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Handtekening .....

Anton de Kom University of Suriname

Faculty of Mathematical and Natural Sciences

Department of Chemistry

Isolation and Identification of Nitrophenols in Diesel Exhaust Particles

A comparative study on the exhaust particles generated from combustion of locally produced diesel and imported diesel

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LIST OF ACRONYMS AND ABBREVIATIONS

DEP	Diesel exhaust particles
GC	Gas Chromatography
FID	Flame Ionization Detector
LoD	Limit of detection
LoQ	Limit of quantitation
t_R	Retention time
US EPA	United States Environmental Protection Agency
OSHA	Occupational Safety and Health Administration
PEL	Permissible exposure limit
DCM	Dichloromethane
NP	Nitrophenol
4NP	4-nitrophenol
2NP	2-nitrophenol
3NP	3-nitrophenol
2M4NP	2-methyl-4-nitrophenol
3M4NP	3-methyl-4-nitrophenol
NCP	Nitrocyclopentane
NaOH	Sodium hydroxide
H_2SO_4	Sulfuric acid
Na_2SO_4	Sodium sulfate
ppm	Parts per million
ppb	Parts per billion
ppt	Parts per trillion

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ABSTRACT

Nitrophenols, classified as one of the most important toxic anthropogenic organic components in the atmosphere, are mainly released through combustion of diesel. Nitrophenols extracted from diesel exhaust particles (DEP), followed by analysis on a gas chromatograph coupled with mass spectrometry (GC-MS) system, showed that the most abundant nitrophenols in DEP are 4-nitrophenol (4NP), 2-methyl-4-nitrophenol (2M4NP), and 3-methyl-4-nitrophenol (3M4NP). These components are believed to cause vasodilation, as well as exhibiting anti-androgenic and estrogenic activity in humans. [1, 3, 10, 13]

The goal of this study was to determine the presence and content of 4NP, 2M4NP, and 3M4NP in DEP samples taken from three types of diesel used in Suriname, on a gas chromatograph coupled with flame ionization detector (GC-FID), equipped with a 5% - Phenyl Methyl Siloxane capillary column.

The GC-FID system was accurately calibrated and validated as suggested by Agilent Technologies Inc. in Analysis of Phenols by Gas Chromatography according to EPA 8040A. The standards that were purchased were of purity >98%. Sampling of DEP from locally produced, imported, and blend diesel was done directly from the exhaust pipe of a diesel engine, through an impinger sampling system containing a solution of 1.0 N NaOH, at a flow rate of 3.0 L/min for 10 minutes, followed by extraction with dichloromethane at pH 2, and subsequent analysis of the concentrated samples on the HP 6890Series Gas Chromatograph equipped with a 5% Phenyl Methyl Siloxane capillary column, and coupled with a 6890 FID.

The nitrophenol components identified through their retention times in both separate standard solutions, and in nitrophenol mix solutions, showed good reliability, with the relative standard deviation of the retention time's mean reported as <0.22%, <0.14%, and <0.12% for 4NP, 3M4NP, and 2M4NP, respectively. The relative standard deviation of the peak area of the nitrophenol components were below 6.29%. The limit of detection (LoD) of the method were determined to be as low as 4.78 ppm, 3.41 ppm, and 2.50 ppm, while the limit of quantitation (LoQ) of the method were determined to be as low as 7.58 ppm, 10.32 ppm, and 14.48 ppm for 4NP, 3M4NP, and 2M4NP, respectively. Recovery % of the nitrophenols in spiked samples, ranged between 77.1% - 93.0%.

However, with the instrument of use, 4NP, 3M4NP, and 2M4NP could not be quantitatively identified in the region <LoD, in any of the samples. Hence why the concentration of these components and the concentration ratios between these components could not be determined, as well as the ratio of nitrophenol content in each type of diesel.

CHAPTER 1: INTRODUCTION

General Description of Areas of Concern

Urban air pollution has become a growing concern for our society. Mainly due to the fact of it affecting the health and wellbeing of humans. A major source of urban air pollution is the exhaust gases generated from automobiles and other combustion vehicles. Of this, a large percentage is exhaust gas generated from the combustion of diesel. [1]

Diesel exhaust is not only a contributor to urban air pollution, but it also has adverse health effects on humans. Emission from diesel motors contains a complex mixture of thousands of organic components, either in the gaseous phase or in particulate form. Diesel exhaust particles (DEP) contain a center core made up of elemental carbon and adsorbed organic compounds. [2] The particles in diesel exhaust, such as the organic components, can be adsorbed easily, and after inhalation can result in deposition in the lungs, because they are very fine in size and have a large surface area. Health risks due to exposure to DEP include acute irritation, allergic rhinitis, bronchial asthma-like diseases, disruption of endocrine function, respiratory and asthma-like symptoms as well as aggravation of allergenic responses to known allergens. Long-term exposure to DEP is known to increase the risk of lung cancer. [3, 4] Diesel exhaust particles (DEP) have an important endocrine-disrupting effect of having a potentially adverse impact on the reproductive system of males. It was previously reported that DEP could suppress spermatogenesis in adult mice, rats, and growing rats. The anti-androgenic effects of DEP could have been partly responsible for the suppression of sperm production since these particles contain carbon nuclei that can adsorb a large variety of chemical organic components such as polyaromatic hydrocarbons (PAHs), nitro-aromatic hydrocarbons, heterocyclics, quinones, aldehydes, and aliphatic hydrocarbons. Some of the organic components found in DEP are known to be strong mutagens and carcinogens, and that they can also induce tumors in the respiratory tracts of animals. The effects of air pollutants that originate from diesel exhaust, on human health, are a serious problem for society, especially in large cities. Vehicles that use diesel, emit up to 2 – 20 times more nitrogen oxides, and up to 30 – 100 times more particles than vehicles that use gasoline. [1, 5]

One group of organic components that are found in DEP are nitrated phenols or nitrophenols. Nitrophenols are a family of aromatic compounds with both nitro (-NO_2) and hydroxyl groups (-OH) connected to a benzene ring. These compounds are among the most important classes of toxic anthropogenic organic compounds that can be found in the atmosphere, and they are of environmental concern, because of their high phytotoxic activity enhanced by long-term chemical and photochemical stability. Nitrophenols are listed as priority pollutants by the US Environmental Protection Agency (US EPA), because of their great potential of carcinogenesis, teratogenesis, and mutagenesis. [6, 7] The study of nitrophenols has become important in the last couple of years for environmental analysis, due to their detriment and vast scale distribution in the environment. The presence of nitrophenols in the environment is related to both natural processes and anthropogenic activity. One major source of emission of nitrophenols to the environment is through the combustion of diesel in automobiles and other combustion vehicles. [8, 9, 10]

Delimitation of the study:

Although there are many sources by which nitrophenols enter the environment, the focus of this study will be the nitrophenols generated from the combustion of diesel and can be found in the exhaust particulates of the diesel. The presence of nitrophenols in the particulate matter of diesel exhaust was previously reported, as well as the effect it could have on human health.

[3, 11] Of the mononitrophenols, 4-nitrophenol (4NP) was found to be the most abundant in DEP compared to its isomers, 2-nitrophenol (2NP) and 3-nitrophenol (3NP). As reported in an earlier study on the presence of nitrophenols in gaseous air samples, 4-nitrophenol was the most abundant among the mononitrophenols, ranging in concentration from 1.2 – 30 ng/m^3. Other nitrophenols were observed in concentrations below 10 ng/m^3. [12] Apart from 4-nitrophenol (4NP), the nitrophenols that were also identified and isolated from diesel exhaust, and were the most abundant are 2-methyl-4-nitrophenol (2M4NP), and 3-methyl-4-nitrophenol (3M4NP). These compounds not only account for a large percentage of the nitrophenols found in DEP, but they were also found to cause vasodilation, as well as exhibiting anti-androgenic activity. One of the nitrophenols that was isolated from DEP, 3-methyl-4-nitrophenol (3M4NP) also exhibited estrogenic activity. [1, 3, 13]

Significance of the Problem

Although there are reasons to believe that the nitrophenols found in DEP could have effects on the health of humans, including causing vasodilation and exhibiting anti-androgenic and estrogenic activity, not enough research has been done on this topic. As for now, there is no international standard or law that states that the maximum limit of emission or exposure to nitrophenols from DEP should not exceed a specific number. But for substances of which there is no international standard or limit, it is common to use the OSHA permissible exposure limits (PELS) for total dust, which is 15 ppm. This limit covers all inert or nuisance dust, whether mineral, inorganic, or organic, not listed specifically by substance name. [14]

It is also not known what the exact amount of nitrophenols emission from diesel combustion is yearly. Especially in Suriname, no previous research has been done on the presence and content of nitrophenols in exhaust particles of diesel that is used in our country, whether that be locally produced or imported. Hence it would be beneficial for society, lawmakers, diesel producers, and diesel importers to know if nitrophenols are also emitted from the combustion of the diesel that is used in our country. And if so, to be informed of the health risks associated with short-term and long-term exposure to nitrophenols, and to propose mitigation measures if it were to be that nitrophenols are indeed found in the diesel that is used in Suriname, and that the nitrophenols content exceed the OSHA PEL.

In this research the focus will be on identifying, and determining the content of the nitrophenols that are the most abundant in DEP, and that were in previous studies identified as vasodilation, anti-androgenic, and estrogenic compounds. These nitrophenols are 4-nitrophenol, 2-methyl-4-nitrophenol, and 3-methyl-4-nitrophenol. These components will be extracted from DEP samples taken directly from diesel exhaust from the exhaust pipe of a diesel engine, after the combustion of three types of diesel: locally produced diesel, imported diesel and a blend. The sampling of the DEP will be done using an impinger (bubbler) sampling system containing a solution of sodium hydroxide, through which exhaust air will be bubbled. Hereafter, extractions with dichloromethane will be done, before acidifying the solution to a pH of 2. Upon extraction with dichloromethane again, and concentrating the subsequent extract, the sample will then be analyzed on its nitrophenols content on a gas chromatography instrument equipped with a flame ionization detector (GC-FID). Studies done on the presence of nitrophenols in DEP, found in the literature, were all done through analysis on GC-MS systems. In this study it will be determined if a GC-FID system is suitable to identify the presence of nitrophenols in DEP samples, due to the unavailability of a GC-MS system.

Problem statement:
Nitrophenols most abundant in DEP have been identified as vasodilation, anti-androgenic, and estrogenic compounds. There has been no previous research in Suriname on the presence of these nitrophenols in the exhaust particles generated from the combustion of diesel that is used in our country; locally produced and imported diesel. Because of the health risks associated with nitrophenols found in DEP, this research would be beneficial for the Surinamese society, lawmakers, and also for those that produce and import the diesel, to know if the combustion of these types of diesel also results in the presence of nitrophenols in the exhaust particles and to what extent.

Key research question formulated for this study:
Are there nitrophenols present in the exhaust particles of locally produced and imported diesel, and if so what is the concentration ratio between the nitrophenols that are found?

Sub-questions that are formulated in support of the key-research question:

1. In regard to the method:
   Is the analysis method using a GC-FID equipped with a 5% - Phenyl Methyl Siloxane capillary GC column suitable for the identification of nitrophenols from DEP?
   What are the best operating conditions of the GC-FID to analyze these components?
   What are the LoD and LoQ of the method?
2. Analysis:
   What is the content of 4-nitrophenol, 2-methyl-4-nitrophenol, and 3-methyl-4-nitrophenol that can be found in the DEP from diesel that is used in Suriname?
   What are the concentration ratios between these nitrophenols in the different DEP samples?

Objectives:
The objective of this study is to identify the presence of nitrophenols in diesel exhaust particles generated from the combustion of diesel that is used Suriname, by using an impinger sampling system, followed by extraction using dichloromethane and analysis of the samples on a gas chromatograph (GC) equipped with a 5% - Phenyl Methyl Siloxane capillary GC column (50.0 m × 320 μm × 0.17 μm), and coupled with a flame ionization detector (FID).

CHAPTER 2: LITERATURE REVIEW

This chapter gives a description of gas chromatography, which is an integral part in this study. As well as some background information on nitrophenols and their presence in DEP, and also a brief description on how previous studies on the presence of nitrophenols in DEP were carried out, and what resulted from those studies.

2.1 Gas Chromatography

Gas chromatography (GC) is an analytical separation technique used to analyze volatile components in the gas phase. A typical GC instrument consists of an injection port, a separation column, a carrier gas flow control equipment, an oven, and heaters for maintaining temperatures of the injection port and the column, an integrator chart recorder, and a detector. A diagram of a typical GC instrument is given in *Figure 1*. In GC, the analytes in a sample are vaporized to separate the different analytes in the sample, and these are then distributed between the stationary phase (separation column) and the mobile phase (carrier gas). To separate the different components in a sample, a sample containing the components of interest is injected into the injection port from where it will be vaporized. The vaporized sample will then be carried through the column by the carrier gas. The column is usually packed with silica and coated with a liquid. The components in the sample that are less soluble in the liquid will elute faster from the column, compared to components that have a higher solubility in the liquid. GC can be used in many different fields such as pharmaceuticals, cosmetics, and environmental toxins. Air samples can also be analyzed using GC. Most of the time, gas chromatography (GC) coupled with a flame ionization detector (FID) is used to determine the components of a given air sample. Although other detectors are also applicable and can be used as well, FID is the most appropriate because of its sensitivity and resolution and also because it can detect very small molecules as well. Gas chromatography coupled with a mass spectrometer (GC/MS) is also another useful method that can determine the components of a given mixture using the retention times and the abundance of the samples. [15, 16]

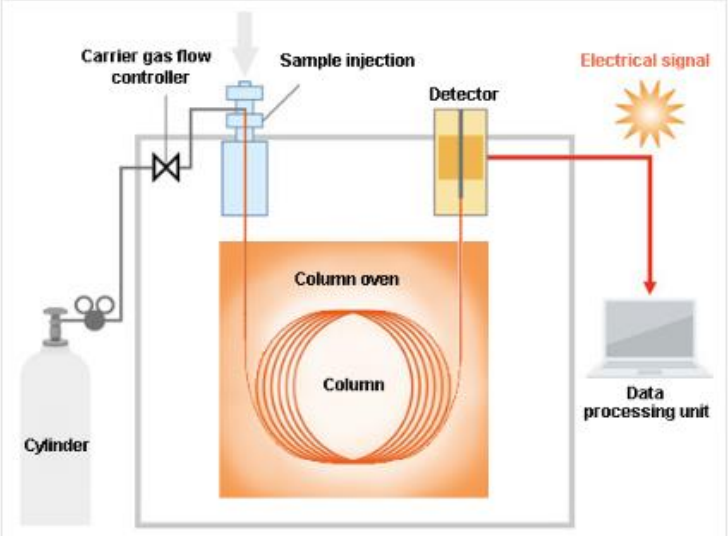

Figure 1. Diagram of a Gas Chromatography Instrument

Injection

For analytical chromatography, the injected volume is typically 0.1 – 2.0 μL of the liquid sample. The vaporized sample is swept by the carrier gas, from the injection port into the chromatography column.

