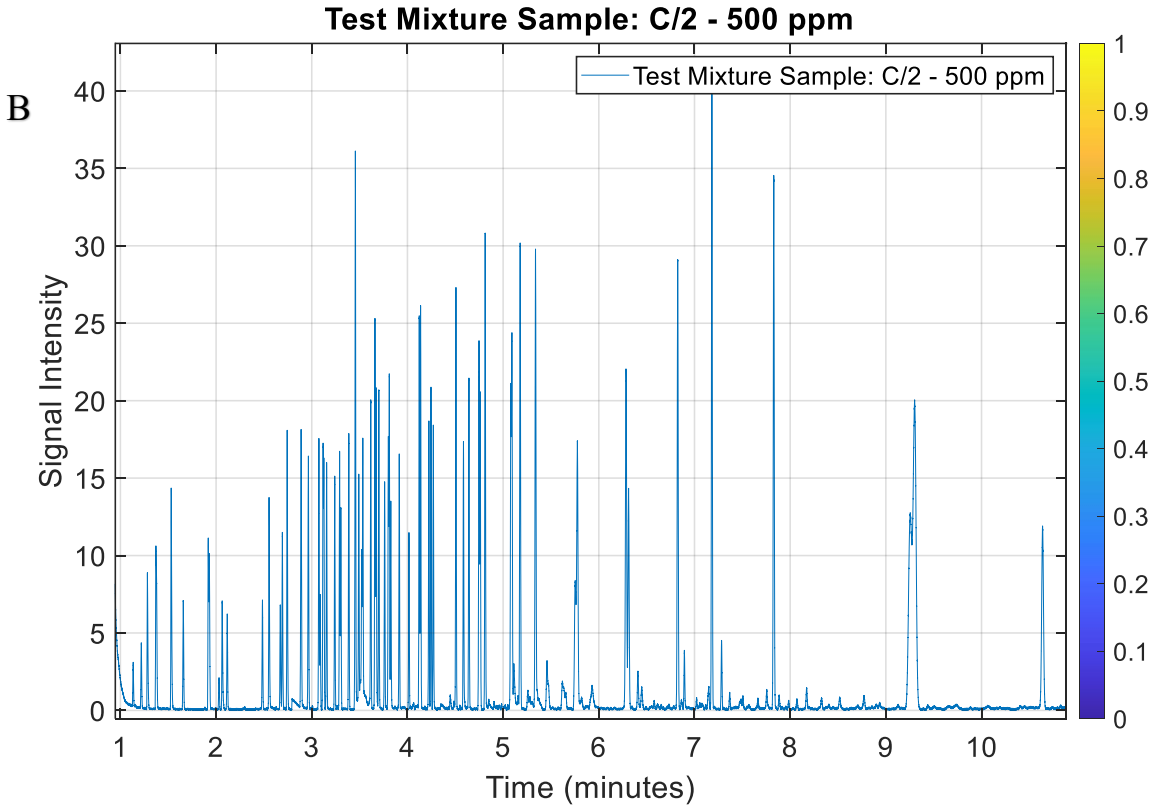
LOD ≈ 8 ppm

This number is the lowest concentration at which the analyte peak has a signal-to-ratio of at least 3:1. It highlights that the method is able to detect nonadecane in this concentration range.

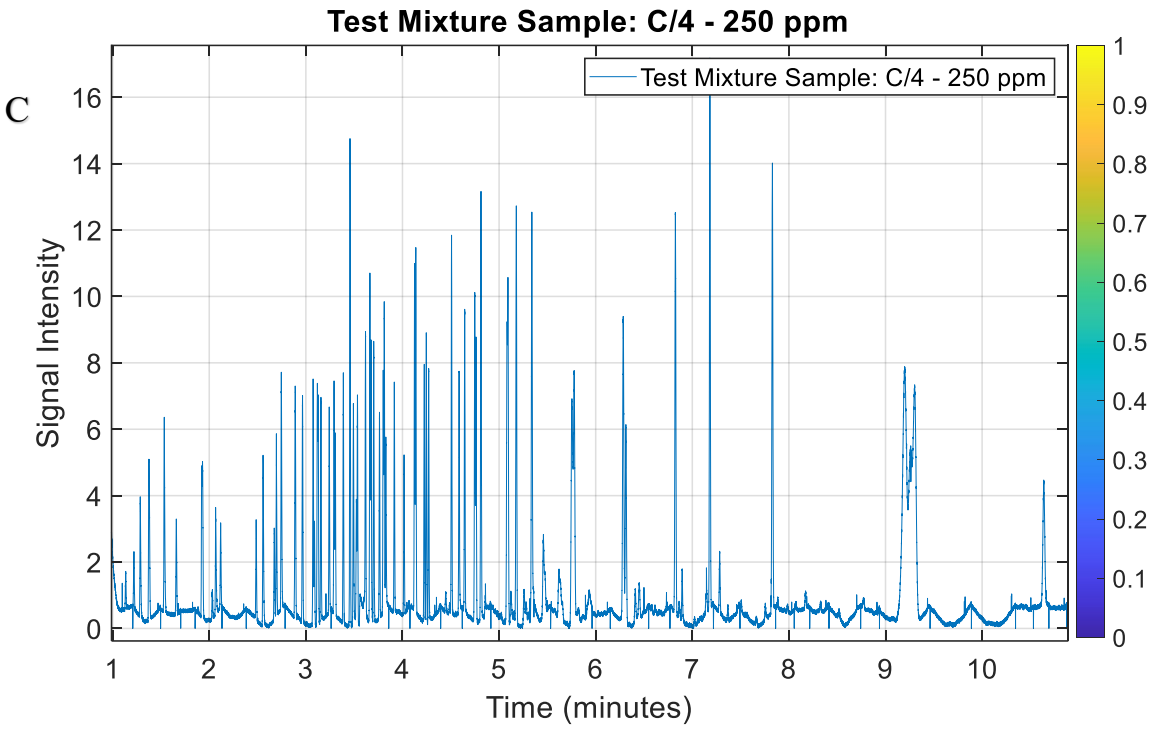
Nevertheless, the 4 ppm sample in Figure 13(I) visually reproduced the same pattern of the peaks, but with significantly low intensities – still detectable.

To conclude this, the limit of detection for the Text Mixture in Intuvo 9000 GC, according to the chromatograms, is around 4 ppm.

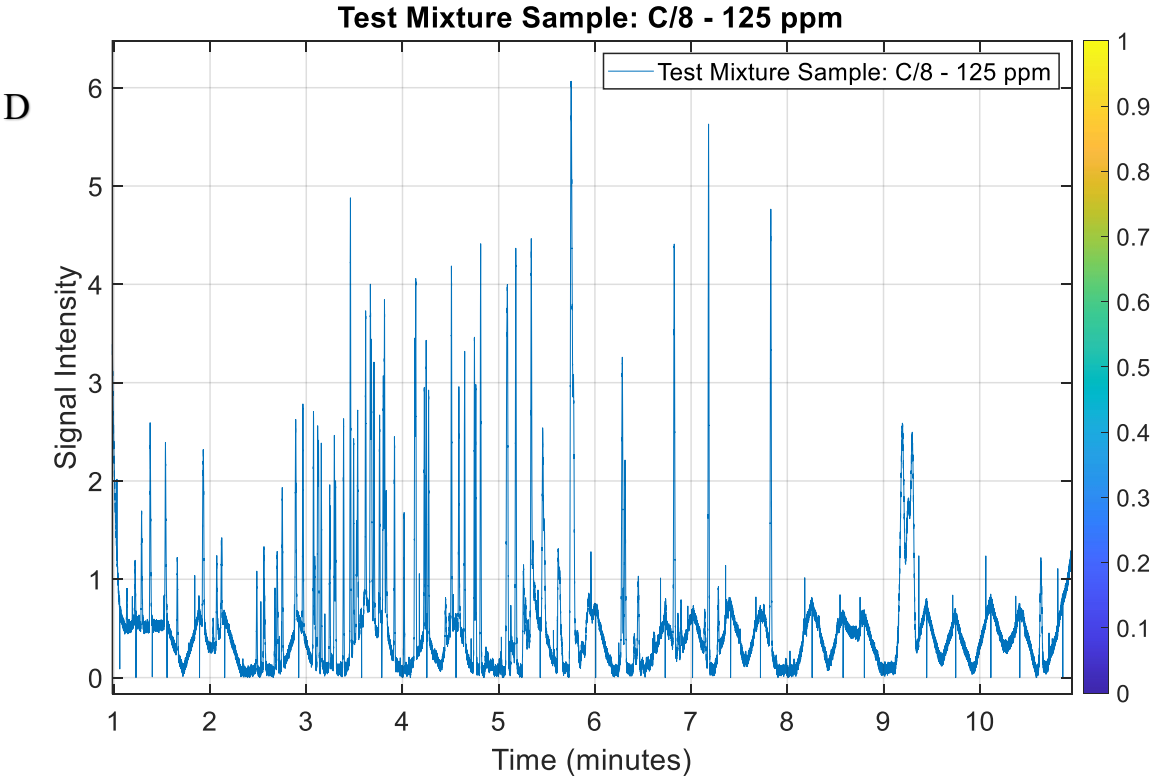

A
Test Mixture Sample: C - 1000 ppm
Test Mixture Sample C - 1000 ppm
Signal Intensity
Time (minutes)


B
Test Mixture Sample: C/2 - 500 ppm
Test Mixture Sample: C/2 - 500 ppm
Signal Intensity
Time (minutes)

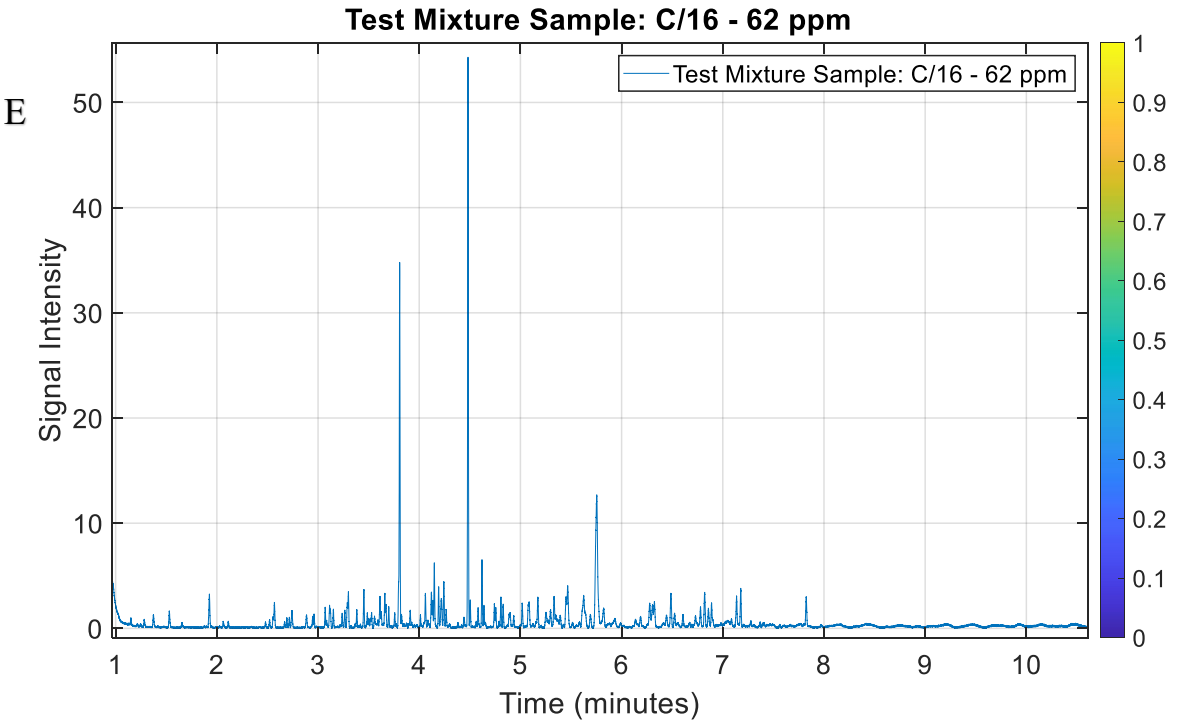
C


Test Mixture Sample: C/4 - 250 ppm
Test Mixture Sample: C/4 - 250 ppm
Signal Intensity
Time (minutes)