In split injection, the sample is introduced in a hot liner where only a part of the sample enters the column, while the rest goes out via the split vent (the sample amount is split). The amount of sample that enters the column depends on the actual volumetric flow that passes the split-point (=column inlet). The proportion of sample that does not reach the column is called the split ratio, and typically varies from 1:50 to 1:600.

In splitless injection, the majority (~80%) of the sample is transferred onto the column, and a little fractionation occurs during injection. This is especially useful for trace analysis. Transfer times are slower and peaks are broader when compared to split injection. During the splitless injection time, the oven is set at a temperature 20°C below the (atmospheric) boiling point of the solvent. The injection time is the time needed to empty the liner volume, which usually is between 60 and 90 seconds. After the injection time has passed, the split-vent is opened and the liner is flushed (this will take out the last molecules of solvent), generating a very sharp solvent peak. At the same time the oven is programmed and the separation starts. [17, 18]

Carrier Gas

The carrier gas used in GC must be a dry, free of oxygen, and chemically inert gas. The most commonly used carrier gasses are helium, hydrogen, and nitrogen. Helium has a large range of flow rates, is compatible with many detectors, and it is also safer to use. Nitrogen and hydrogen are used depending on the desired performance and the detector that is used. On the FID, hydrogen and helium are most commonly used, as they provide a shorter analysis time and lower elution temperatures of the sample due to their low molecular weights and higher flow rates. A carrier gas is required in a GC system to flow through the injector and push the gaseous components of the sample onto the GC column, which leads to the detector. [16]

Column Oven

The oven can be operated by isothermal programming or by temperature programming. In isothermal programming, the temperature of the column is held constant throughout the whole separation process of the sample. For isothermal programming, the optimum column temperature is about the middle point of the boiling range of the sample, and it works best only if the boiling range of the sample is narrow. In temperature programming, the temperature of the column is increased in steps or continuously throughout the separation process. Temperature programming works well for samples with a broad boiling point range. [16]

Separation Column

There are two types of columns; the open tubular columns (capillary columns) and the packed columns. Of the open tubular columns, there are two basic forms; the wall-coated open tubular (WCOT) column and the support-coated open tubular (SCOT) column. WCOT columns are capillary tubes with a thin layer of the stationary phase coated along the column walls. SCOT columns are coated with a thin layer of adsorbent solid on the walls. The adsorbent solid is then treated with a liquid stationary phase. WCOT columns have a greater column efficiency, while SCOT columns are capable of holding a greater volume of stationary phase due to its greater sample capacity. WCOT columns are made of glass, T316 stainless steel, aluminum, copper, or plastics, and each material has its own relative merits depending upon the application. The packed columns are made of glass or metal tubing, densely packed with solid support like diatomaceous earth. These types of columns have a larger diameter than open tubular columns,

due to the difficulty of packing the tubing uniformly, and they also have a limited range of length. This is why packed columns can only achieve about 50% of the efficiency of a comparable WCOT column. Over time, the diatomaceous earth packing is also deactivated due to the semi-permanent adsorption of impurities within the column. [16]
The column that is used for this study is the HP-5 5% Phenyl Methyl Siloxane capillary column, which is a nonpolar column that is applicable for general purposes and a variety of applications. This column has very low column bleeding, and the maximum operable temperature is 325 °C. [19]

Detector

The detector is located at the end of the column and provides a quantitative measurement of the different components in the sample, as they elute from the column. Any property of the gaseous sample, different from the carrier gas, can be used as a detection method. Each detector has two main parts that when used together they serve as transducers to convert the detected property changes into an electrical signal that is recorded as a chromatogram. The first part is the sensor which is placed as close to the column exit as possible to optimize detection. The second is the electronic equipment used to digitize the analog signal so that a computer can analyze the acquired chromatogram. The sooner the analog signal is converted into a digital signal, the greater the signal-to-noise ratio becomes, as analog signals are easily susceptible to many types of interferences. An ideal GC detector is distinguished by several characteristics. The first requirement is that it has adequate sensitivity to provide a high-resolution signal for all components in the sample. The quantity of the sample must also be reproducible and many columns will distort peaks if enough sample is not injected. An ideal column will also be chemically inert and it should not alter the sample in any way. Optimized columns will be able to withstand temperatures in the range of -200 °C to at least 400 °C. Also, such a column would have a short linear response time that is independent of flow rate and extends for several orders of magnitude. Furthermore, the detector should be reliable, predictable, and easy to operate. [16] The detector used in this study is a flame ionization detector (FID). This detector is one of the most generally applicable and most commonly used detectors in gas chromatography. An FID is unresponsive to air, water, carbon dioxide, ammonia, hydrogen sulfide, sulfur dioxide, and most carrier gases used in GC, but it readily responds to compounds containing carbon and hydrogen. In a FID, after the sample exits the column, the sample is directed at an air-hydrogen flame, which creates cations and electrons. A collector electrode is used to collect the created ions and it produces a small current. The electrical conductivity of the flame is very sensitive to the presence of organic vapors and an FID can respond to very small amounts of each component that elutes from a capillary GC column. The use of an FID is advantageous because the detector is unaffected by flow rate, noncombustible gases and water, and this allows the FID to have a high sensitivity and low noise. The unit is both reliable and relatively easy to use. However, this technique does require flammable gas and also destroys the sample. [16]

2.2 Quality Assurance and Method Validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose and quality assurance is the term for quantitative indications that demonstrate whether data requirements have been met. Some specifications for quality assurance and method validation include: sampling requirements, blank values, accuracy, recovery of fortification, precision, repeatability, reproducibility, sensitivity, calibration and linearity, limit of quantitation (LoQ), and limit of detection (LoD). [18]

Sampling requirements

Representative samples must be collected, and the analyte must be preserved after sample collection. Apart from collecting representative samples, acceptable blank values, which contain all components in the sample but the analyte, should be collected. [18]

Field blank

Field blanks can determine contaminants or analytical errors, or bias, stemming from sample collection and analysis. Such contamination may result from bottles and other glassware, sampling equipment and conditions, preservatives, transportation and storage, or the laboratory analysis. A field blank is a sample containing all components except the analyte that has been exposed to the site of sampling and it is taken through all the steps of the analytical procedure. For the analysis of nitrophenols in DEP, a certain volume of DEP will be sucked through a solution of sodium hydroxide in an impinger, from which the nitrophenols will then be extracted and analyzed. The field blank for this method would be, a solution of sodium hydroxide carried and exposed to the site of sampling, and then taken back to the laboratory, and then taken through all the steps of the analytical procedure. The difference between the field blank and the samples would be that DEP was not sucked through the field blank. According to EPA standards, acceptable blanks have target analyte concentrations less than half of the low limit of quantification. [18, 20]

Accuracy

Accuracy is a measure of the difference between the expectation of the test result and the accepted reference value due to systematic method and laboratory error. It is usually expressed as a percentage and it can be evaluated by measuring the recovery of fortification (spike), which is a known quantity of analyte that is added to a sample to test whether the response to a sample is the same as that expected from a calibration curve. [18]

The recovery of fortification (spike) is calculated with:

Eq 1.
$$\%\, recovery = \frac{(C_{spiked\ sample} - C_{unspiked\ sample})}{C_{added}} * 100$$

Precision

Precision is a measure of the closeness of analytical results that are obtained from a series of replicate measurements of the same measure under the conditions of the method. Precision can be measured by the repeatability and reproducibility of analytical results. Repeatability occurs when samples are analyzed on the same day with the same instrument and the same materials by the same analyst in the same laboratory. While reproducibility is represented by any variation in these conditions (e.g. different days, different instruments, different analysts, or different laboratories). Precision is usually measured as the coefficient of variation or relative standard deviation of analytical results obtained from independently prepared quality control standards [18, 21]

Calibration and linearity

The calibration curve relates the response of an analytical method to known concentrations of an analyte. Usually, in a calibration curve, there is a range over which the response is linear to the concentration of the analyte. This is called the linearity, and the calibration curve should follow a straight line, showing that the response is proportional to the quantity of the analyte. A common measure of the linearity is the square of the correlation coefficient (R^2). This value must be close to 1, and when the value is not close to 1 (say, below 0.98) this must be taken as

a warning and it might then be advisable to repeat or review the procedure. Errors may have been made (e.g. in pipetting) or the used range of the graph may not be linear. R^2 can be measured by [18]

Eq 2.

$$R^2 = \frac{[\sum(x_i - \bar{x})(y_i - \bar{y})]^2}{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}$$

Sensitivity

Sensitivity is the capability of responding reliably and measurably to changes in analyte concentration, and sensitivity is equal to the slope of the calibration curve. [18]

Limit of detection (LoD) and limit of quantitation (LoQ)

The detection limit, also known as the lower limit of detection (LoD) is the smallest quantity of an analyte that can be detected and identified with a given degree of certainty, and that is "significantly different" from the blank. The signal of the LoD should be at least 3 times greater than the noise before it is seen as detectable, but still too small for accurate measurements. The lower limit of quantitation (LoQ) is the smallest amount of analyte that can be measured with reasonable accuracy. The LoQ is at least 10 times greater than the noise. [18]

Use of an internal standard

An internal standard is a known amount of a compound that is different from the analyte, and is added to an unknown sample. The use of internal standards is particularly useful for analysis in which the quantity of the samples analyzed or the instrument response, varies slightly from run to run. The use of an internal standard is also desirable for analysis in which sample loss can occur during any step of sample preparation. [18]

2.3 Background Information on Nitrophenols

Nitrophenols are aromatic compounds with both a nitro ($-NO_2$) and hydroxyl group (-OH) connected to a benzene ring. Nitrophenols are one group of organic components that are in diesel exhaust particles (DEP), and are also one of the most important classes of toxic anthropogenic organic components found in the atmosphere. These organic components are of environmental concern, because of their high phytotoxic activity enhanced by long-term chemical and photochemical stability. [7, 9, 10]

The nitrophenols exist in many forms, one being the mononitrophenols. The mononitrophenols exist in three isomeric forms: 2-nitrophenol (or o-nitrophenol), 3-nitrophenol (or m-nitrophenol), and 4-nitrophenol (or p-nitrophenol). Of the mononitrophenols, 3-nitrophenol is less prevalent in the industry compared to 2-nitrophenol and 4-nitrophenol, and the toxicological data on this isomer is also scarce. Between 2-nitrophenol and 4-nitrophenol, 4-nitrophenol is more abundant in the environment, in both the gas and liquid phase, and experimental studies have shown that this isomer is more harmful to animals, and possibly also to humans, compared to 2-nitrophenol. As for this moment, the background levels (when no apparent sources of pollution are present) of the two prevalent mononitrophenols in air are not known, as well as their atmospheric half-lives. In polluted environments, the concentration of nitrophenols would be understandably higher, but due to the fact of nitrophenols exhibiting good stability, combined with their polarity and low vapor pressure, global dispersion of the nitrophenols could happen, through atmospheric precipitation. [7]

The molecular structures of the mononitrophenols, 2-methyl-4-nitrophenol, and 3-methyl-4-nitrophenol are shown below.

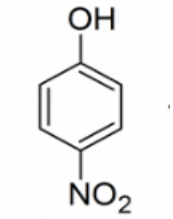

Figure 2. Molecular structure of 4-nitrophenol

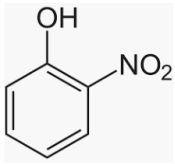

Figure 3. Molecular structure of 2-nitrophenol

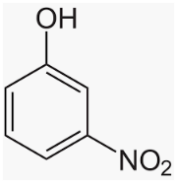

Figure 4. Molecular structure of 3-nitrophenol

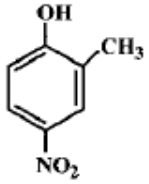

Figure 5. Molecular structure of 2-methyl-4-nitrophenol

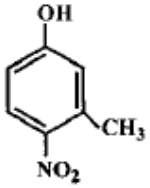

Figure 6. Molecular structure of 3-methyl-4-nitrophenol

Sources of nitrophenols:

The mono-nitrophenols are solely man-made chemicals, with no previous evidence that these compounds are formed through any natural source or any natural process. They either enter the environment directly through production or are formed in the environment through atmospheric photochemical reactions of several aromatic compounds formed from anthropogenic sources. The most likely source of nitrophenols found in air is through direct emission from traffic vehicles, or indirectly via further atmospheric chemistry of traffic-emitted precursors. Especially in urban areas, the most important and biggest source of nitrophenols in air is through emission from vehicular exhaust. In vehicles, nitrophenols are formed as a result of the thermal reaction of fuel with oxides of nitrogen and are then released through the exhaust. Another source of nitrophenols is through homogeneous photochemical reactions of their precursors: benzene, toluene, xylene, and other alkylated mono-aromatics. The exact concentration of nitrophenols that are emitted to the environment from diesel combustion and all of the other sources are however not available as of this moment, but it was found that the nitrophenols in atmospheric air range in the region of nanograms per cubic meter (ng/m^3). [7, 10, 12, 22, 23, 24]

In *Figure 7 and Figure 8*, some of the reactions through which nitrophenols are formed, are given.

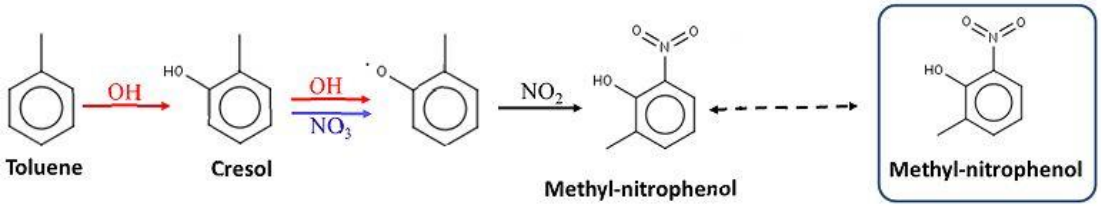

Figure 7. Formation of nitrophenol from toluene (precursor)

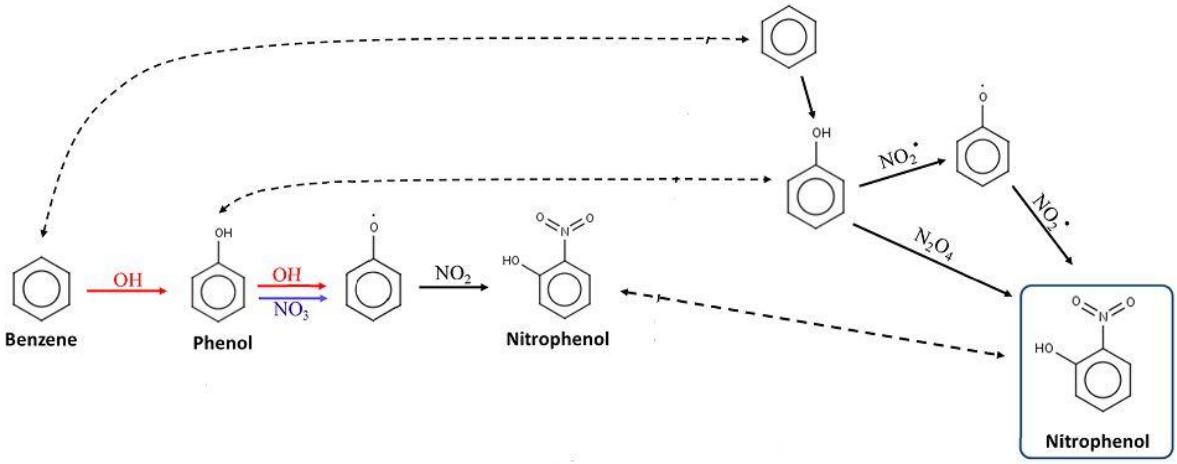

Figure 8. Formation of nitrophenol from benzene (precursor)

2.4 Theory Relevant to the Research Questions

2.4.1 Sampling of Nitrophenols from Air Particulate Matter

A method commonly used for collecting nitrophenols in air is by using an air sampler that uses glass-fiber filters to collect the particulate matter, followed by adsorption cartridges for trapping the volatile components. For multicomponent analysis, however, the best method is by using sample extraction with organic solvent(s) under both acidic and basic conditions. For nitrophenols, which are acidic, the acidic extract is used. In an evaluation of the EPA, a single continuous extraction at a pH of 2 was most efficient for determining both acidic and basic/neutral components in a sample. Additionally, the use of fused silica capillary columns may enhance both the efficiency and detection limits of various components, including nitrophenols, in multicomponent analytical methods. In the studies done on the presence of nitrophenols in DEP, the analyses of the samples were only carried out on GC-MS systems. Detection limit for nitrophenols that were first derivatized before analysis on GC-MS systems, were in the region of 0.5 ppt (ng/L). [3, 7, 23, 25]

2.4.2 Studies on the Presence of Nitrophenols in DEP and Their Health-Effects

The effect a chemical has on your health depends on the dosage and the time that you were exposed to it. The higher the dosage and the longer the exposure time, the more severe the effects on your health will be. [7]

In one of the earliest studies in which nitrophenols were isolated from samples of air particulate matter and vehicular exhaust samples, a method similar to the one used in this research, was applied. During that previous research, a bubbler, otherwise known as an impinger was used. The sampling was done by bubbling vehicular exhaust gas through a 0.5 M 100 ml solution of sodium hydroxide for 15 minutes at a flow rate of 1 L/min. The solution was then extracted twice with ethyl ether, with the aqueous layer then being acidified with sulfuric acid to a pH of 2, and then extracted again with ethyl ether. The collected ethyl ether layer was then washed with distilled water. The resulting solution was dried over anhydrous sodium sulfate, and concentrated, before analysis on GC-MS. The following results were obtained: in samples of air particulate matter the concentrations for 2-nitrophenol and 4-nitrophenol were less than 0.05 – 3.9 µg/g, and 5.1 – 42 µg/g, respectively, while the concentrations for 2-nitrophenol and 4-nitrophenol in a diesel engine were 37 $\mu g/m^3$ and 14.5 $\mu g/m^3$, respectively. The research for the vehicular exhaust was done under engine idling conditions. [26]

In research on the health effects due to exposure to nitrophenols, it was shown that rats that breathed dust of 4-nitrophenol for two weeks, showed signs of a blood disorder that reduced the ability of the blood to carry oxygen to the organs and tissues. However, after the exposure was stopped the abnormalities disappeared after a couple of days. Other experimental studies have also shown that 4-nitrophenol is more harmful than 2-nitrophenol to animals. So it is likely that humans could also develop the same type of blood disorders that these animals did, after continuous exposure to nitrophenols. [7]

In one study, done in Japan, nitrophenols that are responsible for causing vasodilation in rats, were isolated and identified from DEP. These were 4-nitrophenol (4NP), 3-methyl-4-nitrophenol (3M4NP), 2-methyl-4-nitrophenol (2M4NP), 4-nitro-3-phenylphenol (4N3PP) and 3-nitrophenol (3NP). The concentration of the aforementioned compounds in DEP was 15, 28, 32, and 15 mg/kg DEP, respectively. Due to the scarcity of 3-nitrophenol, they were unable to determine its content in DEP, but it was for certain much less than the other nitrophenols. While in another study, regarding the same topics, it was found that for 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol, and 4-nitrophenol the concentration in the sampled DEP was 34, 28, and 15 mg/kg DEP, respectively. It was also proven that these components indeed exhibit vasodilation activity. It was furthermore mentioned that most of the particulate matter in urban ambient air pollutants in Japan is due to diesel exhaust. And it is believed that these nitrophenols could also cause vasodilation in humans, just as in animals. The results obtained from these studies indicated that due to diesel exhaust emission, and in some percentages also through various degradation of pesticides, nitrophenols accumulate in the atmosphere, which could result in serious effects on human health due to disturbance on the cardiovascular system. [11, 27]

Through an improved version of a previously existing method [11], nitrophenols were isolated and identified from DEP, and the concentrations of 4-nitrophenol, 3-methyl-4-nitrophenol, and 2-methyl-4-nitrophenol were 169, 79.1, and 37.4 mg/kg DEP, respectively. The previous method involved successive solvent extractions followed by tedious, and repeated chromatography, which resulted in poor fractionation and a significant loss in accuracy and reliability. The new method, however, involved an acid-base extraction, with in situ derivatization, and analysis by GC-MS, which resulted in significantly higher concentrations of nitrophenols in DEP, than was previously reported. In this study, it was mentioned that the detection limit for derivatized nitrophenols was determined to be 0.5 ppt. [3]

In other studies, it was demonstrated that 3-methyl-4-nitrophenol, isolated from DEP, has anti-androgenic activity on the male reproductive system of rats. This showcases that it is also highly possible that 3-methyl-4-nitrophenol might also have anti-androgenic activity on the male reproductive system of humans. It was also reported that 3-methyl-4-nitrophenol exhibited estrogenic activity. Furthermore, the results suggest that, as a result of diesel exhaust emission or the degradation of various pesticides, the accumulation of nitrophenols in the air and soil could have serious deleterious effects on wildlife and human health through disturbance of endocrine and cardiovascular functions. [1, 13]

CHAPTER 3: METHOD

From previous research on this topic; identification of nitrophenols in DEP, it was gathered that only gas chromatography systems equipped with a mass spectrometer (GC-MS) was used for the analyses of the nitrophenol components in DEP samples. However, due to the unavailability of a working GC-MS at the time this study was carried out, a gas chromatograph equipped with a flame ionization detector (GC-FID) was used to carry out the analysis.
The preparation of samples and solutions, extraction and concentration method did not change from what was initially planned to be done if a GC-MS was used. What did change were the detector type and the capillary column.
This chapter gives an overview of the materials that were needed, and describes the methods that were applied to carry out the experiments.

3.1 Materials

Instruments and chemicals used for sampling:

- Diesel engine (Isuzu)
- Diesel (locally produced, imported and blend)
- 250 mL impingers
- Cooling Box
- Vacuum Pump (Welch-Ilmvac)
- Direct Reading Flow meter (Matheson)
- Tubes
- Funnels
- Glass storage bottles
- 1N sodium hydroxide (NaOH) solution
- Clamp & clamp stand

Materials and equipment for sample analysis:

- Standard laboratory glassware
- 4-nitrophenol (99%, Beantown Chemical)
- 2-methyl-4-nitrophenol (98%, Sigma Aldrich)
- 3-methyl-4-nitrophenol (98%, Alfa Aesar)

  } Standards

- Nitrocyclopentane (99%, Sigma Aldrich) (internal standard)
- Dichloromethane (GFS Chemicals)
- Anhydrous sodium sulfate (Na_2SO_4)
- 10N Sulfuric Acid (H_2SO_4)
- Distilled water
- Glass wool
- Nitrogen gas
- Gas Chromatography instrument (Hewlett Packard HP 6890Series Gas Chromatograph with 6890 FID)
- Analytical balance
- Ring stand
- Universal pH paper
- Separatory funnels
- Volumetric Flasks

- Simple distillation setup

All the standards that were purchased were of GC grade. The reagents were of either GC grade or ACS grade.

3.2 Preparation of Solutions and Standards

Preparation of the nitrophenol calibration standards
The nitrophenol standard materials were purchased as solid crystals. From these the nitrophenol calibration standards were made in the laboratory, using dichloromethane (DCM) as solvent. The calibration standards were diluted from stock solutions, prepared in the laboratory.

Calculations for the prepared solutions are given in Appendix I

A 500 ppm stock solution of 4-nitrophenol (4NP) was prepared by dissolving 0.0255 g; $1.83 * 10^{-4}$ mol in 50 mL DCM.

A 500 ppm stock solution of 2-methyl-4-nitrophenol (2M4NP) was prepared by dissolving 0.0252 g; $1.65 * 10^{-4}$ mol in 50 mL DCM.

A 500 ppm stock solution of 3-methyl-4-nitrophenol (3M4NP) was prepared by dissolving 0.0252 g; $1.65 * 10^{-4}$ mol in 50 mL DCM.

Individual calibration standards of each of the nitrophenol components; 4NP, 2M4NP and 3M4NP, were prepared in the concentrations: 10 ppm and 100 ppm, which were then analyzed on the GC-FID instrument, and from which their respective retention times were confirmed.

A 100 ppm nitrophenol mix working solution was prepared by adding 10 mL 500 ppm 4NP, 10 mL 500 ppm 2M4NP and 10 mL 500 ppm 3M4NP to a 50 mL volumetric flask, and diluting this solution with DCM.

Nitrophenol mix calibration standards of 1 ppm, 2 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm and 80 ppm were prepared from the 100 ppm nitrophenol mix working solution.
The calibration standards, including a blank (DCM) were then analyzed on the GC-FID instrument.

Preparation of nitrocyclopentane (internal standard) solutions:
A 500 ppm stock solution of nitrocyclopentane (NCP) was prepared by diluting 4.6 µL; 0.0050 g; $4.34 * 10^{-5}$ mol in 10 mL DCM.
A 100 ppm working solution of NCP was prepared by diluting 1 mL 500 ppm NCP stock solution to 5 mL with DCM.

Preparation of 1N sodium hydroxide solution (NaOH) and 10N sulfuric acid (H_2SO_4)

The following solutions were prepared every time DEP samples would be taken.

A solution of 1N sodium hydroxide (NaOH) was prepared by dissolving 20 g; 0.5001 moles NaOH in 500 mL of distilled water.

A solution of 10N sulfuric acid (H_2SO_4) was prepared, by diluting 55.40 ml of 96.2% H_2SO_4 in 200 mL of distilled water.

3.3 Method Validation and Identification of Nitrophenol Standards

Gas Chromatography instrument specifications and conditions
A Hewlett Packard (California, U.S.A.) Model HP 6890Series Gas Chromatograph equipped with a 6890 flame ionization detector (FID), and a 6890Series autosampler was used.
The column was a HP-5 5% Phenyl Methyl Siloxane capillary column (50.0 m × 320 μm) with a 0.17 μm film thickness with hydrogen as the carrier gas at a rate of 2 mL/min.
The initial oven temperature was set at 50 °C, then increased to 260 °C at 10 °C/min and then maintained at 260 °C for 5 min; total runtime was 26 minutes.
The injector was set at splitless mode, injector temperature was held constant at 200 °C, and the injection volume was maintained at 1.0 μL.
The FID temperature was kept at 250 °C.
The chromatographic data were analyzed and processed using Agilent Chemstation.

The GC-FID system was calibrated and validated as suggested by Agilent Technologies Inc. in Analysis of Phenols by Gas Chromatography according to EPA 8040A.

To achieve better sensitivity and lower limits of detection and quantitation, using standard gas chromatography (GC) equipment, with a split–splitless injection port and a flame ionization detector (FID), fundamental variables which can lead to a much-improved method performance, were considered. Some important variables to optimize the GC–FID performance are:

- Sample Solvent
- Optimization of Splitless Time
- Initial Oven Temperature Hold
- GC Column Choice
- Carrier Gas Operating Mode
- FID Optimization
- Thermal Gradients

The variables that were considered and investigated to determine under which conditions the analyses of the nitrophenol components would be best.

- The optimum splitless time and initial oven temperature hold were experimentally determined for the best sensitivity
- The carrier gas operating mode was set to constant flow
- To optimize the FID the fuel (hydrogen) to oxidizer (air) ratio was set at 1:11
- The temperature setting was: 50 °C + 10 °C/min → 260 °C

The individual nitrophenol calibration standards were first analyzed under the instrument conditions stated above, and from which the retention times were determined. After confirming the retention times of 4NP, 2M4NP, and 3M4NP on the instrument, nitrophenol mix calibration standards were prepared and analyzed under the same conditions, and their retention times were then compared to those of the individual standards.