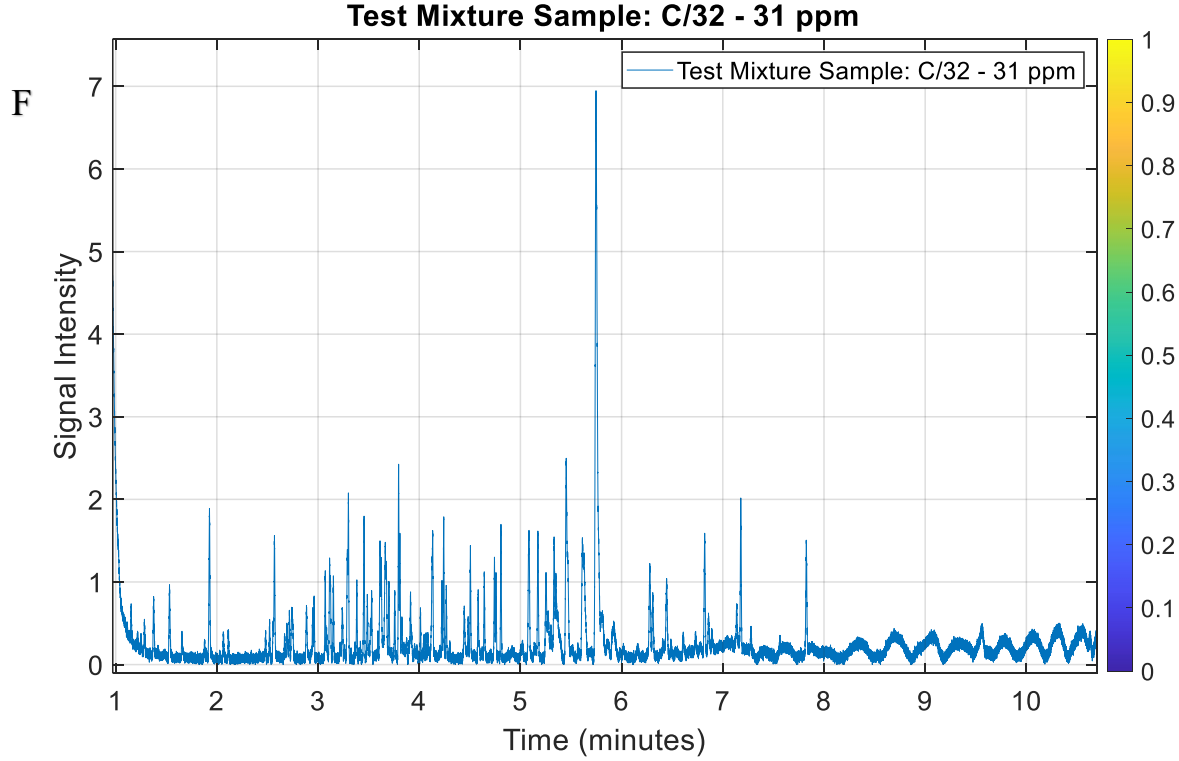
D


Test Mixture Sample: C/8 - 125 ppm
Test Mixture Sample: C/8 - 125 ppm
Signal Intensity
Time (minutes)

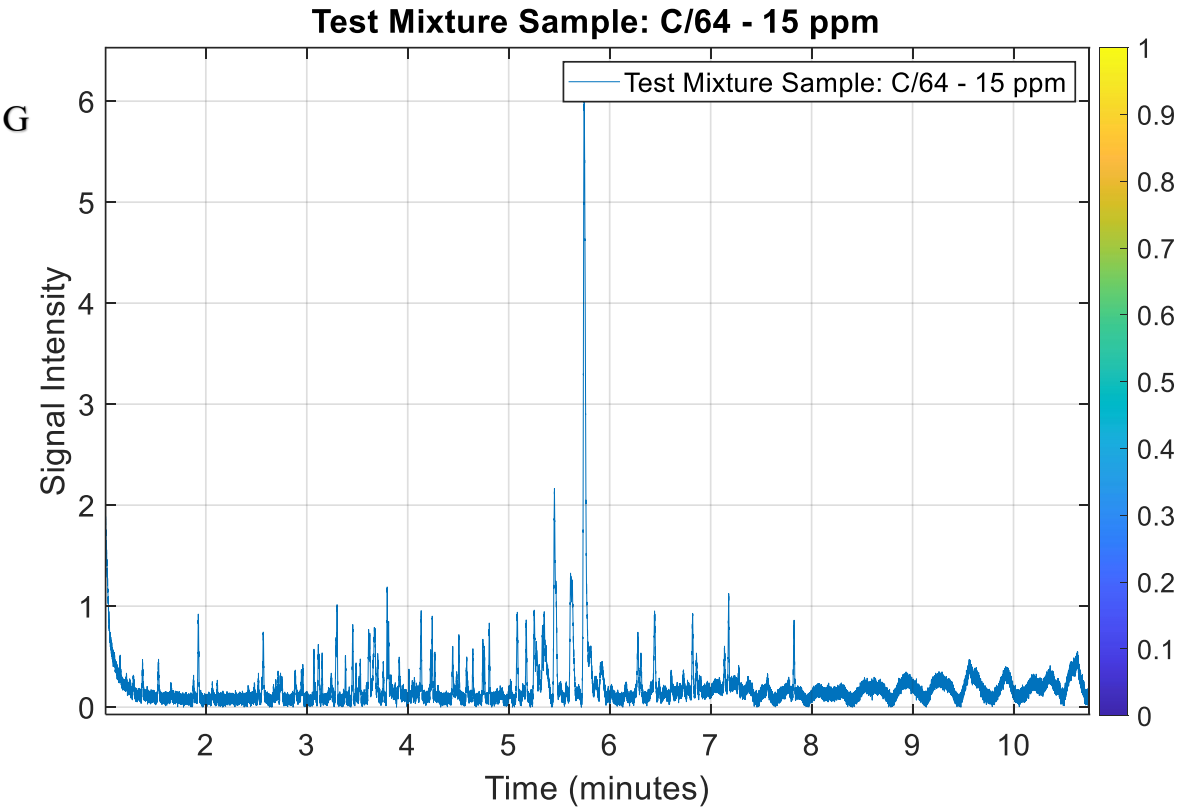
E


Test Mixture Sample: C/16 - 62 ppm
Test Mixture Sample: C/16 - 62 ppm
Signal Intensity
Time (minutes)

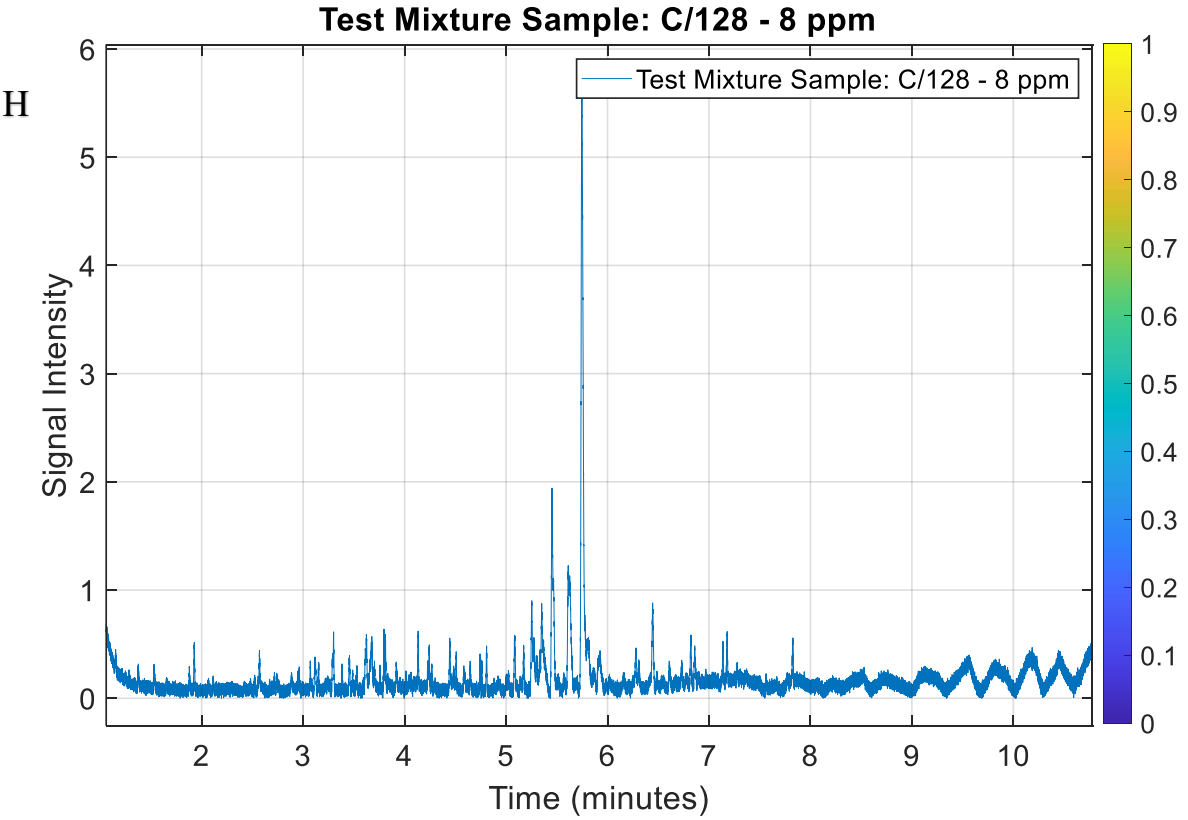
F


Test Mixture Sample: C/32 - 31 ppm
Test Mixture Sample: C/32 - 31 ppm
Signal Intensity
Time (minutes)

G


Test Mixture Sample: C/64 - 15 ppm
Test Mixture Sample: C/64 - 15 ppm
Signal Intensity
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10

H


Test Mixture Sample: C/128 - 8 ppm
Test Mixture Sample: C/128 - 8 ppm
Signal Intensity
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10

I

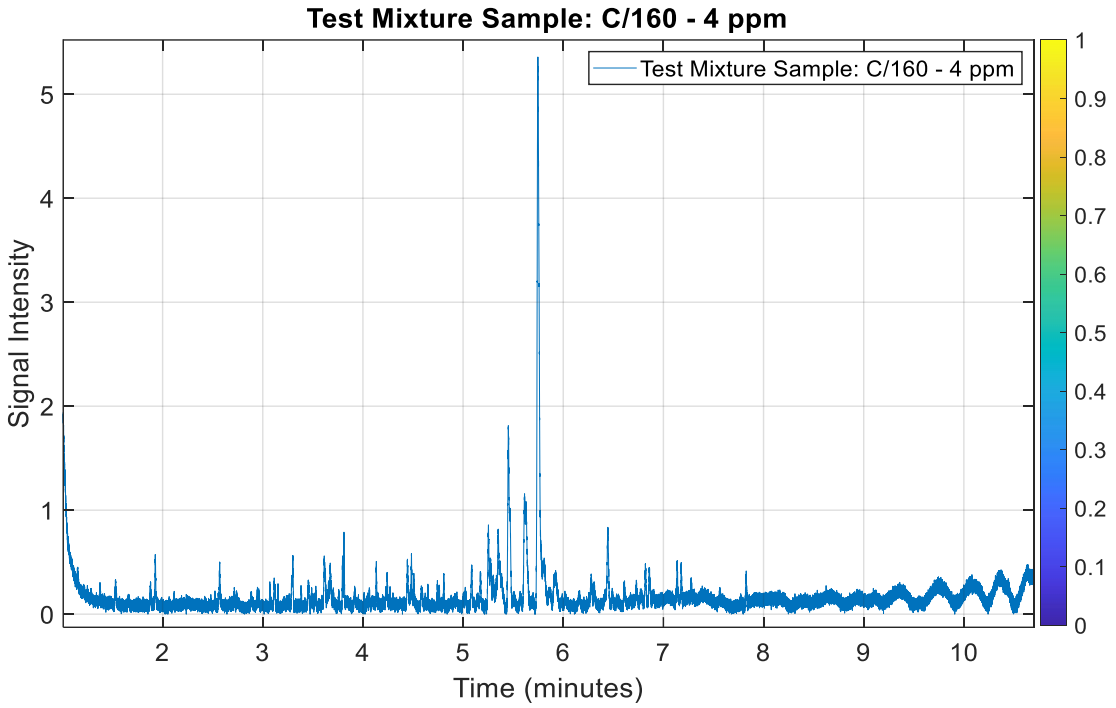



**Figure 13.** Concentrations for the Test Mixture at concentrations of 1000 ppm (A), 500 ppm (B), 250 ppm (C), 125 ppm (D), 62 ppm (E), 31 ppm (F), 15 ppm (G), 8 ppm (H), and 4 ppm (I).