The parameters and requirements, such as calibration and linearity, limit of quantitation (LoQ), limit of detection (LoD), accuracy (recovery of fortification), precision, repeatability, and sensitivity, for quality assurance and method validation were carefully considered and determined.
The chromatograms of all solutions were processed and analyzed in Chemstation, and to plot the calibration curves of the nitrophenol components and to determine the above mentioned parameters, Excel was used. To determine the parameters, such as LoD and LoQ by regression analysis, and to validate the data, statistical and data analysis tools in Excel were used.

The LoD and LoQ were determined from the calibration plots and regression analysis. To determine the LoD and LoQ, the slope (s) of the regression line and the standard deviation of the y-intercept (σ) should be known. And to calculate the LoD and LoQ, the following equations were used. [28, 29, 30]

Eq 3. $$LoD = 3.3 * \sigma/S$$

Eq 4. $$LoQ = 10 * \sigma/S$$

The coefficients 3.3 and 10 are called expansion factors and are obtained assuming a 95% confidence level.

Accuracy of the method was determined by spiking blank solutions with the nitrophenol mix standards. The recovery of fortification (spike) was determined by [18]

Eq 1. $$\%\, recovery = \frac{(C_{spiked\ sample} - C_{unspiked\ sample})}{C_{added}} * 100$$

3.4 Sampling Procedures, Sample Preparation and Analysis

The methodology was adapted from: (Valkenburg C. et al., 1989 and Norbert F. et al., 2018) [26, 31]

Across multiple studies done on the presence of nitrophenols in air and exhaust air, air samplers with glass-fiber filters for collection of the particulate matter, were used. Followed by sample extraction with organic solvent(s) under both acidic and basic conditions. Sampling of DEP on glass fiber filters, followed by extraction of the nitrophenols at a pH of 2, showed to be the most efficient. Another method that is used to sample nitrophenols from exhaust air, is by bubbling the exhaust air through a solution of sodium hydroxide, using an impinger sampling system. The collected sample is then extracted with an organic solvent to remove components, other than the phenolic compounds in the sample, followed by extraction with the same organic solvent, but under acidic conditions (pH ~2), to extract the nitrophenols from the sample. Due to the unavailability of the equipment needed for the first method, which was shown to be the most efficient, the second method using an impinger sampling system, followed by extraction of the nitrophenols with an organic solvent, was applied in this study. [3, 7, 23, 25, 26]

Preparation of sampling solutions and sampling equipment
The 1N NaOH sampling solution and 10N H_2SO_4 solution were either prepared the day before or the morning before going to the field.
On each day before the sampling took place, a test was done on the vacuum pump and flow meter to check whether the flow rate remained constant at 3 L/min. This was done by regulating the intake air that goes through the flow meter, sucked by the vacuum pump. With the two

regulators, one on the vacuum pump and one on the flow meter, the flow rate was held constant at 3 L/min throughout the whole sampling process.
Before taking any sample on the sampling days, the diesel engine was also always completely flushed, to remove any remaining diesel that was still in the engine. After flushing the engine, the diesel form which DEP samples would be taken, was then burned.

See Appendix 1, Figure 23, for the sampling setup

Sampling technique and collection of samples

To compare the concentration of nitrophenols in DEP, samples were taken from three different types of diesel: locally produced diesel, imported diesel, and diesel that is a blend. This third type of diesel that was purchased (not self-made); the blend, could be diesel that is locally produced or diesel that is imported or diesel that is a mixture between locally produced and imported diesel. Most likely this diesel is a blend between imported and locally produced diesel. However it is not known what the ratio of imported diesel to locally produced diesel is in the blend. Each time this type of diesel is purchased, it could be of a different composition.
Of each type of diesel, 4 samples were collected; in total 12 samples. These samples were taken on 6 days, between 9:30 AM and 11:00 AM, and on each day a field blank was taken as well. The samples were taken directly from the diesel exhaust after the combustion of the diesel before it mixed with the surrounding air. The diesel engine, which was in an excavator, was operated under engine idling conditions. At the end of the exhaust pipe of the excavator, a funnel was placed connected to an impinger with a tube, which in turn was connected to the flow meter and the vacuum pump. After turning on the engine, a sample of the diesel exhaust air was sucked through 200 mL of 1N NaOH solution in the impinger. The collection of each sample lasted 10 minutes, and the flow rate was set at 3 L/min. Each sample consisted of 30 L of DEP that was sucked through the NaOH solution in the impinger. Because the solubility of the nitrophenols in solutions of sodium hydroxide is not known, these sampling conditions were based on and adapted from a previous study [25], in which exhaust air was sampled through a solution of 0.5 N 100 mL NaOH, at a flow rate of 1 L/min for 15 minutes.
On each sampling day, 2 samples of DEP were collected, as well as 1 field blank (100 mL NaOH solution), which was taken to the site of sampling and exposed to the surroundings. The container with the field blank was opened during the time the sampling took place and then sealed off after sampling was completed. After completion of the sampling, the samples and the field blank were then taken back to the laboratory to be further treated, on the same day.

Sample preparation, sample extraction and analysis

The field blank was first treated, to minimize contamination from the samples to the field blank. The field blank (100 mL NaOH solution) was transferred from the container into a separatory funnel and the solution was extracted with dichloromethane (2 x 25 mL). The dichloromethane layer was discarded in the container with halogenated chemical waste, and the aqueous layer was collected. This solution was made acidic; pH 1 – 2, with 35 mL 10N H_2SO_4. The acidic solution was then transferred into a separatory funnel and was extracted with dichloromethane (2 x 35 mL). The dichloromethane layers were collected, transferred into a separatory funnel again, to which distilled water (50 mL) was added. The solution was lightly swirled, and the dichloromethane layer was collected, while the water layer was discarded through the sink after diluting the solution to a neutral pH.
Before any extraction procedure, each DEP sample (200 mL NaOH solution) was divided into two equal aliquots of 100 mL NaOH solution from 1 sample. These 2 split samples (e.g. aliquot

1A and aliquot 1B) were then treated as individual samples and were treated in the same manner as the field blank, as described above.

To all the samples and the field blank, anhydrous Na_2SO_4 (~20 g) was added, after the extraction steps, to remove any remaining water droplets in the solutions, and these were left to "dry" until the next day. The flasks containing the solutions were wrapped in aluminum foil and were stored in a cool dark place (below 20°C); a refrigerator, for 20 hours. The following day the dichloromethane solutions were filtered with glass wool, to separate the liquid from the Na_2SO_4 crystals. After filtration, the liquid extracts were concentrated by distillation to reduce the volume to 3 – 5 mL. Simple distillation of the solvent was done, in place of using a rotary evaporator or a rotavapor, to remove most of the solvent from the solution. Dichloromethane has a boiling point of 39.5 °C, while the boiling points of the nitrophenol components are all above 200 °C, so the method of simple distillation worked well in this case. After distillation, nitrocyclopentane (0.1 mL 100 ppm) was added to the remaining solutions in the flasks. The solutions were swirled and were then transferred into test tubes, to furthermore concentrate the solutions to 1 mL; almost "dryness", under a gentle stream of nitrogen gas. The strongly concentrated samples (1:70) were then transferred into GC vials and were analyzed under previously given gas chromatography conditions, on the HP 6890Series Gas Chromatograph equipped with a HP-5 5% Phenyl Methyl Siloxane capillary column, and.coupled with a 6890 FID, in the splitless mode.

The chromatograms of the samples were processed and analyzed in Chemstation, while the calculations and data analysis were done in Excel.

CHAPTER 4: RESULTS AND DISCUSSIONS

In this chapter the results obtained throughout this whole study are given, as well as the discussions on those results that were obtained.

4.1 Results of Method Validation

Method validation was done through a series of tests that proofs any assumptions on which the analytical method is based and established, as well as documenting the performance characteristics of the method, demonstrating whether the method fits for this particular analytical purpose. For this research, calibration and linearity, accuracy and recovery, precision, limit of quantitation (LoQ), limit of detection (LoD), and sensitivity were determined.

4.1.1 Calibration

The results of determining the retention time (t_R) of the different nitrophenol standards in

- separate solution (individual)
  and in
- mix calibration solution

is presented in *Table 1*. The t_R of a compound is not fixed as many factors can influence it even when using the same instrument and column. These include the gas flow rate, the length of the column, temperature differences in the oven and column, and column degradation. The length of the column that was used was 50.0 m, while the gas flow rate was held constant at 2 mL/min. As for these conditions, they were the same throughout all the analyses in this research.
Due to certain limitations in this research, the nitrophenols were identified by retention time, and not by spectroscopic techniques (MS, IR, NMR). The retention time was investigated under the same analysis conditions, described in *Chapter 3*, in both the individual nitrophenol standard solutions and in the nitrophenol mix calibration solutions. The retention times of the nitrophenols in the mix calibration solutions were confirmed when it was compared to the retention time of the individual standards in the separate solutions. A matching was made between the respective nitrophenol retention time in the separate standard solution and in the mix calibration solution. The retention times of the nitrophenol components became evident in both the separate solutions and the mix calibration solutions because when analyzed under the same conditions, the same component always elutes at relatively the same retention time.

Table 1. Retention time of the nitrophenol standards in the separate solutions and in the mix calibration solutions

		4-nitrophenol	**3-methyl-4-nitrophenol**	**2-methyl-4-nitrophenol**
Retention Time [minutes]	Separate solution (100 ppm)	19.725	20.379	20.606
	Separate solution (10 ppm)	19.740	20.388	20.620
	10 ppm mixed solution	19.764	20.396	20.632
	20 ppm mixed solution	19.779	20.410	20.645
	40 ppm mixed solution	19.796	20.425	20.662
	60 ppm mixed solution	19.811	20.439	20.675
	80 ppm mixed solution	19.825	20.452	20.687
	2 ppm mixed solution	19.885	20.475	20.694

The retention time of the internal standard nitrocyclopentane was also determined under the same analysis conditions. The t_R of nitrocyclopentane was between 11.75 and 11.80 min.

4.1.2 Results for Drawing the Calibration Curves

The response of the instrument to the different concentrations of nitrophenol mix calibration solutions is presented in the tables below. In *Table 2* the retention time of the nitrophenol mix calibration solutions for each prepared concentration is given. The peak area at each concentration of nitrophenol standard was determined, which is proportional to the amount of the compound (=concentration) that is present. In *Table 3A* the peak area of each concentration of 4-nitrophenol (4NP), 3-methyl-4-nitrophenol (3M4NP) and 2-methyl-4-nitrophenol (2M4NP) calibration standard is presented, and these were used to plot *Figure 9 and 10.*

Table 2. Retention time of the nitrophenol mix calibration standards (I)

		4-nitrophenol	**3-methyl-4-nitrophenol**	**2-methyl-4-nitrophenol**
Retention Time [minutes]	10 ppm mixed solution	19.764	20.396	20.632
	20 ppm mixed solution	19.779	20.410	20.645
	40 ppm mixed solution	19.796	20.425	20.662
	60 ppm mixed solution	19.811	20.439	20.675
	80 ppm mixed solution	19.825	20.452	20.687
	2 ppm mixed solution	19.885	20.475	20.694
Mean retention time [minutes]		19.810	20.433	20.666
Standard deviation of the mean of the retention time		0.043	0.029	0.024
Relative standard deviation of the mean of the retention time		0.22%	0.14%	0.12%

Table 3A. Peak area of the nitrophenol mix calibration standards (I)

Concentration of nitrophenol mix calibration standard [ppm]	**Peak Area**		
	4-nitrophenol (4NP)	**3-methyl-4-nitrophenol (3M4NP)**	**2-methyl-4-nitrophenol (2M4NP)**
80	576.97	813.50	767.68
60	398.59	574.79	549.17
40	285.56	420.03	401.77
20	123.70	193.90	190.20
10	56.27	95.76	92.62
2	13.90	19.91	23.33

The chromatograms of the nitrophenol mix calibration standards can be found in Appendix II

The 1 ppm nitrophenol mix standard that was initially prepared and was to be used in the calibration plots, was replaced by a 2 ppm nitrophenol mix standard.

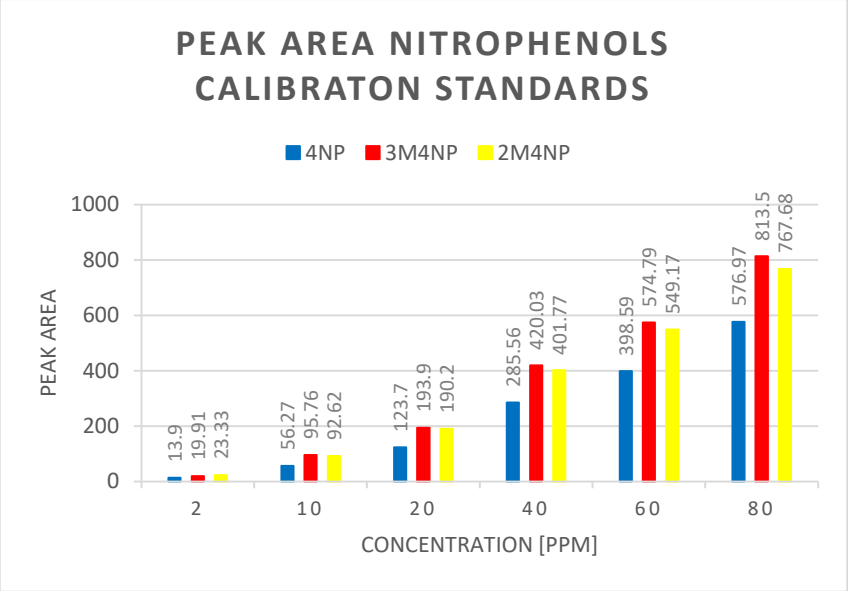

Figure 9. Peak Area of Nitrophenol Mix Calibration Standards (I)

In *Figure 9* the peak area of the nitrophenol mix calibration standards is plotted against the concentration of the components, to give a visualization of the difference in peak areas of 4NP, 2M4NP, and 3M4NP, at each of the prepared concentrations. Comparing the peak areas of each of the standards, it is seen that at each concentration, except the 2 ppm standard, 3M4NP has the largest peak area, followed by 2M4NP and 4NP, respectively. In the 2 ppm standard, 2M4NP has the largest peak area, followed by 3M4NP and 4NP. The standards of 10 ppm, 20 ppm, 40 ppm, 60 ppm, and 80 ppm were all prepared and analyzed on the same day, while the 2 ppm standard was prepared and analyzed later on, when the results for the 1 ppm standard had to be omitted, because the signals of the nitrophenol components at this concentration could not be quantified.

In *Figure 10* the peak area of the nitrophenol compounds is plotted against its corresponding concentration in the mix calibration standards. This resulted in calibration plots for each of the nitrophenol standards. In *Table 3B* the calibration plot equations for the nitrophenol compounds are given.

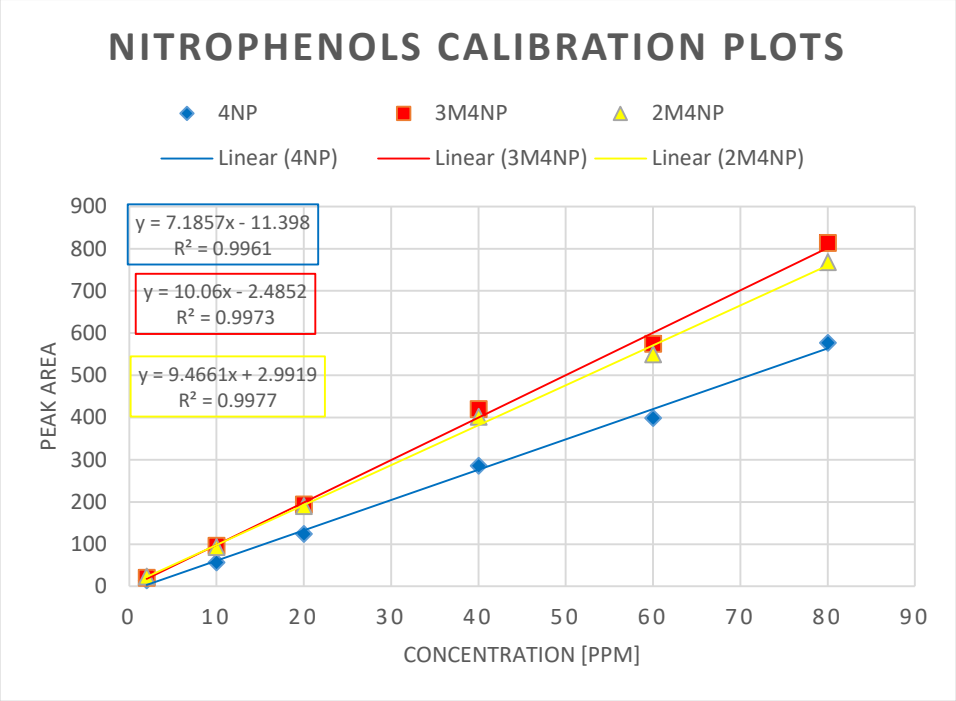

Figure 10. Nitrophenols Calibration Plots (I)

From the plots given in *Figure 10*, it is obvious that all calibration plots have different slopes. The steeper the slope, the stronger the response (signal) of the instrument is to concentration changes of the analyte. A steeper slope also indicates better sensitivity of the instrument to that specific analyte, as the sensitivity is determined from the slope of the calibration plot.

Table 3B. Data of the nitrophenol calibration plots (I)

	4-nitrophenol (4NP)	**3-methyl-4-nitrophenol (3M4NP)**	**2-methyl-4-nitrophenol (2M4NP)**
Y-function	y = 7.1857x – 11.398	y = 10.06x – 2.4852	y = 9.4661x + 2.9919
R-squared value	R^2 = 0.9961	R^2 = 0.9973	R^2 = 0.9977

2M4NP has the highest R^2, which means that the linear model that was computed for the calibration plots of the nitrophenol components fits the data of the 2M4NP standards the best. This means, that in comparison to 4NP and 3M4NP, for 2M4NP the observed values (peak areas) of the standards are the closest to the fitted values of the calibration plot of this component.

4.1.3 Precision and Reliability

In terms of the retention time of the nitrophenol components, the results are reliable. Because at each of the prepared concentrations of the standards, the nitrophenol components elute at relatively the same retention time. The relative standard deviation of the mean of the retention time are 0.22%, 0.14%, and 0.12% for 4NP, 3M4NP, and 2M4NP, respectively. *(See Table 2)* A small relative standard deviation indicates that the data is tightly clustered around the mean. For GC analysis the relative standard deviation of the retention time of the analyte should not be higher than 0.5%. [32] And for all nitrophenol components, this percentage is not exceeded,

this means that regarding the retention time of 4NP, 3M4NP and 2M4NP, the results are reliable and repeatable.
The relative standard deviation of the mean of the retention time of the nitrophenol components, as is given in *Table 2*, reflects not a large amount of variation, it is only less variable for the different nitrophenol components. From least to most variable in retention time are ranked: 2M4NP, 3M4NP and 4NP, with retention time of 20.669 min ± 0.12%, 20.432 min ± 0.14%, and 19.804 min ± 0.22%, and respectively.
And in terms of the peak area of each of the nitrophenol components at the prepared concentrations, the results are also reliable. With the coefficient of determination; R^2 of the calibration plots of the nitrophenol components >0.996, the computed model fits all calibration plots well, and the results of the response from the GC-FID instrument are reliable. The relative standard deviation of the response from the instrument (peak area), computed in Excel were determined to be below 6.29% for each of the nitrophenol components.

Another set of nitrophenol mix calibration solutions was prepared. These were analyzed under the same analysis conditions as before, but were prepared on another day and from another nitrophenol mix stock solution. When compared to the first set of calibration solutions, the stock solution that was prepared for these standards had a higher concentration. This was done to be less wasteful with the solvent; dichloromethane. Hence the volume of stock solution was reduced, but the same amount of moles of nitrophenol components were dissolved in the solvent. The volume of working stock solution and the prepared calibration standards were also reduced, but they were of the same concentration as in the first set. In *Table 4* the retention times of these nitrophenol mix calibration solutions are given.

Table 4. Retention time of the nitrophenol mix calibration standards (II)

		4-nitrophenol	**3-methyl-4-nitrophenol**	**2-methyl-4-nitrophenol**
Retention Time [minutes]	10 ppm mixed solution	19.571	20.166	20.387
	20 ppm mixed solution	19.571	20.167	20.389
	40 ppm mixed solution	19.569	20.166	20.388
	60 ppm mixed solution	19.568	20.166	20.388
	80 ppm mixed solution	19.573	20.171	20.395
Mean retention time [minutes]		19.570	20.167	20.389
Standard deviation of the mean of the retention time		0.002	0.002	0.003
Relative standard deviation of the mean of the retention time		0.01%	0.01%	0.02%

In *Table 5A* the response (peak area) of the instrument to the different concentrations of the nitrophenol components is given.

Table 5A. Peak area of the nitrophenol mix calibration standards (II)

Concentration of nitrophenol mix standard [ppm]	**Peak Area**		
	4-nitrophenol (4NP)	**3-methyl-4-nitrophenol (3M4NP)**	**2-methyl-4-nitrophenol (2M4NP)**
80	421.3	614.4	707.3
60	315.3	455.4	528.1
40	192.1	303.6	350.1
20	91.5	162.5	185.2
10	58.2	114.7	133.4

The data given in *Table 5A* was used to plot *Figure 11 and Figure 12*. In *Figure 11* the peak area of the nitrophenol mix calibration standards is plotted against the concentration to give a visualization of the difference in peak areas of 4NP, 2M4NP and 3M4NP, at each of the prepared concentrations.

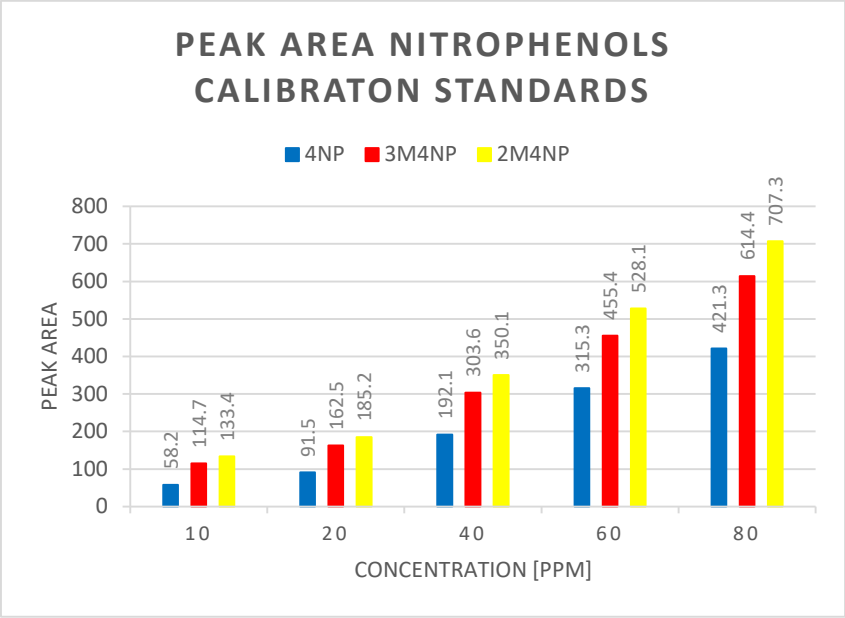

Figure 11. Peak Area of Nitrophenol Mix Calibration Standards (II)

In *Figure 12* the peak area of the nitrophenol compounds is plotted against its corresponding concentration in the nitrophenol mix calibration standards, from which calibration plots for each compound was computed. In *Table 5B* the calibration plot equations for the nitrophenol compounds are given.

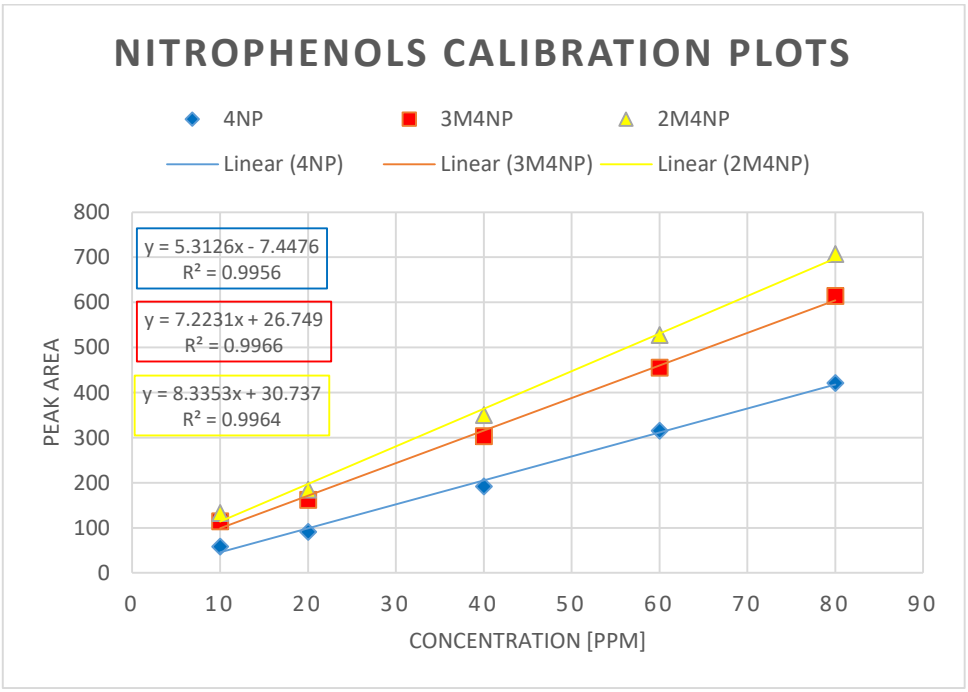

Figure 12. Nitrophenols Calibration Plots (II)

The calibration plots in *Figure 12* all have different slopes. The steeper the slope of the plot is, the stronger the response (signal) of the instrument is to changes in concentration of the analyte, which also indicates better sensitivity of the instrument to the analyte.

Table 5B. Data of the nitrophenol calibration plots (II)

	4-nitrophenol (4NP)	**3-methyl-4-nitrophenol (3M4NP)**	**2-methyl-4-nitrophenol (2M4NP)**
Y-function	y = 5.3126x - 7.4476	y = 7.2231x + 26.749	y = 8.3353x + 30.737
R-squared value	R^2 = 0.9956	R^2 = 0.9966	R^2 = 0.9964

The relative standard deviation of the mean of the retention time for this set of nitrophenol calibration standards are respectively 0.01%, 0.01%, and 0.02% for 4NP, 3M4NP, and 2M4NP. *(See Table 4)*
For this set of calibration standards, the retention time of 4NP, 3M4NP, and 2M4NP were determined to be 19.570 min ± 0.01%, 20.167 min ± 0.01%, and 20.389 min ± 0.02%, respectively. The standard deviation of the mean of the retention time of the nitrophenol components, also reflects not a large amount of variation. For GC analysis the acceptable relative standard deviation of the retention time of the analyte is 0.5% or less. [32] For all nitrophenol components, this is not exceeded, which means that in regards to the retention time, the results are reliable.
The coefficient of determination (R^2) of the calibration plots are higher than 0.995 and the relative standard deviation of the response from the instrument (peak area), computed in Excel were determined to be below 5.41% for each of the nitrophenol components.