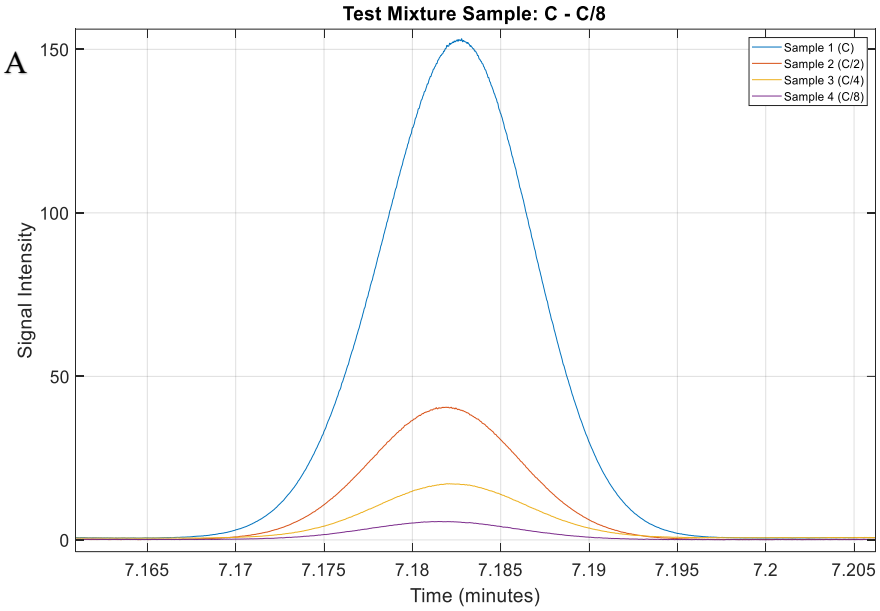



**Figure 14A.** Zoomed-in peaks of Nonadecane at concentrations for the Test Mixture: 1000 ppm, 500 ppm, 250 ppm, 125 ppm with the retention time remaining consistent around 7.18 minutes.

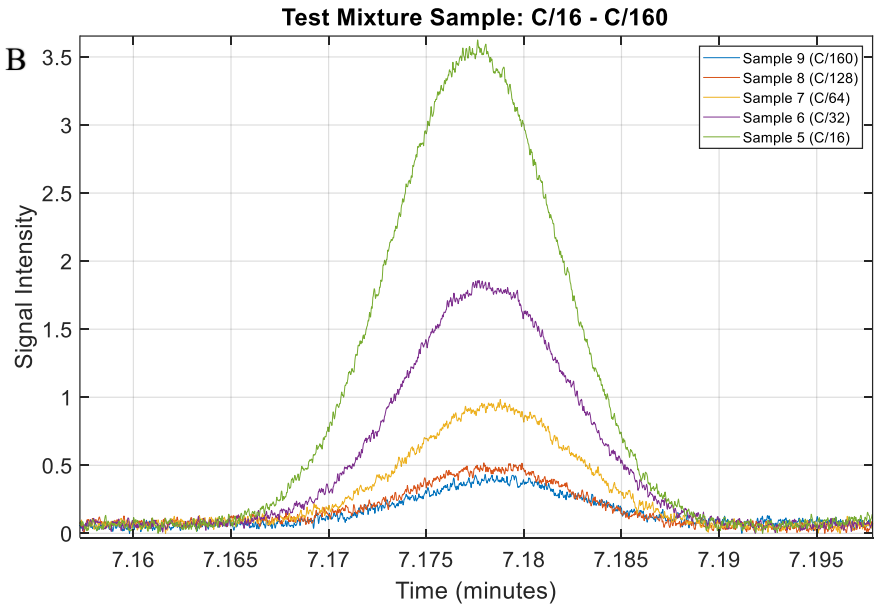



**Figure 14B.** Zoomed-in peaks of Nonadecane at concentrations for the Test Mixture: 62 ppm, 31 ppm, 15 ppm, 8 ppm, and 4 ppm with the retention time remaining consistent around 7.18 minutes.

# Chapter 3. Analysis of Contaminated Water Using Solid Phase Extraction and One-Dimensional Gas Chromatography with Quadrupole Mass Spectrometry

## 3.1 Introduction

In this chapter, the goal was to analyze the impact of SPE cartridges on extraction efficiency and volatile and semi-volatile organic components in water samples and diesel extracts. Along with it, methanol and hexane extracts were compared to determine the solvents' selectivity for analyte recovery.

## 3.2 Experimental

### 3.2.1 Overview of the analysis

Three main steps of the analytical workflow- the first step is to use different SPE cartridges to extract the contaminated water compounds. The second step is eluting with methanol and hexane solvents. The third - using GC-qMS to identify and compare extracted components to fulfill each other.

The ability of GC-qMS to provide mass spectral identification and chromatographic separation of analytes led to its selection as an analytical instrument for the research in the first place. Table 4 shows optimized conditions used to run samples in GC-qMS.

| **Injector** | |
|---|---|
| Temperature | 250 °C |
| Sample size | 0.1µL and 0.2 µL |
| **Column** | |
| Dimension | 30m x 0.25mm x 0.25µm |
| Stationary phase | Rxi-1MS |
| Flowrate | 1.0 ml/min |
| **Column Temperature Program** | |
| Initial temperature | 40 °C |
| Initial hold time | 1.5 min |
| First program rate | 4 °C/min |
| Final temperature | 240 °C |
| Final hold time | 1 min |

**Table 4.** GC-qMS Operating Conditions.

## 3.3 Results and discussions

### 3.3.1 Various types of extracts and choosing the cartridges

The Shell Diesel sample was analyzed to provide a baseline for hydrocarbon identification. C-18, silica, $NH_2$, and Florisil cartridges, along with various types of extracts, were tested to analyze sorbent selectivity. Some results are shown in Figure 15. The chromatogram results illustrated that the C-18 cartridge and methanol solvent were more effective, with the most peaks and the broadest retention range.

Other cartridges, like silica, reproduced fewer peaks, extracted more polar compounds, which means matrix interferences were less co-eluted (Figure 15 B). It has been noted before in urine example that silica-based SPE cartridge effectively extracts preferably polar analytes [17].

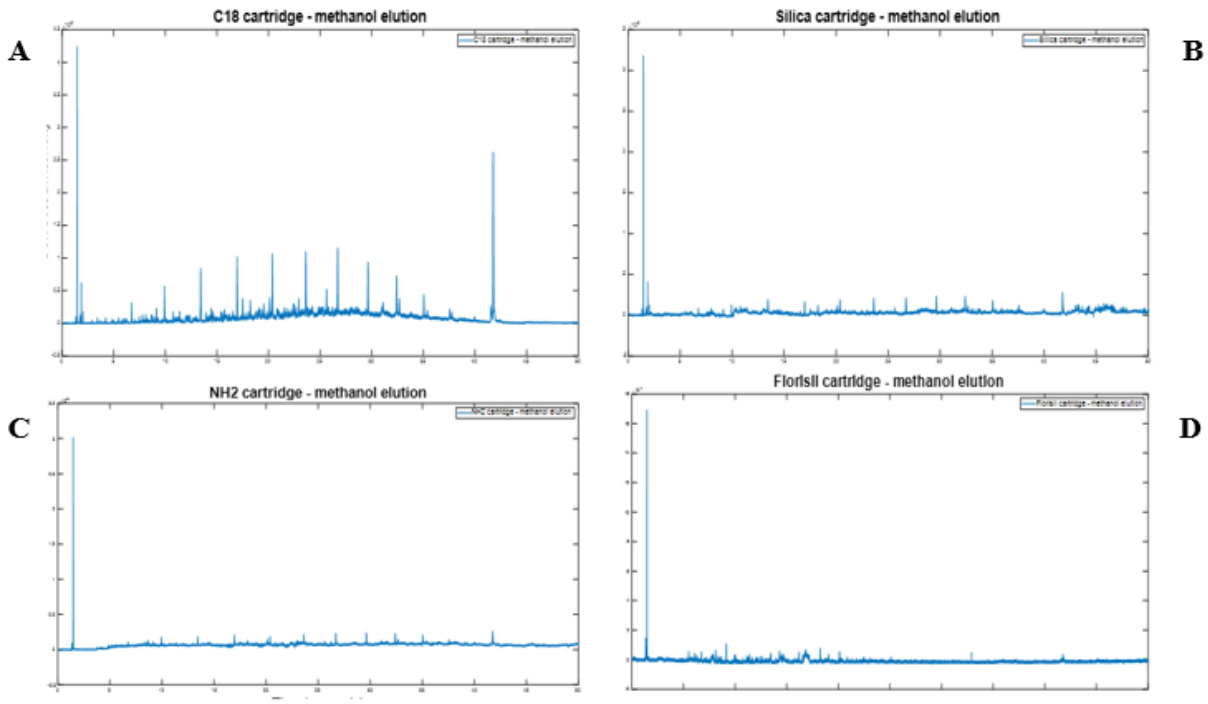



**Figure 15.** Choosing the type of cartridge.
Methanol elution with C-18 (A), Silica (B), NH2 (C), and Florisil (D) cartridges.

The chromatograms produced by the $NH_2$ and Florisil cartridges showed low peak intensity, confirming limited recovery during elution also shown in Figure 15 C and D.

### 3.3.2 Choosing the concentration of the original sample in the Contaminated Sample

In order to find the ideal injected concentration of the original sample in the Contaminated Sample, three different volumes were tested: 1 mL, 0.5 mL, and 0.25 mL. Three overlayed chromatograms in Figure 13 illustrate that: high intensity peaks at 1 mL and 0.5 mL showed high sensitivity and intensity, and signal loss at 0.25 mL indicates that some low sensitive components were not found. In order to keep repeatable peak resolution, 1 mL was chosen as the optimal injection volume (Figure 16).

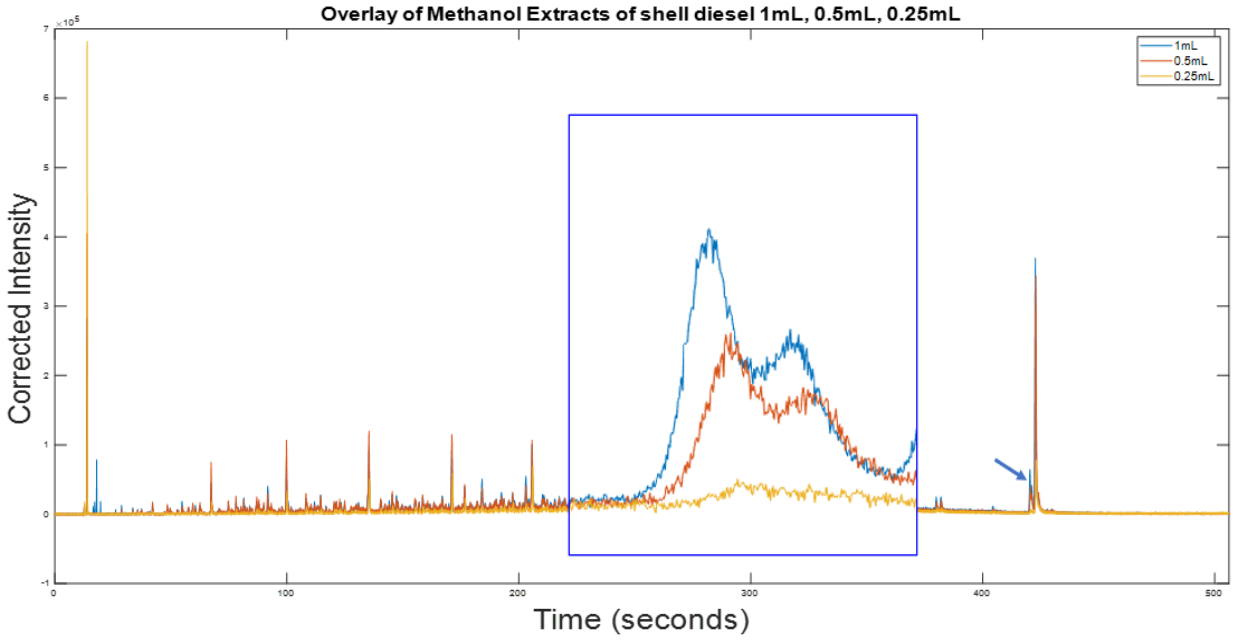



**Figure 16.** Methanol extracts with different concentrations of shell diesel. 1 mL-blue, 0.5 mL-red, 0.25 mL-yellow.

### 3.3.3 Comparison of Methanol and Hexane Extracts

Two solvents of different polarity, such as methanol (polar) and hexane (non-polar), were used to observe If all of the compounds on the SPE cartridge efficiently eluted (Figure 17). Both eluents were analyzed keeping the same GC-qMS conditions to determine the ability to recover the wide spectrum of analytes retained during extraction.