In terms of achieving replicate results for the second set of calibration standards compared to the first set of calibration standards, it is seen that the retention time of each of the nitrophenol components has shifted. The second set of calibration standards had a shorter retention time compared to the first set. In *Table 6* the retention times of the nitrophenol components are compared. In the second set, the relative standard deviation of the mean of the retention time of the nitrophenol components were smaller in comparison to the first set, meaning that they were more tightly clustered around the mean retention time. The first set however had retention times that were closer to that of the individual standards. *(See Table 1)*
The internal standard (NCP) that was analyzed during these analyses, also had a shorter retention time at 11.51 min. In comparison to when the first analyses were carried out, NCP eluted from the column around 11.77 min. (*See Appendix II, Figure 17 and 18).* When comparing the retention times of all components, including the internal standard, it is seen that the retention times of all components have shortened with 0.2 – 0.3 min in the second set of standards.
Although the conditions on the GC-FID were the same on both days that the analyses of the calibration standards were carried out, a difference in retention time for all components is seen. Differences in the retention time for the same component, when using the same instrument and the same column, can be due to a change in the gas flow rate, the length of the column, temperature differences in the oven and the column, and column degradation. In this case the difference in retention time for all components could be due instrument variation, as well as due to column degradation.

Table 6. Comparison of the retention time of the nitrophenol components

	4-nitrophenol		**3-methyl-4-nitrophenol**		**2-methyl-4-nitrophenol**	
	(I)	**(II)**	**(I)**	**(II)**	**(I)**	**(II)**
Relative retention time	19.804 min ± 0.22%	19.570 min ± 0.01%	20.432 min ± 0.14%	20.167 min ± 0.01%	20.669 min ± 0.12%	20.389 min ± 0.02%

Table 7. Comparison of the peak area of the nitrophenol components

Concentration [ppm]	**Peak Area**					
	4-nitrophenol (4NP)		**3-methyl-4-nitrophenol (3M4NP)**		**2-methyl-4-nitrophenol (2M4NP)**	
	I	**II**	**I**	**II**	**I**	**II**
80	576.97	421.30	813.50	614.40	767.68	707.30
60	398.59	315.30	574.79	455.40	549.17	528.10
40	285.56	192.10	420.03	303.60	401.77	350.10
20	123.70	91.50	193.90	162.50	190.20	185.20
10	56.27	58.20	95.76	114.70	92.62	133.40

The results from the response of the instrument (peak area) to the different concentrations of 4NP, 3M4NP, and 2M4NP in the calibration solutions could however not be replicated. In *Table 7* the peak area of the nitrophenol components at each prepared concentration are compared. The 2 ppm solution is not compared, because the solution of this concentration from the second set of standards, did not give signals that could be quantified. *(See Appendix II, Figure 10)*
The relative standard deviation of the response of the instrument for 4NP, 3M4NP, and 2M4NP, are reported as <27.7%, <22.8%, and <25.5%, respectively.

4.1.4 LoQ, LoD and Sensitivity

Determining the parameters from a calibration curve

To demonstrate that the method was "fit for purpose", the limit of quantitation (LoQ) for this method, using the HP 6890Series Gas Chromatography instrument equipped with an HP-5 5% Phenyl Methyl Siloxane capillary column and a 6890 FID, was determined to be 10 ppm, from visual observations of the calibration curve of the nitrophenol standards. The limit of detection (LoD), which is the lowest possible concentration of analyte that can be detected and which is significantly different from the noise, was determined to be 2 ppm, by this method.
After analyzing the 1 ppm nitrophenol mix standard that was initially prepared, this concentration could not be accepted as the LoD, because the S/N ratio requirement was not met, and the difference between the LoQ and LoD would be too large. A higher concentration of nitrophenol mix standard was prepared, at a concentration of 2 ppm. At this concentration the signals of the nitrophenol standards were sharp, and the S/N ratio requirement of 3:1 for the LoD was met.

Determining the LoD and LoQ by regression analysis

In *Table 8* the LoD and LoQ, calculated by regression analysis, are given. The LoD and LoQ were determined for both sets of nitrophenol mix calibration standards.

Table 8. LoD and LoQ by regression analysis

	Compound	LoD [ppm]	LoQ [ppm]
1st set of standards	**4-nitrophenol (4NP)**	6.04	18.31
	3-methyl-4-nitrophenol (3M4NP)	5.73	17.37
	2-methyl-4-nitrophenol (2M4NP)	5.37	16.23
2nd set of standards	**4-nitrophenol (4NP)**	4.78	14.48
	3-methyl-4-nitrophenol (3M4NP)	3.41	10.32
	2-methyl-4-nitrophenol (2M4NP)	2.50	7.58

The LoD and LoQ, determined from regression analysis of the first set of nitrophenol standards, resulted in LoD and LoQ values that were significantly higher than what was determined from the calibration curves, while regression analysis of the second set of standards resulted in LoD and LoQ values that were much closer to the determined LoD and LoQ from the calibration curves.

From the calibration curves of the nitrophenol mix standards, and assuming that the response of the instrument (y) is linearly related to the concentration of the standard (x), the function can be expressed as: y = a + bx; which is the y-function given in *Table 3B and Table 5B*. From this model, the sensitivity (b) can be determined.

Table 9. Sensitivity of the nitrophenol compounds

	4-nitrophenol (4NP)	3-methyl-4-nitrophenol (3M4NP)	2-methyl-4-nitrophenol (2M4NP)
Y-function (I)	y = 7.1857x – 11.398	y = 10.06x – 2.4852	y = 9.4661x + 2.9919
Sensitivity	7.19	10.06	9.47
Y-function (II)	y = 5.3126x - 7.4476	y = 7.2231x + 26.749	y = 8.3353x + 30.737
Sensitivity	5.31	7.22	8.34

In GC, sensitivity is usually defined as the peak height-to-noise ratio. The sensitivity of the detector to the nitrophenol components is given in *Table 9*. From calibration plot equations of the nitrophenol components, the sensitivity of the FID to each component was determined. The results showcase that to 3M4NP the detector has the highest sensitivity, followed by decreasing sensitivity to 2M4NP and 4NP.
When the second set of calibration standards were analyzed, the sensitivity of the FID to each nitrophenol component was determined again. These results showcase that the sensitivity of the detector to the nitrophenol components are from highest to lowest: 2M4NP, 3M4NP and 4NP. The sensitivity determined from these calibration standards are in comparison to the first set also lower. Although the same conditions on the instrument system were applied for both sets of standards.

4.1.5 Accuracy and Spike Recovery

To test the extraction method and to determine the accuracy, the recovery percentages of the nitrophenol components in spiked solutions were determined. Two solutions of 100 mL 1.0 N NaOH (blanks; containing no amount of nitrophenols) were spiked with respectively, 1 mL 10 ppm nitrophenol mix standard and 1 mL 80 ppm nitrophenol mix standard. The 10 ppm and 80 ppm nitrophenol mix standards were prepared from the first 100 ppm nitrophenol mix working solution. The two spiked solutions were then treated as if they were samples from the field, and the extraction, distillation, and concentration procedures as described in *Chapter 3* were followed, before analyzing the samples on the GC-FID.

The results are given in *Table 10A and Table 10B.* The recoveries of the spikes were calculated using the calibration plots of the first set of standards.

Table 10A. Results 10 ppm spiked solution

	Peak area			
	10 ppm nitrophenol mix standard	**10 ppm nitrophenols spiked solution**	**Spike recovered [ppm]**	**% Recovery**
4-nitrophenol (4NP)	56.27	51.56	$\frac{51.56 + 11.398}{7.1857} = 8.76$	$\left(\frac{8.76}{10}\right) * 100\% = 87.6\%$
3-methyl-4-nitrophenol (3M4NP)	95.76	78.93	$\frac{78.93 + 2.4852}{10.06} = 8.09$	$\left(\frac{8.09}{10}\right) * 100\% = 80.9\%$
2-methyl-4-nitrophenol (2M4NP)	92.62	91.01	$\frac{91.01 - 2.9919}{9.4661} = 9.30$	$\left(\frac{9.30}{10}\right) * 100\% = 93.0\%$

For the solution in which 10 ppm nitrophenol mix was spiked, the recovery percentages of the nitrophenol components were between 80.9% and 93.0%. The recovery % of 2M4NP was the highest, while 3M4NP had the lowest recovery %.

Table 10B. Results 80 ppm spiked solution

	Peak area			
	80 ppm nitrophenol mix standard	**80 ppm nitrophenols spiked solution**	**Spike recovered [ppm]**	**% Recovery**
4-nitrophenol (4NP)	576.97	439.97	$\frac{439.97 + 11.398}{7.1857} = 62.81$	$\left(\frac{62.81}{80}\right) * 100\% = 78.5\%$
3-methyl-4-nitrophenol (3M4NP)	813.50	618.20	$\frac{618.20 + 2.4852}{10.06} = 61.70$	$\left(\frac{61.70}{80}\right) * 100\% = 77.1\%$
2-methyl-4-nitrophenol (2M4NP)	767.68	590.82	$\frac{590.82 - 2.9919}{9.4661} = 62.10$	$\left(\frac{62.10}{80}\right) * 100\% = 77.6\%$

The recovery percentages of the nitrophenol components in the 80 ppm spiked solution were much lower compared to the 10 ppm spiked solution. In the 80 ppm spiked solution the recovery % ranged between 77.1% and 78.5%. The recovery percentage for 4NP was the highest, while 3M4NP had the lowest recovery percentage.

The 10 ppm and 80 ppm standards used for the spiked samples were made from the 100 ppm nitrophenol mix working stock solution prepared two weeks prior. The stock solutions and the working stock solution were stored in test tubes, wrapped with aluminum foil, and stored in the refrigerator, away from sunlight, to prevent degradation of the components in the solutions. Standard stock solutions should be good for up to 6 months if they are stored away from light and if they are kept in a cool place. Taking into account that degradation of the nitrophenol components did not occur, the recovery of 77.1% - 78.5%, and 80.9% – 93.0% nitrophenol

spikes in the 80 ppm, and 10 ppm spiked sample respectively, sample loss may have occurred during the steps in the extraction, distillation, and concentration procedure. Spiking was done in the very beginning. The first initial solution; 100 mL 1N NaOH, was spiked and the samples were then taken through the whole extraction, distillation, and concentration procedure. To minimize losses as much as possible, each step in the procedure was carefully investigated and deemed necessary, and the use of minimal glassware in the process was taken into consideration.

4.2 Analysis Results

4.2.1. Sampling

Each week 2 samples and 1 field blank were taken in one day. Sampling was done over the course of 6 weeks, in end February – mid March, and in May. From each type of diesel 4 samples were taken, which were then split into 2 equal aliquots (A and B). These were then treated as individual samples, followed by analysis on the GC-FID under analysis conditions as described in *Chapter 3*. In *Table 11* the results from the sampling are given.

Table 11. Results of Sampling

Number of samples	Volume of each sample	Sampling days	Sampling time
12 DEP Samples • no 1 – 4 (A and B) from locally produced diesel; • no 5 – 8 (A and B) from blend diesel; • no 9 – 12 (A and B) from imported diesel	200 mL 1N NaOH solution with 30L of DEP	6 sampling days, with 2 samples/ 4 split samples each day	10 min
6 Field Blanks	100 ml 1N NaOH solution		

4.2.2 Qualitative Analysis

Results day 1 of sampling

The chromatograms of field blank 1 and samples 1A, 1B, 2A, and 2B are given in *Figure 13 – 17*. The signals in these chromatograms are more prominent, compared to the chromatograms of the calibration standards, because these solutions were all concentrated. Concentrating these solutions meant that most of the solvent was removed from the solution. This resulted in the components that were left in the solutions to be more concentrated and when analyzing the samples, the signals from these components would be amplified in the chromatogram.

The internal standard NCP elutes from the column at t_R 11.77 minutes, hence 4-nitrophenol (4NP), 3-methyl-4-nitrophenol (3M4NP), and 2-methyl-4-nitrophenol (2M4NP) were expected to elute around respectively t_R 19.7 – 19.8 min, 20.4 – 20.5 min, and 20.6 – 20.7 min. The chromatograms of samples 1A and 1B are predominantly the same in the region t_R 19.0 – 21.0 minutes. Samples 1A and 1B are aliquots from the same DEP sample. Chromatograms of samples 2A and 2B, which are aliquots from DEP sample 2, are also predominantly the same in the region t_R 19.0 – 21.0 minutes. Comparing the chromatograms of samples 1A, 1B, 2A, and 2B to field blank 1, no apparent signals are observed around t_R 19.7 – 19.8 min, 20.4 – 20.5 min, and 20.6 – 20.7, from which the presence of the nitrophenol components can be determined. However, at t_R 20.8 min there is a sharp peak in the field blank and the samples.

And although there was a chance that this signal could have been caused by the presence of 2M4NP, which had a retention time of 20.6 – 20.7 minutes in the mix calibration standards, this was soon ruled out. With the help of the *Figure 18 and 19* below, it is explained why this signal at t_R 20.8 minutes is not due to the presence of 2M4NP in the samples, but rather an impurity that was found in the solvent DCM.

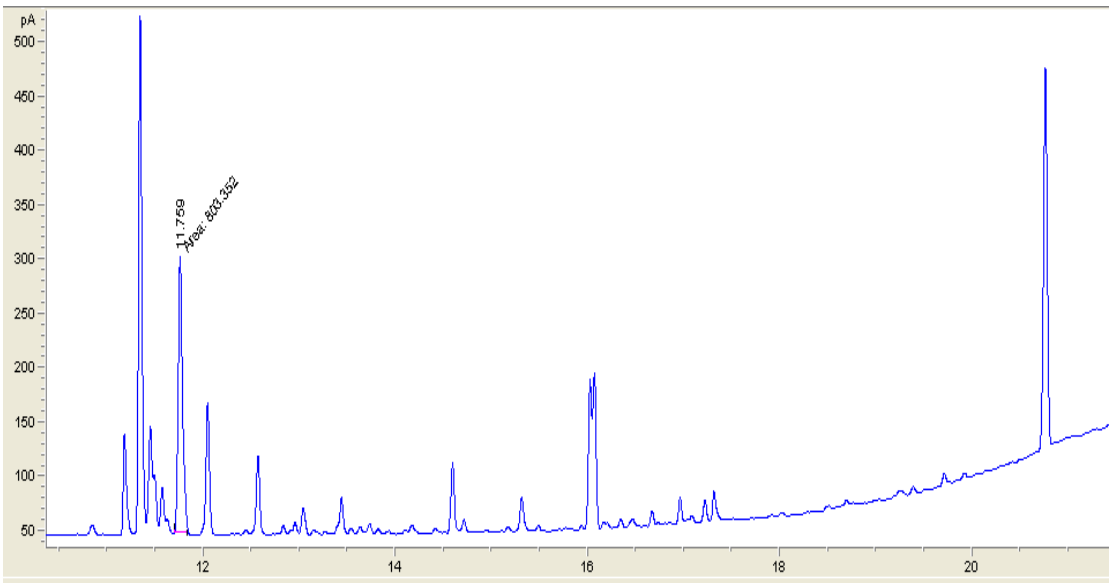

Figure 13. Chromatogram of Field blank 1

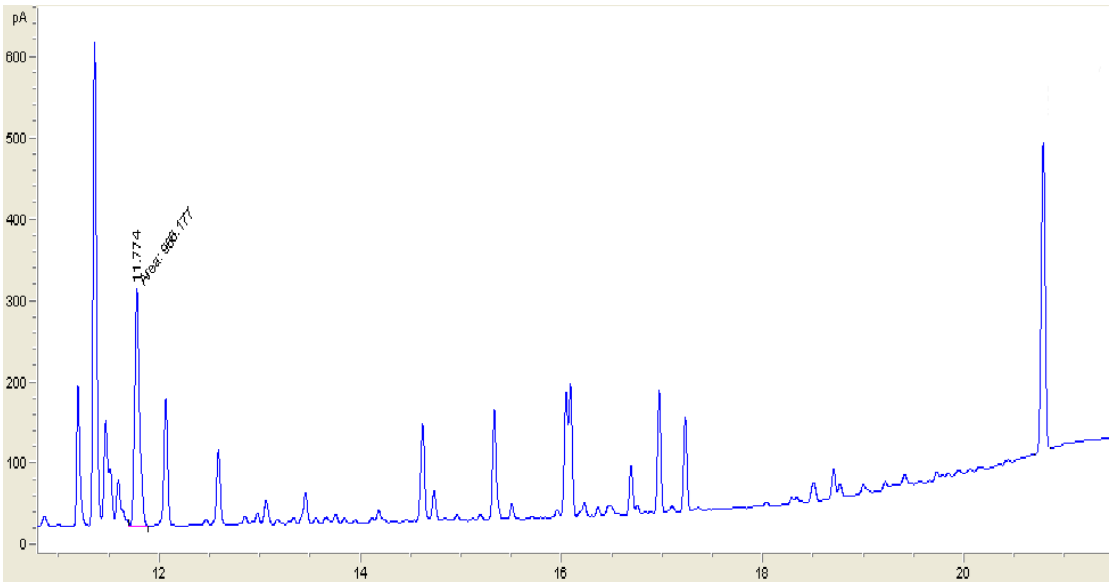

Figure 14. Chromatogram of Sample 1A

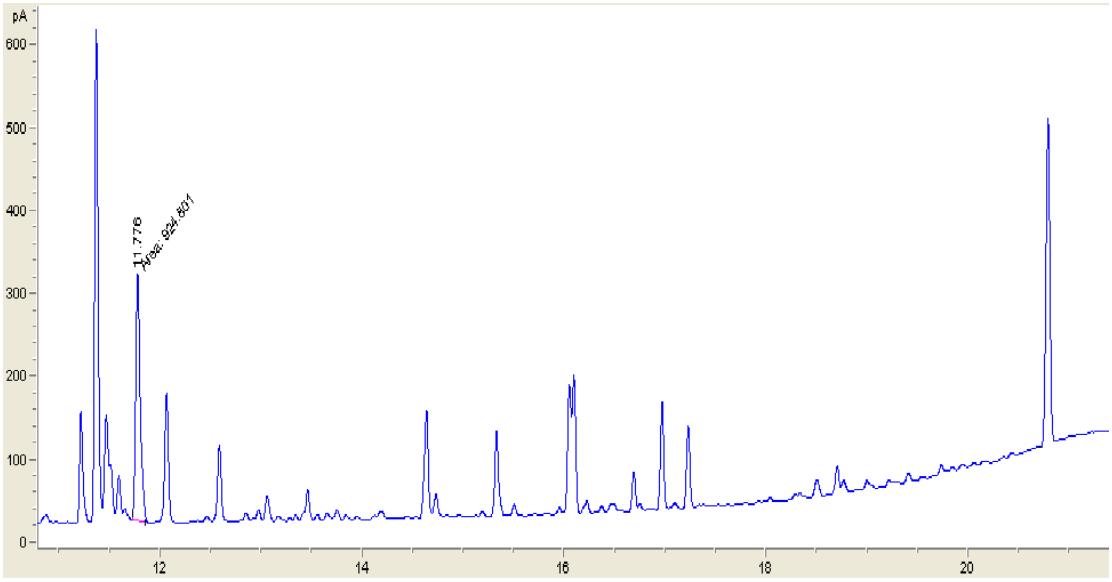

Figure 15. Chromatogram of Sample 1B

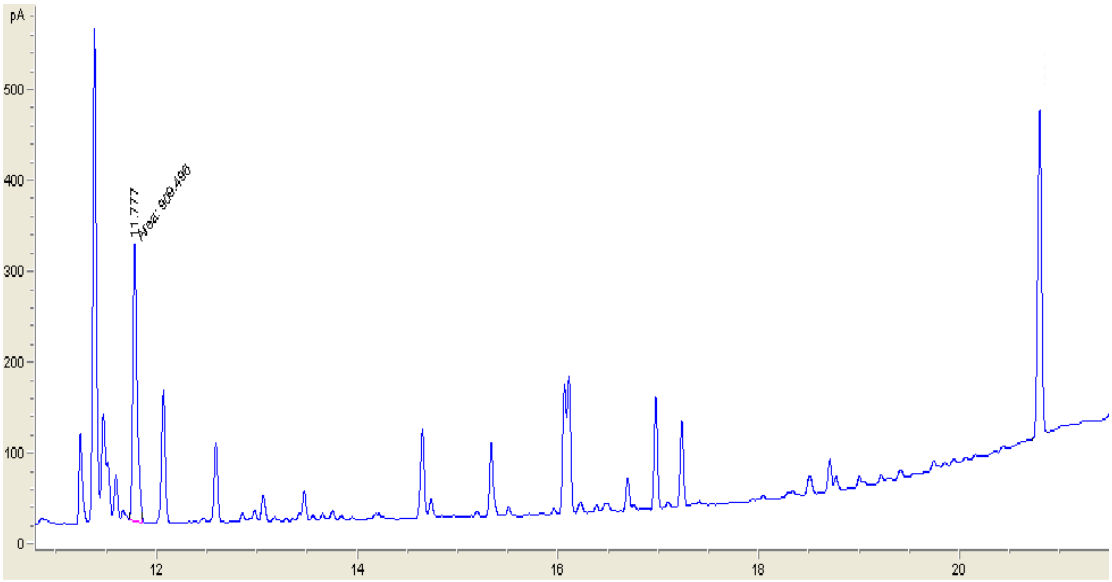

Figure 16. Chromatogram of Sample 2A

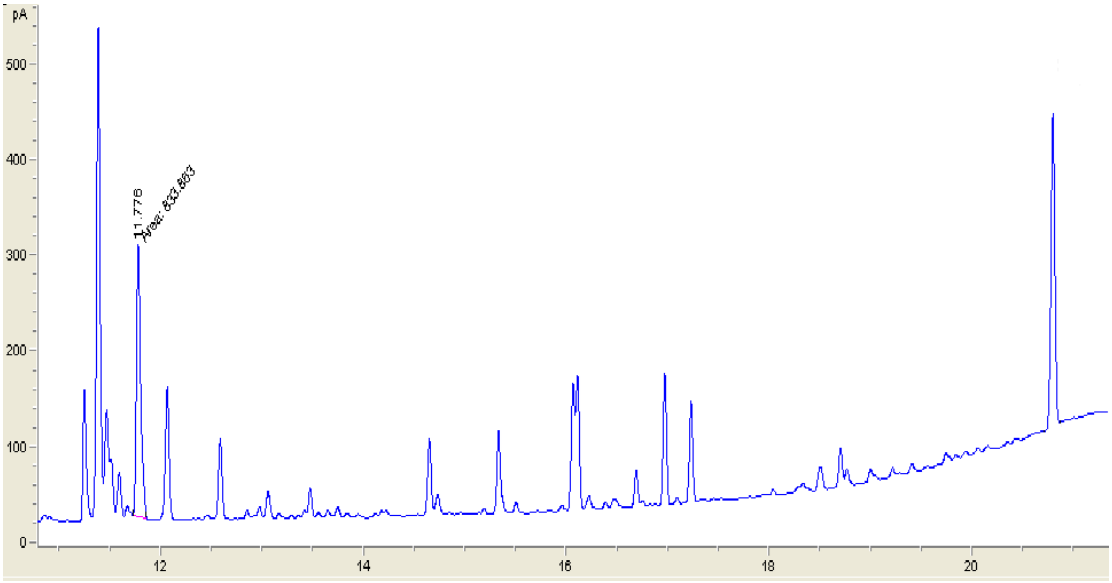

Figure 17. Chromatogram of Sample 2B

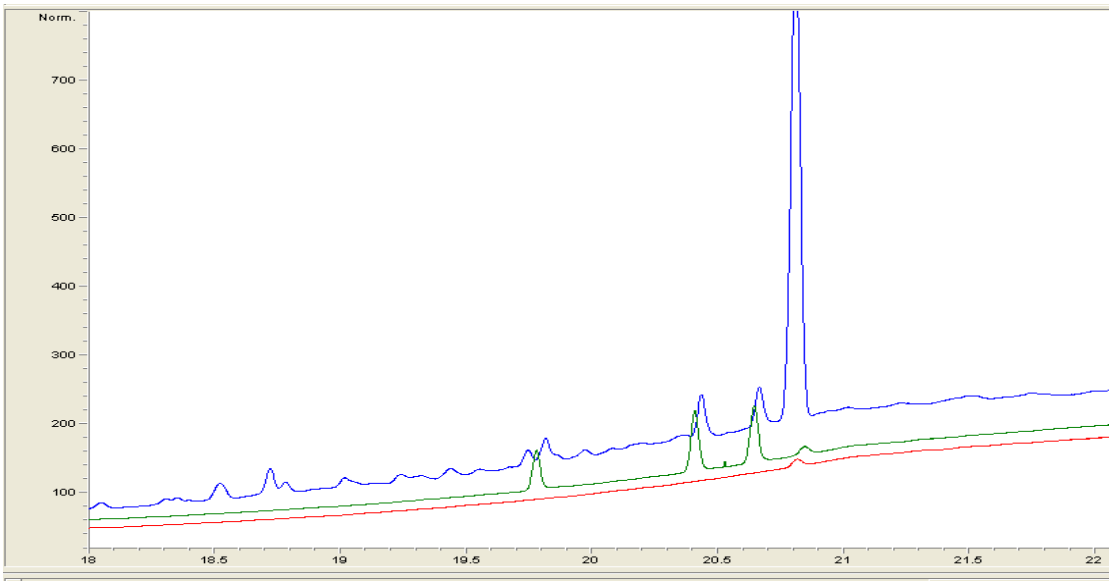

Figure 18. Chromatograms of spiked sample (blue), nitrophenol mix calibration standard (green) and DCM Blank (red)

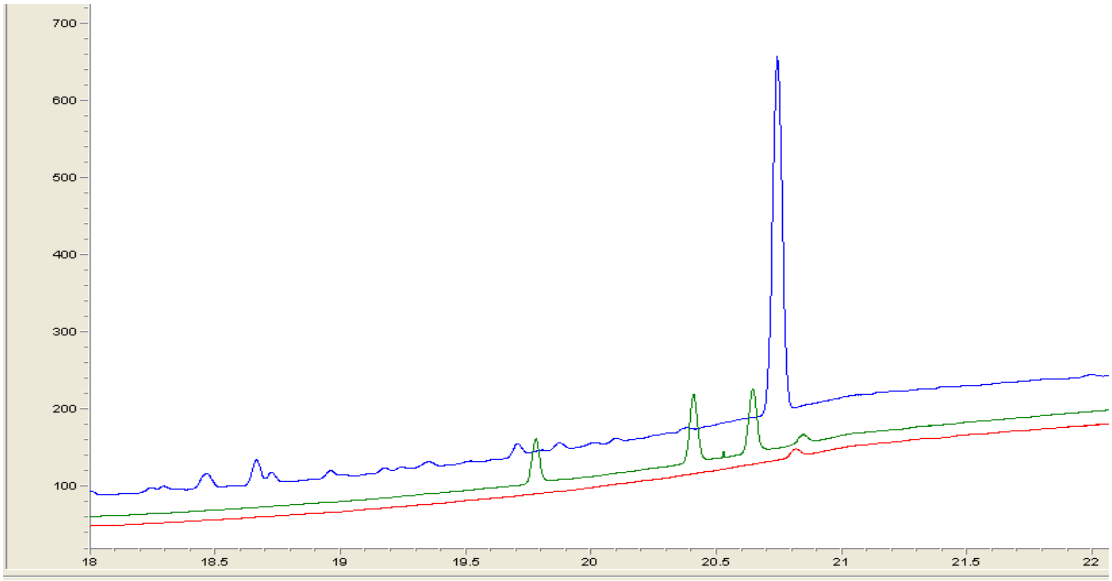

Figure 19. Chromatograms of field blank 1 (blue), nitrophenol mix calibration standard (green) and DCM Blank (red)

In *Figure 18* the chromatograms of a nitrophenol mix spiked sample, a nitrophenol mix calibration standard, and a blank are given. In the chromatogram of the blank, a small signal at t_R 20.8 min is seen. This is an impurity in the solvent itself. The blank consist of nothing but the solvent; in this case dichloromethane (DCM), that is analyzed. In the chromatogram of the mix calibration standard, the signal for the impurity is seen again, right after the signal of 2M4NP. In this chromatogram, the signal has shifted a little, compared to the chromatogram of the blank. The last chromatogram in this figure is of a spiked sample. This sample was spiked at the very beginning and it was taken through all the steps in the extraction, distillation, and concentration procedure, as if it was a sample from the field, before it was analyzed on the GC-FID. In this chromatogram, the signals for the nitrophenol standards are identified at their respective retention times, although the peaks have shifted a little. Right after the signal of 2M4NP, there is a larger peak. This is the signal of the impurity that was found in the solvent. The reason for it being more prominent and amplified in this chromatogram is because this solution was strongly concentrated (1:70), while the other two solutions were not. The signal at t_R 20.8 min is seen back in the chromatograms of the field blank and the samples. *(See Figure 13 – 17).*
In *Figure 18* the chromatograms of field blank 1, a nitrophenol mix calibration standard, and a blank are given. In field blank 1, the signal of the impurity returns at t_R 20.8 min returns. Comparing the chromatograms in *Figure 18 and 19*, it becomes evident that the signal at t_R 20.8 min is not due to the presence of 2M4NP, but rather an impurity.
In the chromatograms of the following field blanks and samples this signal returns again (*See Appendix III*), meaning that this impurity is always present, due to it being present in the solvent dichloromethane (DCM). Purity level of the DCM that was used was 99.5 % (GLC Assay). [33]

The chromatograms of the rest of the samples and field blanks are given in Appendix III

Results day 2 of sampling
The chromatograms of field blank 2 and samples 3A, 3B, 4A, and 4B are given in *Appendix III, Figure 19 – 23, page 64 – 66*. In these samples, there is also a signal at t_R 20.8 minutes. And as was discussed before, this signal was caused by the impurity found in the DCM. Apart from that signal, there are no clearly detectable signals for the nitrophenol compounds, at their

respective retention times, in the chromatograms of field blank 2, and samples 3A, 3B, 4A, and 4B. The chromatograms of these samples and field blank 2 are zoomed in on the region where the signals of 4NP, 3M4NP, and 2M4NP would be expected. In these chromatograms, the signal for the internal standard NCP is not shown, but NCP was also added to these solutions, and it eluted from the column at 11.76 min.