The methanol extract showed a dense chromatogram with a broad combination of analytes that covered the retention range from 2 to 45 min. Early-eluting peaks around 2-10 min correspond to small, more polar molecules; meanwhile, semi-volatile aromatics appeared as overlapping peaks in the middle of the chromatogram between 10 – 30 min. Peaks after 30 min illustrate heavier hydrocarbons. The methanol extract effectively elutes polar and nonpolar compounds from the cartridge.

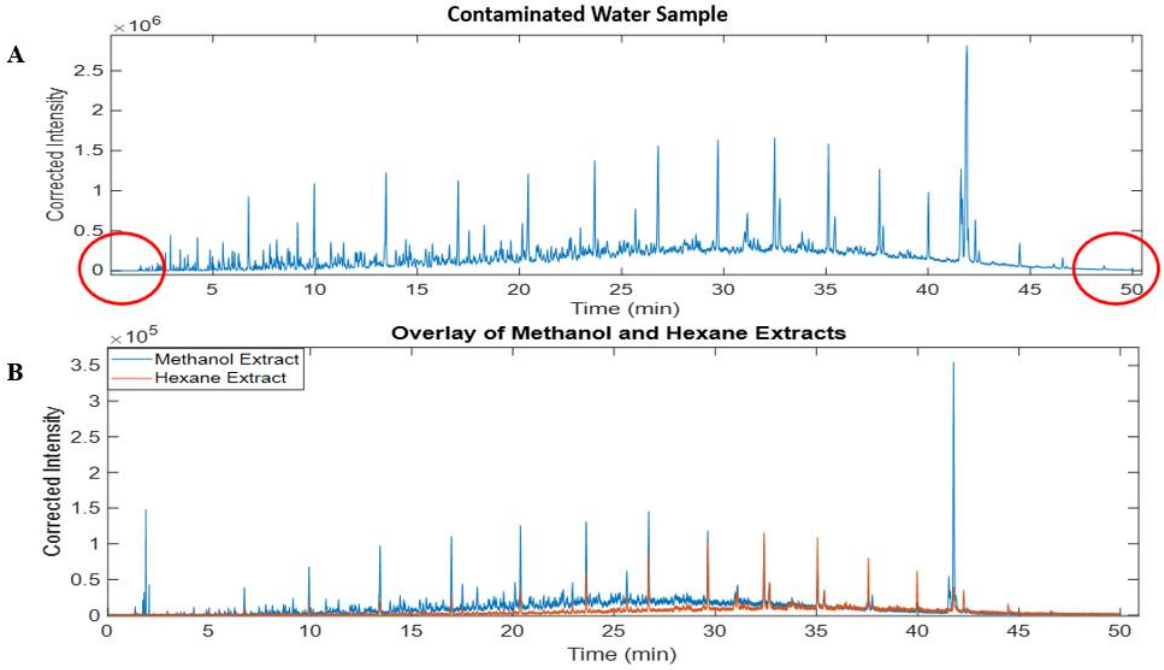



**Figure 17.** Methanol and Hexane extracts. Contaminated Water Sample (A) and overlay of Methanol and Hexane Extracts (B).

A simpler chromatogram was produced by the hexane extract, where most of the intense peaks showed up between 20 and 45 minutes. It indicates nonpolar aliphatic hydrocarbons, which are mostly common in petroleum leftovers. In contrast, polar analytes could not be eluted from the cartridge by hexane, it can be observed by the lack of early peaks on the chromatogram.

After analyzing both chromatograms, many co-eluting and unresolved peaks were seen in the mid-retention area. The baselines went back to zero as circles with red in Figure 12 A, highlighting that the overlapping peaks were caused because of the complexity of the sample rather than technical challenges. In this case, to improve separation and compound identification, higher resolving power and an additional separation dimension in need for complex mixtures.

# Chapter 4. Analysis of Contaminated Water Using Solid Phase Extraction and Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry

## 4.1 Comparison 1D-GC-qMS to 2D GC×GC-MS

The resolution of one-dimensional gas chromatography combined with quadrupole mass spectrometry (1D-GC-qMS) was not enough to separate every component of the complex mixture. Despite the fact that 1D-GC-qMS showed useful qualitative information on the chromatogram, multiple unresolved and co-eluting peaks were seen in the chromatogram. It can be seen in Figure 14 that, particularly in the middle retention area, hundreds of overlapping analytes need more separation. In this case, Comprehensive Two-Dimensional Gas Chromatography combined with Time-of-Flight Mass Spectrometry (GC×GC-TOFMS) was used to resolve the limitations.

The GC×GC-TOFMS uses a thermal modulator to connect two columns with different selectivity (nonpolar×polar) and increases peak capacity, plots 3D chromatograms [18] to easily identify groups of compounds, for example, alkanes, cycloalkanes, and aromatics (Figure 18). Once the sample is in the $^{1}$D column, its compounds are sorted by volatility, and the $^{2}$D column helps to cluster analytes by polarity and functional group interactions.

Peak capacity is significantly increased by orthogonal separation. The TOFMS`s collection rate helps to get a more precise splitting of overlapping signals. So, analytes are gathered by chemical groups - easy to identify and recognize patterns in the comprehensive two-dimensional chromatogram that GC×GC-TOFMS creates. This and more advantages of using the 2D GC were discussed in the literature before [19].

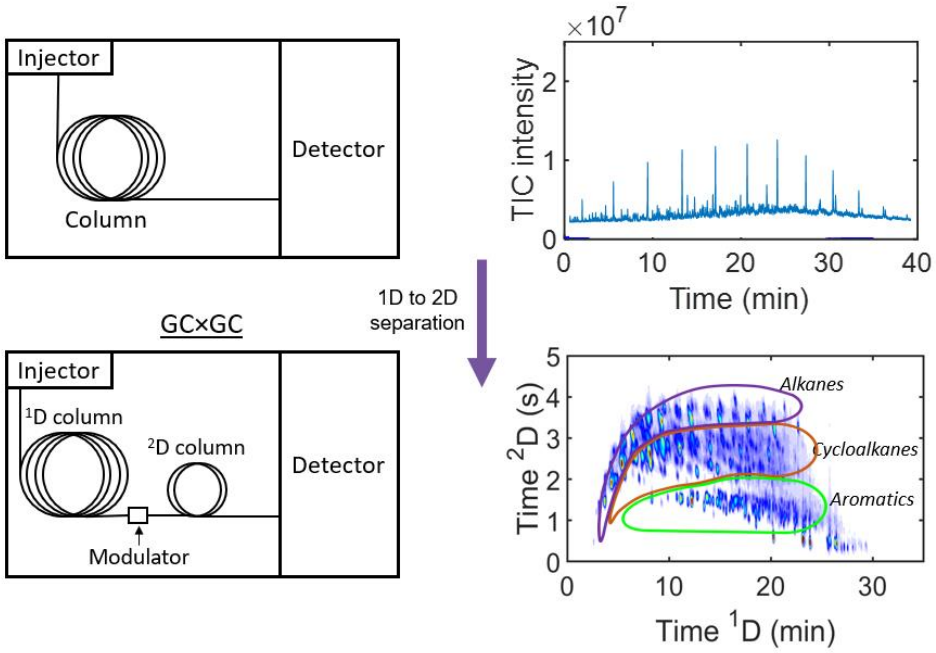



**Figure 18.** Limitations of GC-MS and need for GC×GC-TOFMS.

## 4.2 Experimental

### 4.2.1 Comparison of the Original Sample to the Methanol and Hexane Extracts

Using GC×GC-TOFMS, the contaminated water sample and its methanol and hexane extracts were analyzed to determine the ability of each extraction to complement each other and observe if the different compounds could be retained onto the SPE cartridge.

All samples for this analysis were prepared using the same procedures as described in Chapter 3. The temperature program started at 40°C, holding for 1.5 min, and increased by 4°C/min up to 240 °C. Other important column configurations are shown in Table 5. The collection rate on TOFMS was set to be 100 Hz within 44-335 m/z mass channels.

| **Injector 1** | |
|---|---|
| Temperature | 250 °C |
| Split ratio | 1:1 or 20:1 |
| Sample size | 0.1 or 0.2 µL |
| **Column 1** | |
| Dimension | 30m x 0.25mm x 0.25µm |
| Stationary phase | Rxi-17Sil MS |
| Flowrate | 2.0 ml/min |
| **Column 1 Temperature Program** | |
| Initial temperature | 40 °C |
| Initial hold time | 1.5 min |
| First program rate | 4 °C/min |
| Final temperature | 240 °C |
| Final hold time | 1 min |
| **Column 2** | |
| Dimension | 1.5m×0.18mm×0.18µm |
| Stationary phase | Rxi-1Sil MS |
| Modulation Period | 4 sec |
| **Column 2 Temperature Program** | |
| Secondary Oven Temperature Offset temperature | 5 °C |
| Modulator Temperature Offset temperature | 18 °C |
| Transfer Line Temperature | 285 °C |

**Table 5.** GC×GC Operating Conditions with Reverse Column Configuration Polar $^{1}$D × Non-Polar $^{2}$D.

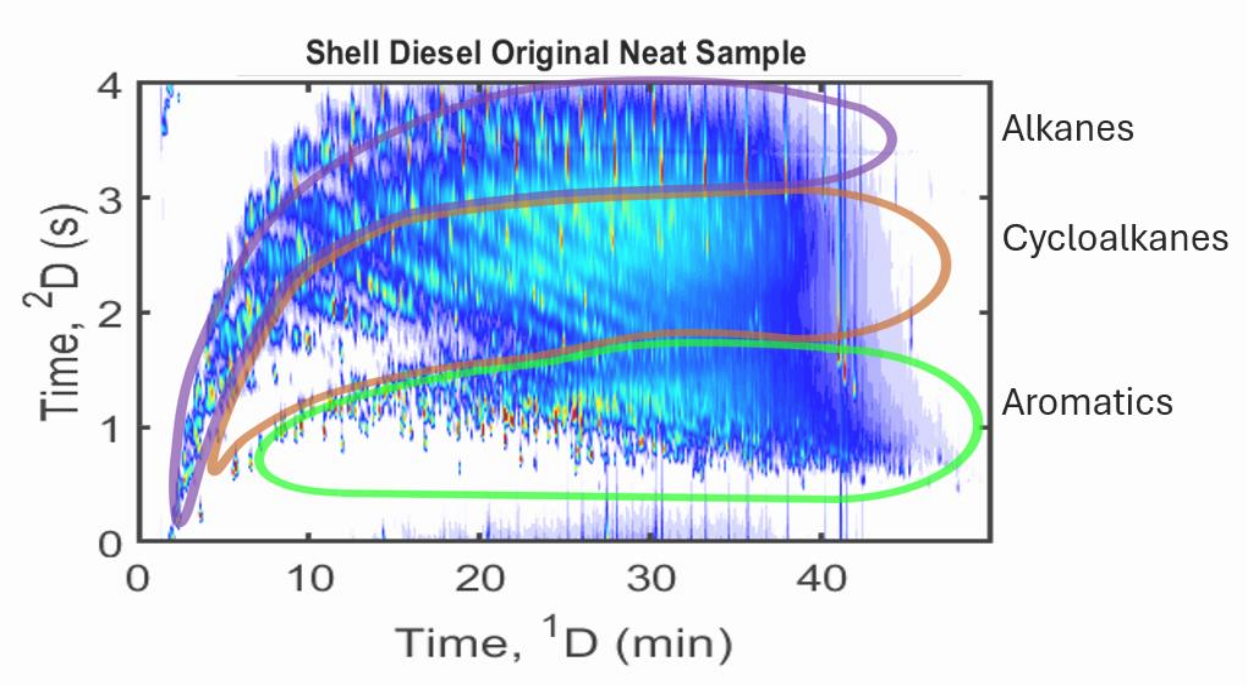



**Figure 19.** Original Shell Diesel Contaminated Sample chromatogram

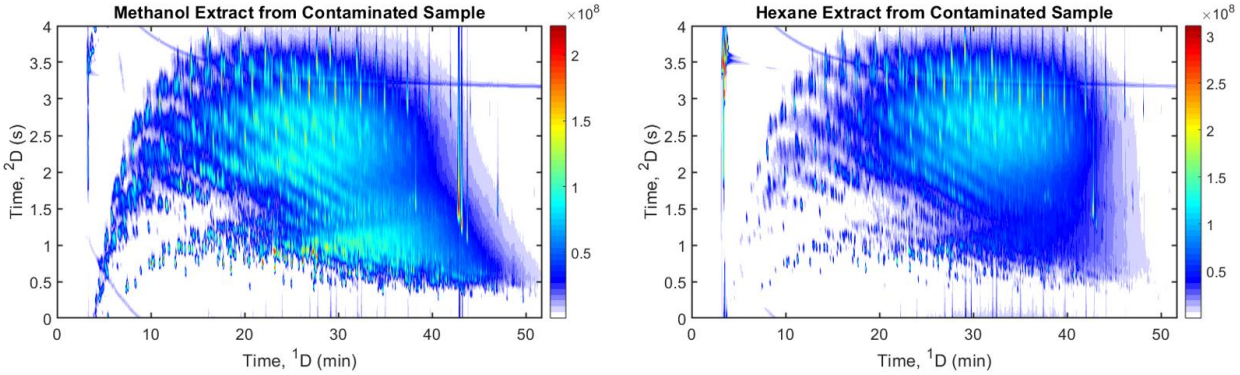



**Figure 20.** Hexane and Methanol Extracts from Contaminated Sample chromatograms.

The 2D chromatograms (Figures 19, 20) showed that the Hexane Extract, Methanol Extract and Original Shell Diesel Contaminated Sample have significant differences. The original sample illustrated dense regions of unresolved peaks, proving it is a very complex mixture with numerous overlapping hydrocarbons. Figure 20 shows the effectiveness of SPE, which decreased matrix

interference while keeping the target analytes, and it can be observed by the more distinct and separable patterns displayed on chromatograms.

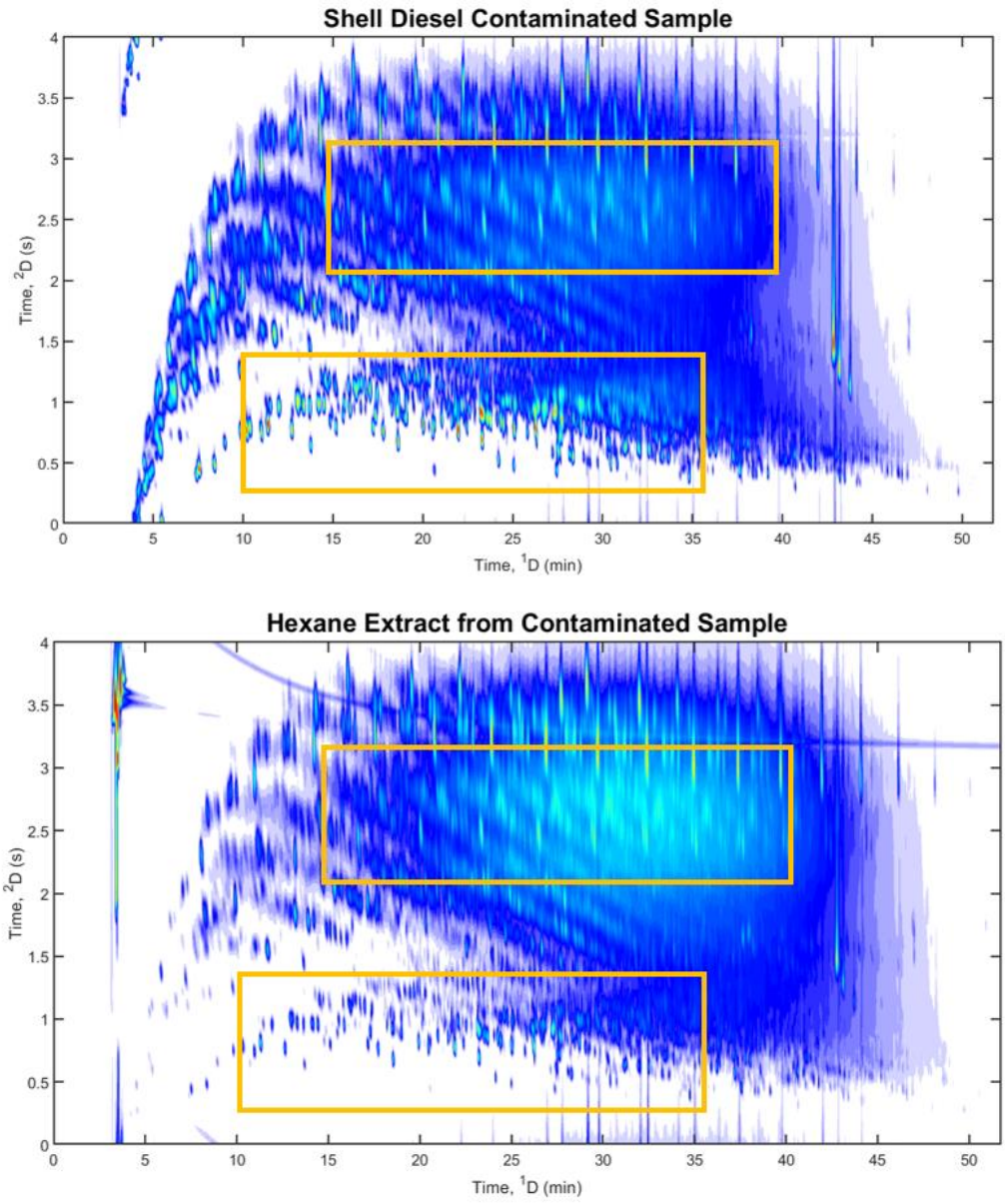



**Figure 21.** Comparing the Original Shell Diesel Sample and the Hexane Extract from the Contaminated Sample.

The Hexane Extract in Figure 21 reflected the top portion of the diesel chromatogram, where mostly nonpolar aliphatic hydrocarbons and aromatic rings are concentrated. Hexane preferably

eluted the hydrophobic components of the analytes, as shown by two-dimensional chromatogram patterns between 15 and 40 minutes in 1D and from 2 to 3 seconds in 2D. But the intensity is significantly lower in the lower left part of the chromatogram, which is connected to polar species, and some zoomed-in peaks can be seen in Figure 22. Proving that hexane is effectively used as a nonpolar eluent.

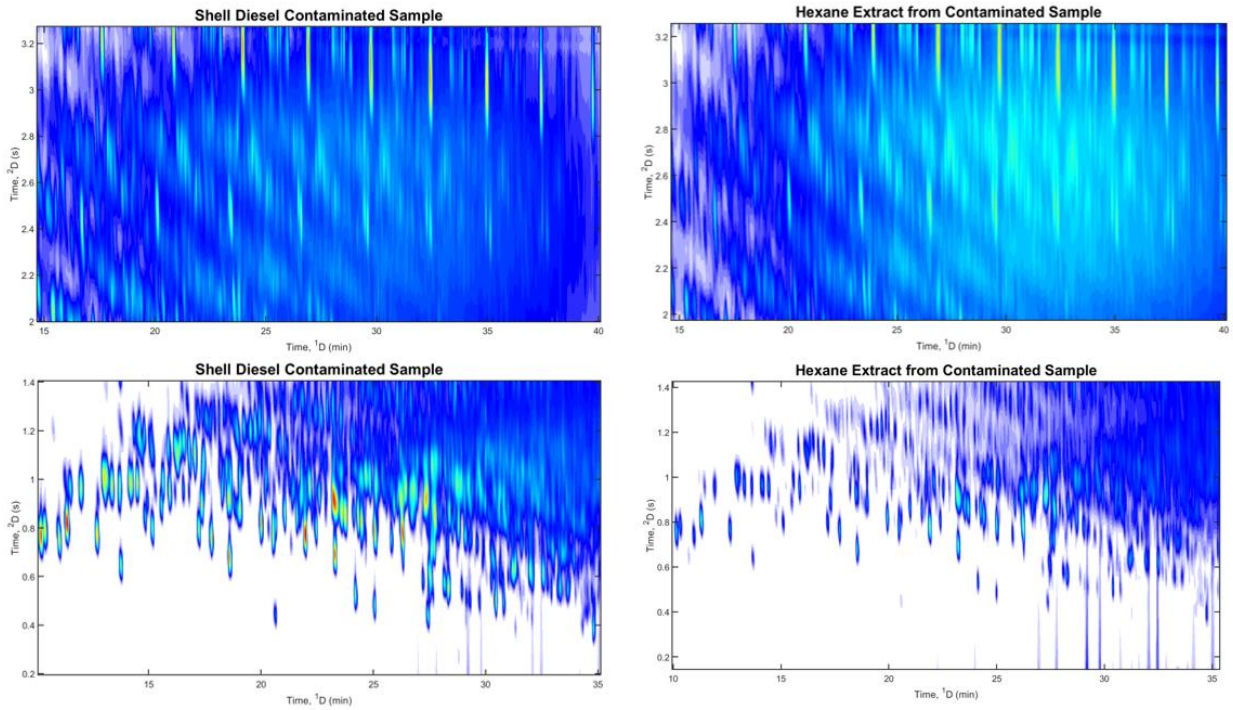



**Figure 22.** Zoomed-in highlighted areas comparing the Original Sample and the Hexane Extract from the Contaminated Sample.

In contrast to Hexane Extract, the distribution of patterns in Methanol Extract (Figure 23) is more extensive and complex along the second dimension, highlighting more specifically polar compounds. Those compounds, like aldehydes, short-chain esters, or ketones, can be found in the lower retention time area between 0.5 and 1.5 seconds in 2D, which were missing during hexane extraction. Zoomed-in areas are shown in Figure 24.

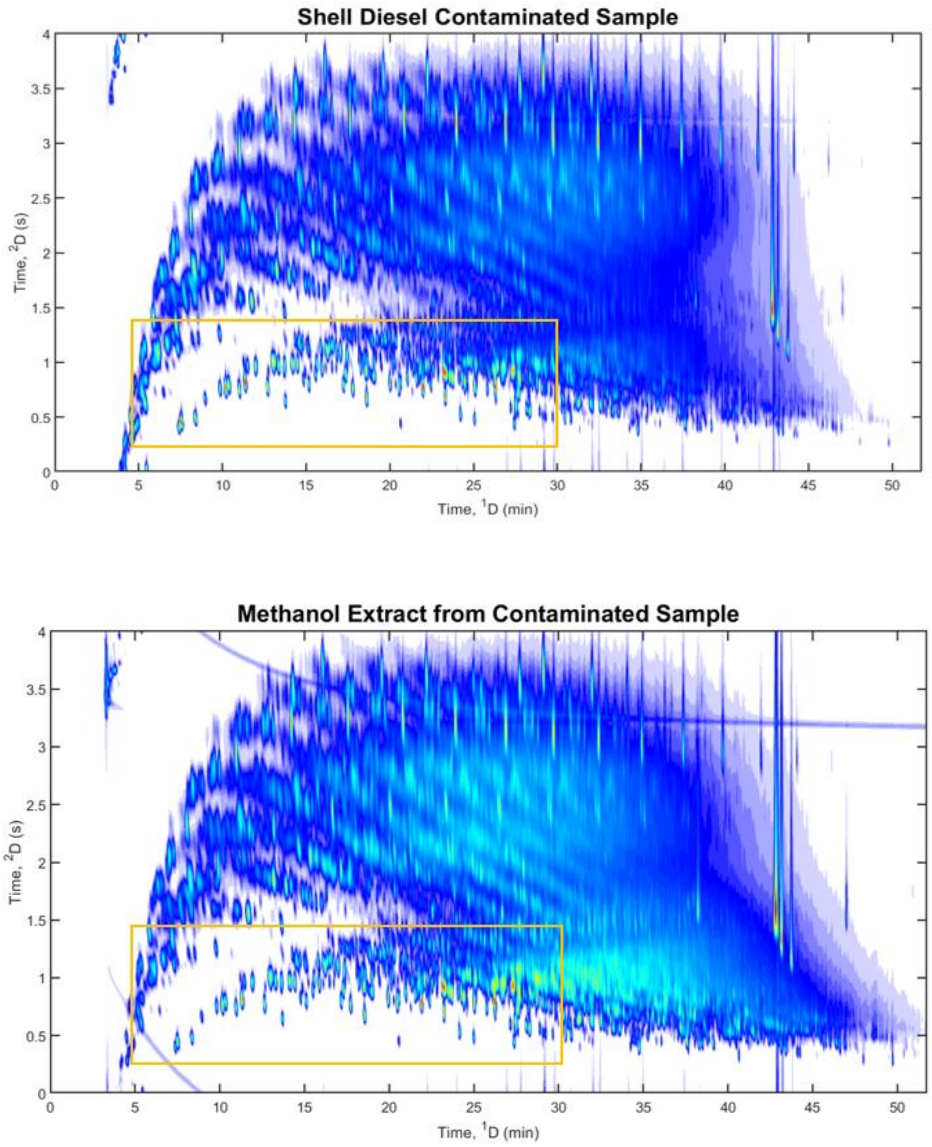



**Figure 23.** Comparing the Original Sample and the Methanol Extract from the Contaminated Sample.

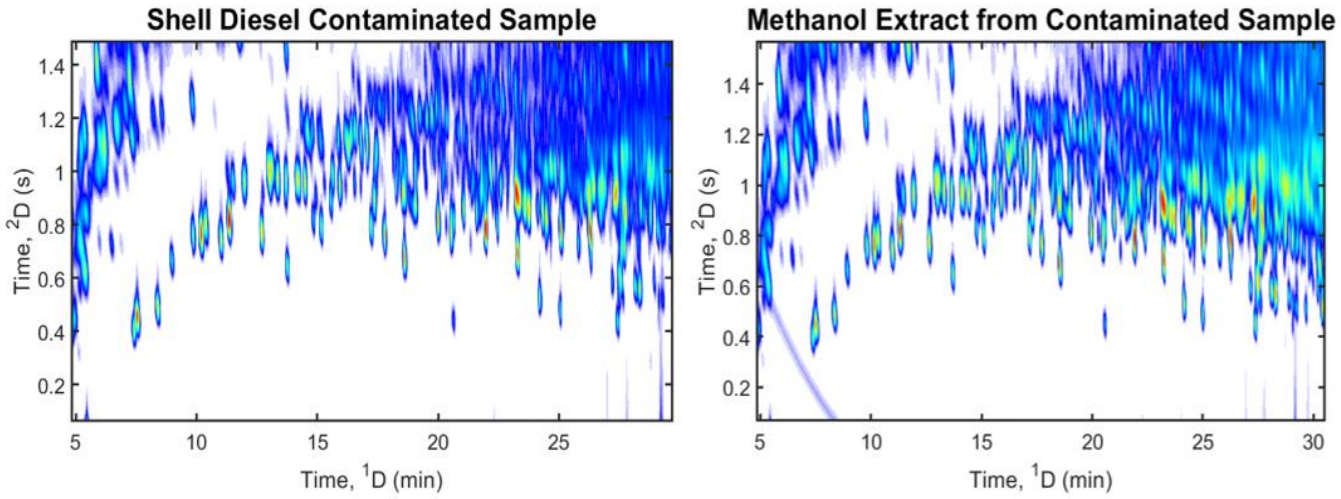



**Figure 24.** Zoomed-in highlighted areas comparing the Original Sample and the Methanol Extract from the Contaminated Sample.

Overall, the Methanol eluent extracted both polar and non-polar analytes, resulting in the closest chemically complete chromatographic fingerprint, versus the Hexane eluent effectively recovered nonpolar hydrocarbons. This comparison further shows the complementary nature of the two extracts to show a complete picture of the original sample (Figure 25).

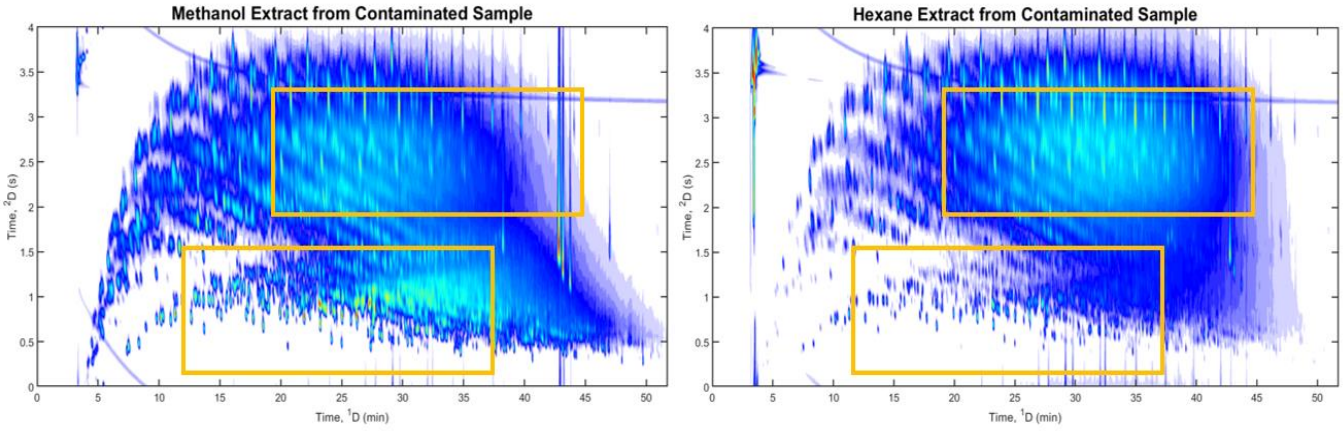



**Figure 25.** Comparing Methanol Extract and Hexane Extract from Contaminated Sample.

### 4.2.2 Principal Component Analysis of the Original Sample and the Extracts

Principal component Analysis (PCA) of the Original Sample and Extracts was used primarily to analyze reproducibility of replicates, and reflect compositional differences between the samples in the GC×GC- TOFMS. All resolved integrated peak areas of the compounds were mean-centered and normalized.

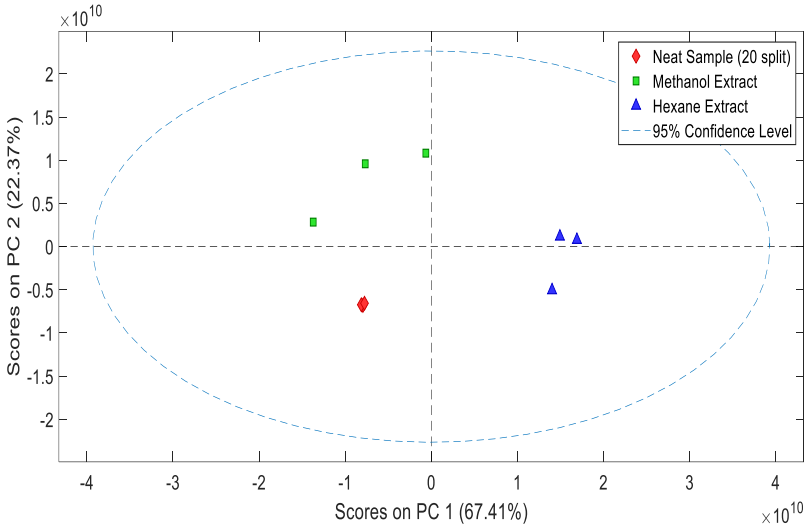



**Figure 26.** Principal Component Analysis (PCA).

The Methanol Extract, Hexane Extract, and the contaminated water all formed clear grouping/clustering in the resulting PCA score plot (Figure 26). The first principal component (PC1) is 67.41% of all the variance, and the second principal component (PC2) captured an additional 21.6%. Since hydrocarbons, phenols, background organics, and other products mixed together, the Original Sample covered the largest area. It proves the greatest chemical complexity and a wide compounds spectrum. While eliminating high matrix interferences, the Methanol Extract was able to recover the majority of polar analyte classes and some non-polar compounds, as shown by its tight clustering, which is slightly offset from the Original Sample. Even though

compounds of the Hexane Extract were tightly clustered, it looked further away from the other clusters, which indicates a narrower nonpolar hydrocarbons portion.

### 4.2.3 Mass-to-charge ratio selectivity in GC plots

The ChromaTOF software (LECO Corp.) was used to create mass-to-charge ratio(m/z) selective plots to help with the better visualization and identification of chemical classes in the complex GC×GC-TOFMS chromatograms. To reduce overlap and highlight the compositional differences between the samples, this method separates specific fragment ions for certain chemical classes. The selective ion channels were used as follows: 1- m/z = 57,71, and 85 for n- and branch alkanes;2- m/z = 124,138,151, and 164 for dicycloalkanes; 3 – m/z = 128,141,155,169, and 183 are for naphthalenes.

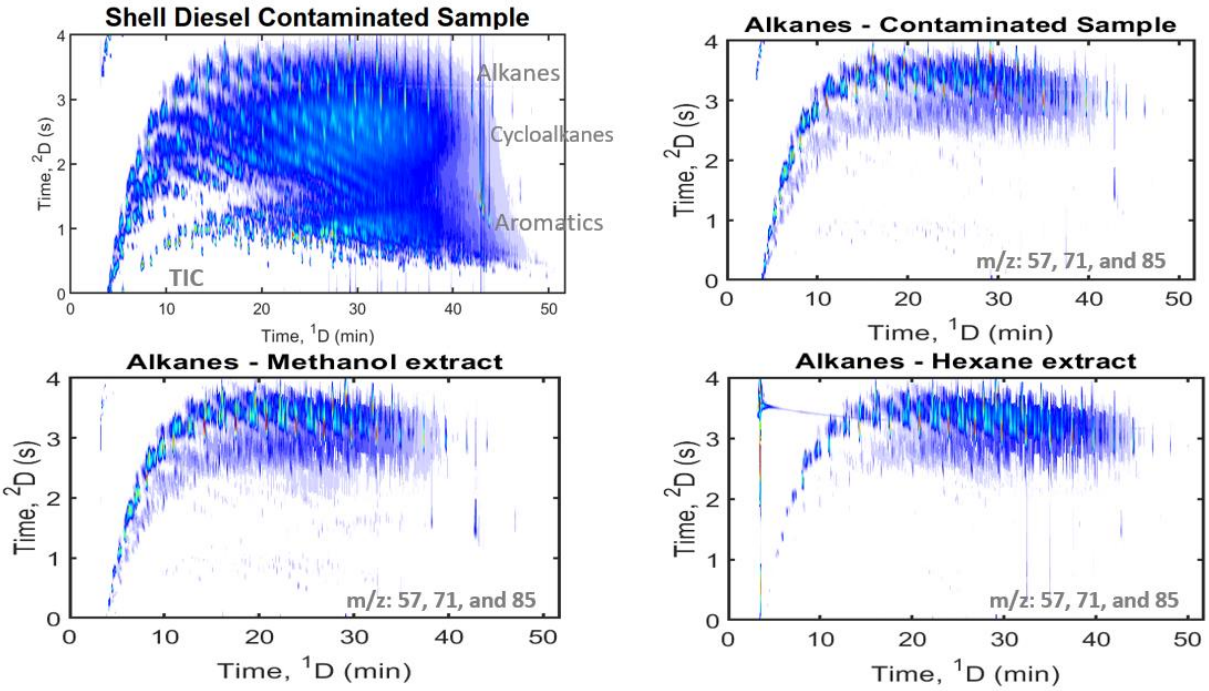



**Figure 27.** Analytical Ion Chromatograms – Alkanes.

Figure 27 illustrates mass channels 57, 71, and 85 and the Original Contaminated sample chromatogram to visually compare with. With less sharp peaks and a wider dispersion. The Contaminated water sample formed a similar, but weaker pattern with partial aliphatic hydrocarbon decomposition. The Hexane Extract mostly highlighted the high intensity non-polar compounds. The Methanol Extract recovered almost the whole alkane pattern, short and long chain hydrocarbons, therefore offering a broader presentation of the hydrocarbon fingerprints in the Original Contaminated Sample.

An important intermediate polarity analyte in the diesel spectrum, dicycloalkanes are separated by the mass channels 124, 138, 151, and 164 illustrated in Figure 28. The Shell Diesel reference demonstrates a dense middle retention range between 12 and 38 minutes in 1D and between 1.5 and 3.5 seconds in 2D. And it can be seen that this region was reproduced by both the Methanol and Hexane extracts. The Contaminated Sample reflects this area with less concentrated perspective and wider dispersion.

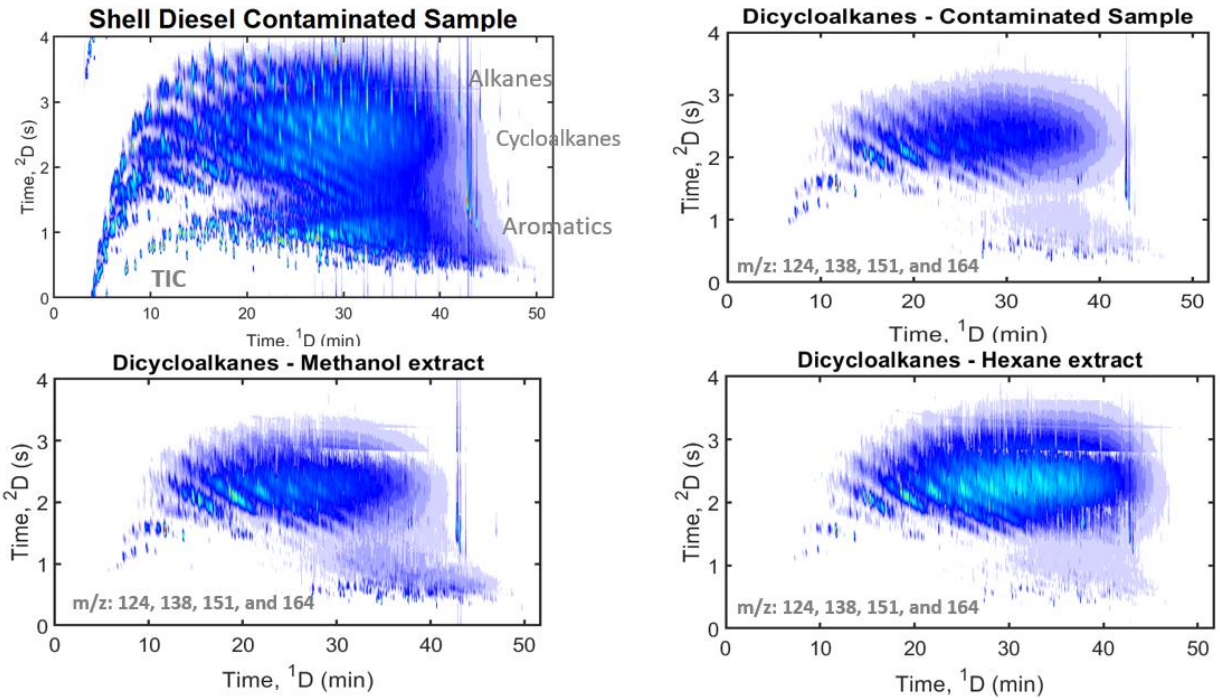



**Figure 28.** Analytical Ion Chromatograms – Dicycloalkanes.

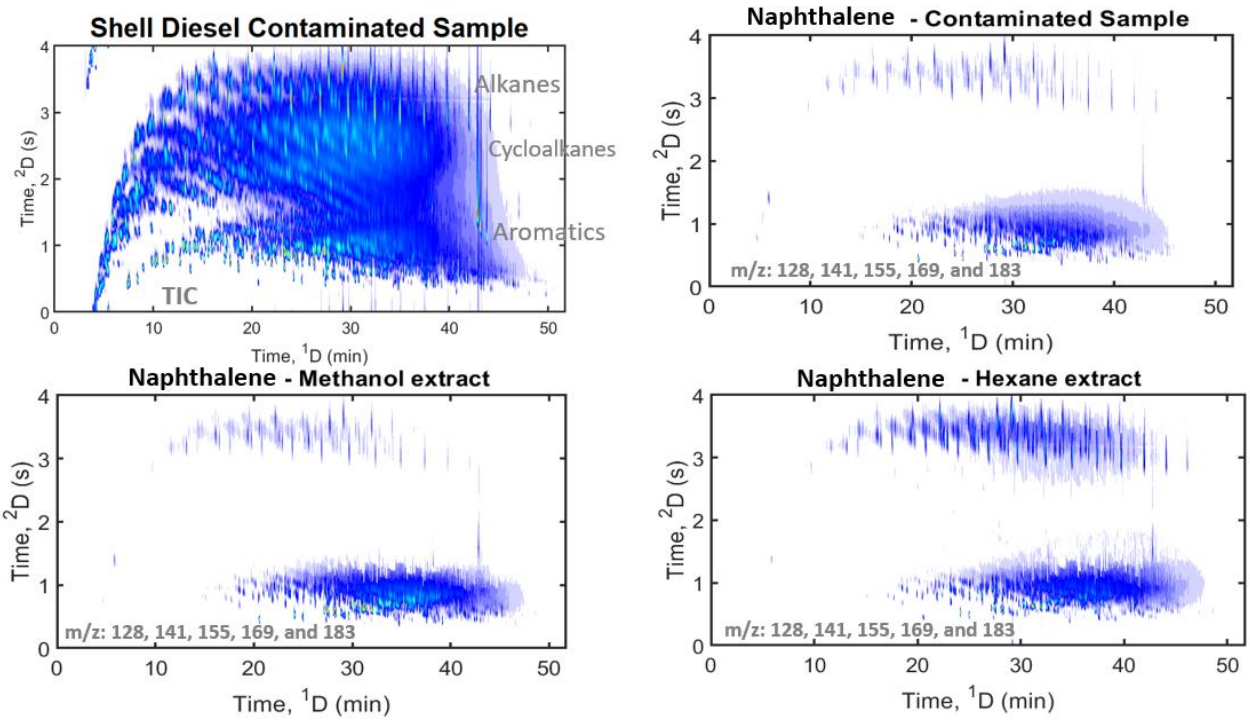



**Figure 29.** Analytical Ion Chromatograms – Naphthalene.

Mass channels of 128,141,155,169, and 183 detected into chromatograms in Figure 29 were less intense in all samples; nevertheless showed signs of semi-volatile aromatic compounds. The higher signal distribution in both dimensions showed that the Methanol Extract reproduced a wide range of naphthalenes. Whereas the Hexane Extract included more other species as well and less concentrated napthalenes.

The chemical composition in the Contaminated Water sample is more precisely captured by the Methanol elution. According to the mass selective ion chromatograms, it reproduces a broader range of compound classes from oxidized aromatics to saturated hydrocarbons.

## 4.3 Results and discussion

### 4.3.1 Chemometric and Compound Identification Analysis Results

A tile-based Fisher Ratio (F-ratio) analysis was used to statistically identify areas of the GC×GC chromatograms with the most significant differences within Shell Diesel, Contaminated Water, and Methanol Extract. This method segments the chromatogram into smaller retention times known as "tiles" and then compares the variation of signal intensity within each tile and groups [20].

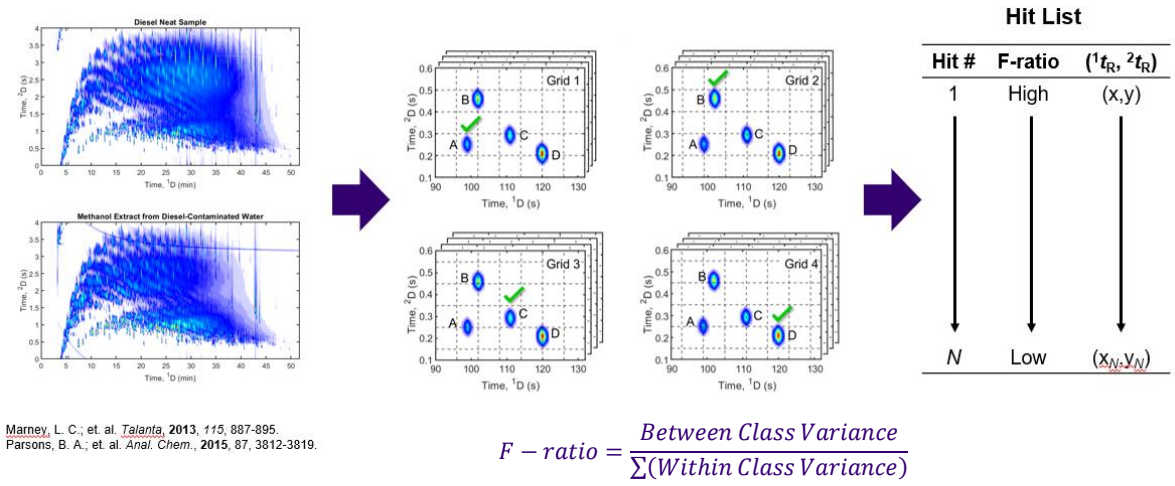



**Figure 30.** Tile-Based Fisher Ratio (F-ratio) Analysis.

The tiles with higher F-ratio values are associated with certain areas where the methanol extract differs from the diesel, and a low F-ratio indicates an area where they are similar. The detected signal class-to-class variation was divided by the total of the signal within class variance to get the F-ratio (equation in Figure 30).

All chromatograms were normalized and color-coded to visually analyze the difference (Figure 31). Chromatographic areas where samples are statistically different are identified using the tile-based F-ratio method. Initially, a total of 2593 tiles were identified as statistically different. Once noise and slight retention time shifting, which cause false positive signals and redundant hits

were eliminated, a list of 1216 significant tiles resulted, from which one typical mass channel of 120 was chosen to observe closely in different replicates.

Within each replicate of the Original Neat Shell Diesel sample (Figure 32 A) and the Methanol Extracts (Figure 32 B), the peak location illustrates notable reproducibility. Nevertheless, the Methanol Extract peaks show lower intensity, suggesting a loss of some compounds during the extraction process. The signal intensities of the Methanol Extracts are around half that of the Original Neat Sample replicates. A quantitative comparison bar chart in Figure 33 supports these observations.

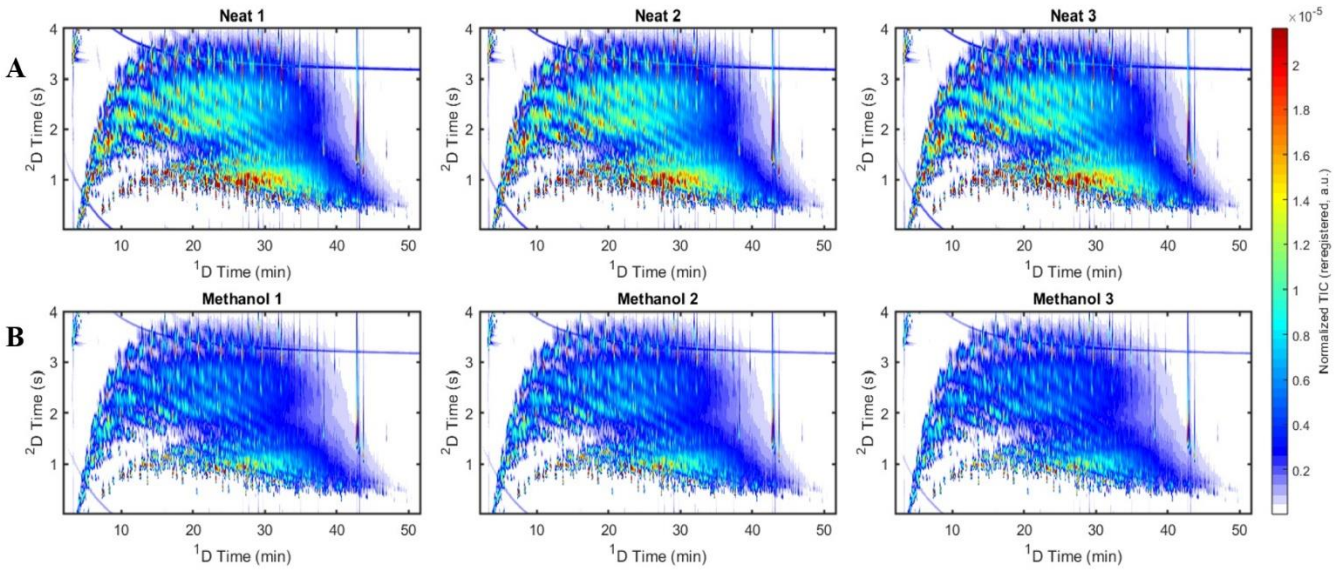



**Figure 31. GC**×GC-TOFMS chromatograms comparing three replicates of (A) Original Neat Shell Diesel and (B) Methanol Extract.

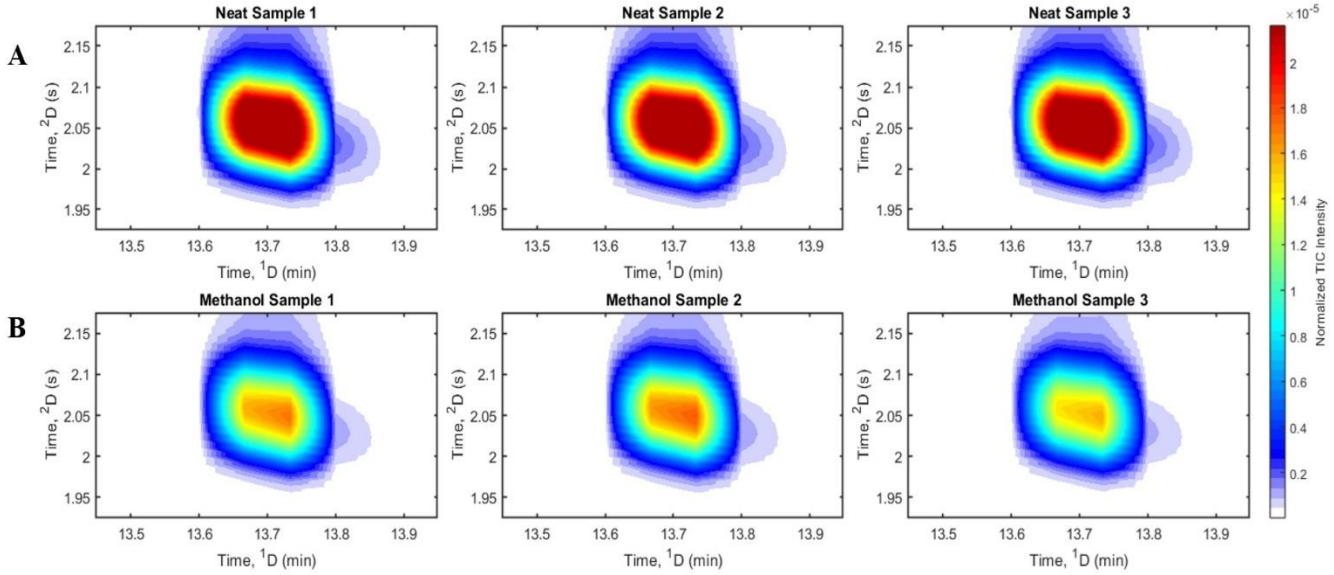



**Figure 32.** Zoomed-in m/z 120 peak of the 1-ethyl-2-methylbenzene in GC×GC-TOFMS chromatograms comparing three replicates of (A) Original Neat Shell Diesel and (B) Methanol Extract.

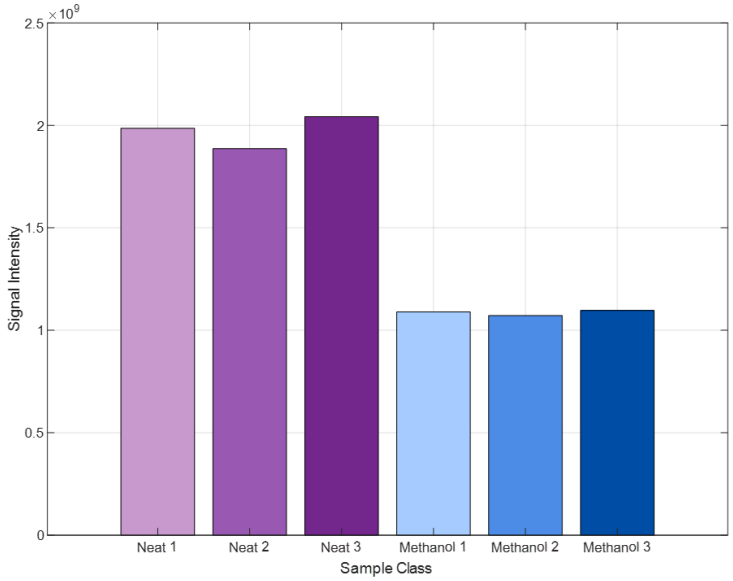



**Figure 33.** Peak Intensity Comparison of Original Neat Samples vs Methanol Extracts.

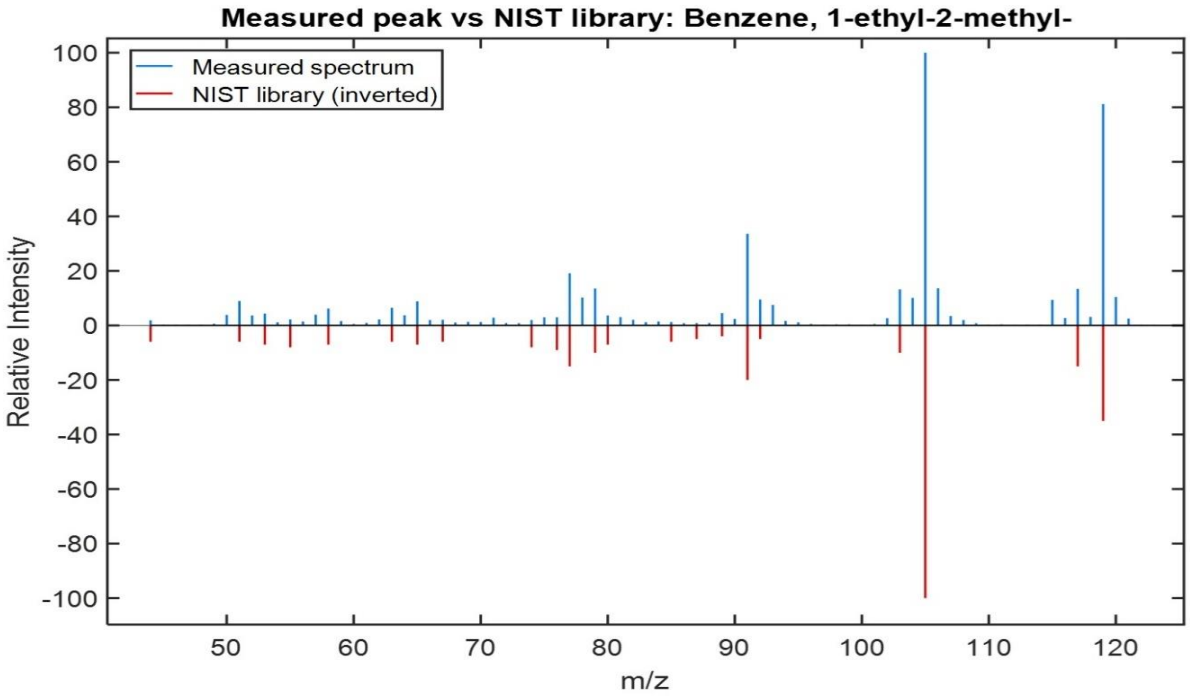



**Figure 34.** Comparison of the 1-ethyl-2-methylbenzene data and the NIST library data of m/z = 120.

The identification of 1-ethyl-2-methylbenzene was confirmed when the laboratory spectrum almost matches the NIST library spectrum, as comparatively illustrated in Figure 34, showing prominent peaks at m/z 105 and 120. Specific areas, such as alkanes, smaller aromatics, and larger aromatics abundances in the Original Neat Sample, and the two Extracts are summarized in Figure 35. The GC×GC plots were used to identify these zones, and quantitative comparisons were made by integrating their total signal intensities. Table 5 illustrates the numerical values for the related area.

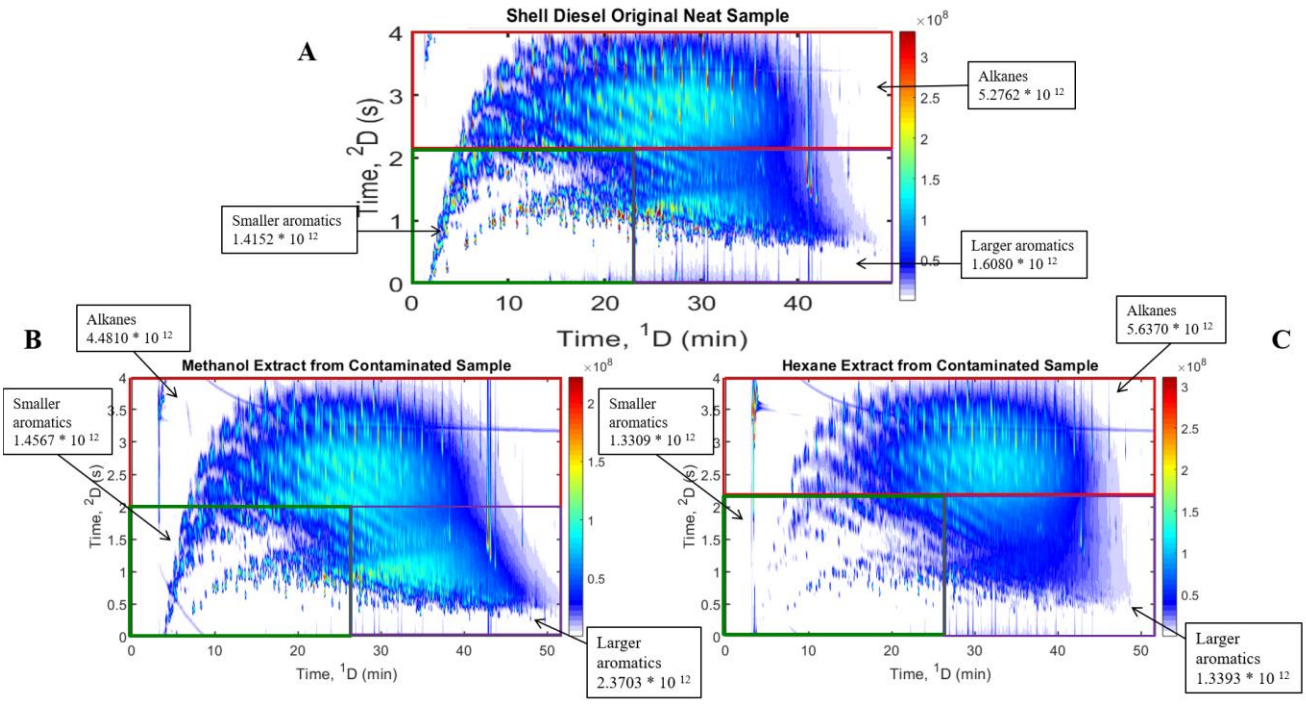



**Figure 35.** GC×GC-TOFMS chromatograms show class areas for alkanes, smaller and larger aromatics in the (A) Original Neat Shell Diesel, (B) the Methanol Extract, and (C) the Hexane Extract with signal intensities for each area. Compound classes are represented by red, green, and blue integrated zone boxes.

| | **Alkanes** | **Smaller aromatics** | **Larger aromatics** |
|---|---|---|---|
| **Neat Original Shell Diesel** | $5.2762 \times 10^{12}$ | $1.4152 \times 10^{12}$ | $1.6080 \times 10^{12}$ |
| **Hexane Extract** | $5.6370 \times 10^{12}$ | $1.3309 \times 10^{12}$ | $1.3393 \times 10^{12}$ |
| **Methanol Extract** | $4.4810 \times 10^{12}$ | $1.4567 \times 10^{12}$ | $2.3703 \times 10^{12}$ |
| Normalized total TIC for each sample chromatogram: $8.3128 \times 10^{12}$ | | | |

**Table 6.** Integrated signal intensities for hydrocarbon classes in Neat Original Shell Diesel and Methanol, Hexane Extracts.

The neat sample showed the most extensive distribution in alkanes, followed by larger and then smaller aromatics. The Hexane Extract retained this pattern, yielding slightly increased alkane number, but showing almost the same number of aromatic groups. Alternatively, the Methanol Extract balanced distribution was as follows: alkane abundance reduced to ~ 0.85 of the Original Neat sample, larger aromatics rose significantly (1.48), and smaller aromatics were still comparable (1.10). These ratios, in Table 6, highlight that Methanol Extract favors the more polar, bigger aromatic molecules, whereas Hexane Extract performs as a non-polar solvent that recovers alkanes mostly. Overall, it supports the trend seen in GC×GC chromatograms and statistical analysis earlier.

| | **Alkanes** | **Smaller aromatics** | **Larger aromatics** |
|---|---|---|---|
| **Neat Original Shell Diesel** | 1 | 1 | 1 |
| **Hexane Extract** | 1.0494 | 1.0082 | 0.8334 |
| **Methanol Extract** | 0.8492 | 1.1022 | 1.4764 |

**Table 7.** Relative abundance ratios of alkanes, smaller and larger aromatics compared to Original Neat Diesel.

# Chapter 5. Conclusion and Future Directions

In this thesis work, Solid Phase Extraction with Gas Chromatography with Quadrupole Mass Spectrometer and Comprehensive Two-Dimensional Gas Chromatography with time-of-flight mass spectrometry were used to analyze a contaminated water sample with diesel. 1D chromatography results showed that the compounds overlapped mostly in the middle of the chromatogram, making it difficult to identify and distinguish. Adding two-dimensional GC-TOFMS produced more structured chromatographic patterns. This clustered visually hydrocarbons like alkanes, aromatics, and cycloalkanes.

The comparison of extracts showed that hexane reproduced a diesel pattern and identified nonpolar aliphatic hydrocarbons. Methanol Extract reproduced closely the Contaminated Water Sample compound. These results were proved further by Solid Phase Extraction and tile-based statistical analysis. Overall, the results highlight the effectiveness of GC×GC-TOFMS when coupled with SPE cartridges for the identification of contaminated complex water samples. F-ratio analysis detected and reduced the chemical difference areas. Additionally, PCA outlined the difference between the Original Neat Diesel Sample and the Extracts.

Future research might aim to minimize background analyte contamination and improve unstable hydrocarbons, using Solid Phase Microextraction (SPME) as a solvent - free alternative to SPE. One more direction might be: to group samples using chemical patterns rather than individual components by using Partial Least Squares Discriminant Analysis (PLS-DA) on the GC×GC-TOFMS data.

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