Results day 3 of sampling

As mentioned before, the diesel that was used to take DEP samples 5 and 6 from, is a blend, hence the results for these samples could vary from sample to sample. The chromatograms of field blank 3 and these samples are given in *Appendix III, Figure 24 – 28, page 66 – 68*. NCP elutes from the column at 11.75 min. The signals of 4NP, 3M4NP, and 2M4NP would be expected to appear at their respective retention times of 19.7 – 19.8 min, 20.4 – 20.5 min, and 20.6 – 20.7 min. However, from the chromatograms of samples 5A, 5B, 6A, and 6B, and comparing them to field blank 3, no visible signals for the nitrophenol compounds are seen. The signal at t_R 20.8 min, is from the impurity in the solvent.

Results day 4 of sampling

Samples 7 and 8 were also taken from DEP of diesel that is a blend. While adding the internal standard to the solutions, a mistake was made in the volume of internal standard that was supposed to be added to each solution. Instead of adding 0.1 mL of 100 ppm NCP to the solutions, 0.5 mL 100 ppm NCP was added. This mistake was only realized after the samples had been analyzed. The addition of a larger volume of NCP to the sample resulted in a higher concentration of NCP in the final concentrated solution that was analyzed. This resulted in stronger signals and larger peak areas for NCP in the chromatograms of field blank 4, and samples 7A, 7B, 8A, and 8B, compared to the signal and peak area of NCP in the previous samples and field blanks. The chromatograms of these samples and field blank 4 are given in *Appendix III, Figure 29 – 33, page 69 – 71*. The retention time of NCP in these samples is also around 11.75 min. The peaks of 4-nitrophenol, 3-methyl-4-nitrophenol, and 2-methyl-4-nitrophenol would be expected at t_R 19.7 – 19.8 min, 20.4 – 20.5 min, and 20.6 – 20.7 min. Comparing the chromatograms of the field blank and the samples, there are no apparent signals for the nitrophenol components, at the aforementioned retention times. The signal at t_R 20.8 min, is from the impurity in the solvent.

It is important to note that the type of diesel that was used to sample samples 5 – 8, was from a blend. The company that sells this type of diesel imports its diesel but also purchases locally produced diesel. The diesel that is purchased at any of the local service stations by this company can thus be fully imported diesel or fully locally produced diesel or a blend of imported and locally produced diesel. However, it is not known what the ratio of imported to locally produced diesel is in the blend. These differences can cause irregularities in the results from the samples taken from this type of diesel. Each time diesel is bought at any of their service stations, the diesel can be of a different composition and quality. Samples 5 and 6 were sampled in mid March, while samples 7 and 8 were taken in the beginning of May. Assuming that these are from different batches, no significant difference can be seen in the chromatograms at the retention times of the nitrophenol components.

Results day 5 of sampling

From here on out a different batch of dichloromethane (DCM) was used for the extraction procedure of the nitrophenols. This batch of DCM was different from the one that was used before. The first batch of DCM had run out, which is why another batch of DCM was used

from this point on. In the chromatograms it can be seen that this DCM did not have the contamination at t_R 20.8 min. The chromatograms of field blank 5 and samples 9A, 9B, 10A, and 10B are given *in Appendix III, Figure 34 – 38, page 71 – 73.* The signals of 4NP, 3M4NP, and 2M4NP were expected to appear at t_R 19.7 – 19.8 min, 20.4 – 20.5 min, and 20.6 – 20.7 min, as the signal of NCP appeared at 11.75 min. However, for none of these components, there are signals observed in the chromatograms. In the chromatograms of field blank 5 and samples 9A, 9B, 10A, and 10B, there appears to be a signal at t_R 19.4 min. This signal could not have been due to the presence of 4NP, because the difference in retention time between this signal and the retention time of standard 4NP is huge. Also, not a large shift in retention time of NCP is observed, and as seen in *Figure 34*, the signal at t_R 19.4 min also appears in the field blank with almost similar peak area as in the samples, meaning that the concentration of that component would be almost the same in all samples and the field blank. This would make it unlikely that the peak at t_R 19.4 min, would be due to the presence of 4-nitrophenol in the samples.

Results day 6 of sampling

Before analyzing the field blank and the samples on this day, a concentrated blank solution; only DCM, was analyzed, because there were some doubts that the signal at t_R 19.4 min, that was seen in the last samples and field blank, could be from 4NP. The concentrated DCM (blank solution) had a concentration factor of 10 and was concentrated under a gentle stream of nitrogen gas. The chromatogram of the concentrated DCM is given in *Appendix III, Figure 39*. After analyzing the concentrated DCM, some huge interferences were seen in the region between t_R 18.5 min and 20.0 min. This could have been caused by contamination, during any step in the concentration process or transfer of the solution into the GC vial. The field blank and samples 11A, 11B, 12A, and 12B were analyzed on the same day the concentrated DCM was analyzed. These were analyzed after analysis of the concentrated DCM. The chromatograms of field blank 4 and these samples and are given in *Appendix III, Figure 40 – 44, page 74 – 76*. The interferences seen in the chromatogram of the concentrated DCM seemed to continue in the chromatograms of the field blank and sample 11A as well. The interferences could have been due to contamination in the concentrated DCM, which would then be transferred into the field blank and sample 11A. The same pipette that was used to transfer the concentrated DCM to the vial was also used to transfer the solutions of the field blank and sample 11A into their vials. Even though the pipette was rinsed twice after each use, the contamination in the DCM could have been transferred to the following solutions. The doubts there were about whether the signal was from 4NP, were later cleared when the chromatograms of the samples were compared to a DCM blank (not concentrated). *See Figure 45.*

From the retention time, it was confirmed that the signal was due to a contamination in the DCM. In samples 11A, 11B, 12A, and 12B, there are also no signals for 4NP, 3M4NP, and 2M4NP, at their respective retention times. NCP eluted from the column at 11.75 min.

4.2.3 Quantitative Analysis

Samples from DEP of locally produced diesel

The samples that were taken from DEP of locally produced diesel are samples 1 – 4. Field blank 1 was taken on the day samples 1 and 2 were taken, and field blank 2 was taken on the day samples 3 and 4 were taken. For the chromatograms of these field blanks and samples *see Figure 11 – 15, page 34 – 36 and Appendix III, Figure 19 – 23, page 64 – 66.*

In all the chromatograms of these field blanks and samples, there are no quantifiable signals for 4NP, 3M4NP, and 2M4NP, which were expected to elute from the column at respectively 19.7 – 19.8 min, 20.4 – 20.5 min, and 20.6 – 20.7 min, from which the peak area could be

determined. The exact concentration of these components in the DEP samples from locally produced diesel could hence not be determined. However, from the results, it is assumed that in these samples the content of 4NP, 3M4NP, and 2M4NP was less than the LoQ and LoD of this method.

Samples from DEP of a blend

Samples 5 – 8 were taken from DEP of diesel that is a blend. As mentioned before this diesel could be imported or locally produced or a blend between locally produced and imported diesel. Samples 5 and 6 were taken on one day, with field blank 3, while samples 7 and 8 were taken on another day with field blank 4. Samples 5 and 6 are from the same batch, and it is assumed that samples 7 and 8 are from another batch, different from the one from which samples 5 and 6 were taken. The chromatograms of these field blanks and samples can be found in *Appendix III, Figure 24 – 33, page 66 – 71.* From the chromatograms of these samples and comparing them to the chromatograms of their respective field blanks, it is obvious that there are also no signals for 4NP, 3M4NP, and 2M4NP significant enough to be quantified. Because the peak area of the aforementioned components could not be determined, and hence their exact concentration in the samples, it is assumed that the content of these components in the samples is less than the LoD and LoQ of this method.

Samples of DEP from imported diesel

Samples 9 – 12 were taken from DEP of diesel that is imported. Field blank 5 was taken on the day samples 9 and 10 were sampled, while field blank 6 was taken on the day samples 11 and 12 were sampled. The chromatograms of these field blanks and samples can be found in *Appendix III, Figure 34 – 38 and 40 – 44, page 71 – 76.* The concentration of the nitrophenol components in these samples could also not be determined, because there are no apparent signals for these components that can be quantified. In field blank 6 and sample 11A there were also some interferences in the region were the signals for 4NP, 3M4NP, and 2M4NP were expected, caused by possible contamination. Because the exact content of 4NP, 3M4NP, and 2M4NP could not be determined due to the aforementioned reasons, it is assumed that in these DEP samples as well, the content of 4NP, 3M4NP, and 2M4NP is less than the LoQ and LoD of this method.

Limitations

Due to the unavailability of some of the equipment used in the existing sampling and preparation method, some modifications were made. Instead of using a digital flow meter and an air sampler, a direct-reading flow meter and a vacuum pump used to suck the diesel exhaust through the solution in the impinger were used. Also because of the unavailability of a rotary evaporator, to concentrate the solutions with, simple distillation was applied. The method of distilling most of the solvent from the solution worked well because the difference in boiling point between DCM (the solvent) and the nitrophenols was more than 100 °C. The boiling point of DCM is 39.5 °C, while the nitrophenols begin to boil and decompose at temperatures above 200 °C. Although these changes to the sampling and preparation method, may not have directly resulted in the poor determination of the nitrophenols in the samples, it should be noted that these changes were made to an existing method, in replacement of that which was unavailable.

To identify and determine the content of 4-nitrophenol, 2-methyl-4-nitrophenol, and 3-methyl-4-nitrophenol in samples of DEP, the use of a gas chromatography instrument, equipped with mass spectrometry (GC-MS) was recommended, as these were used in the previous studies

done on the presence of nitrophenols in DEP. However, due to the unavailability of a working GC-MS at the time the analyses in this study were carried out, a gas chromatography instrument equipped with a flame ionization detector (GC-FID) was used instead. The column that was used in the previous studies was a 5%–Phenyl Arylene 95%–Dimethylpolysiloxane capillary column, such as the ZB-5ms, which due to the phenyl-arylene bonded phase improves resolution of aromatic compounds, and reduced activity for acidic and basic compounds leads to better quantitation with these columns. These columns are especially useful for the analysis of aromatics and semi-volatile mixtures containing acids, bases, and neutrals. [3]

In this study a HP-5 5% Phenyl Methyl Siloxane capillary column was used. Although this column is applicable for general purposes, and it should have low column bleeding, it was not the right column to determine the presence of nitrophenol components in DEP samples. The instrument combined with the HP-5 column and FID was semi-quantitative, as it was possible to detect the nitrophenol components in the standards quantitatively up to concentrations as low as 10 ppm.

CONCLUSION

The operating conditions of the instrument (Hewlett Packard HP 6890Series Gas Chromatograph coupled with 6890 flame ionization detector (FID)) for the analysis of the nitrophenol components was accurately validated and calibrated as suggested by Agilent Technologies Inc., in Analysis of Phenols by GC according to EPA 8040A.

The relative retention time of the components 4NP, 3M4NP, and 2M4NP, were determined to be 19.804 min ± 0.22%, 20.432 min ± 0.14%, and 20.669 min ± 0.12%, respectively. These were confirmed from the retention time of the individual standards which were 19.725 – 19.740 min for 4NP, 20.379 – 20.388 for 3M4NP, and 20.606 – 20.620 min for 2M4NP. The results from these calibration standards were shown to be reliable, as well as fitting the computed calibration plots well. 2M4NP fitted the model for the computed calibration plot the best.

The calibration check done with a second set of nitrophenol mix standards showed reliable results, with standard deviations of the mean of the retention time to be 0.01%, 0.01%, and 0.02% for 4NP, 3M4NP, and 2M4NP, respectively. Although these eluted faster from the column, compared to the first nitrophenol mix standards, the shift of the signals are accounted to instrument variation and degradation of the column, because the same shift could also be seen with the signals of the internal standard. The results, in terms of the peak area of the nitrophenol mix standards could however not be replicated. The standard deviation of the peak area of the components is reported as <27.7%, <22.8%, and <25.5%, for 4NP, 3M4NP, and 2M4NP, respectively.

The LoD of the method were determined to be as low as 2.50 ppm, 3.41 ppm, and 4.78 ppm, for 2M4NP, 3M4NP, and 4NP, respectively, while the LoQ of the method were determined to be 7.58 ppm, 10.32 ppm, and 14.48 ppm, for 2M4NP, 3M4NP, and 4NP, respectively. TheGC-FID showed the best sensitivity to 3M4NP, followed by 2M4NP and 4NP.

The nitrophenol standards purchased as solid material were of GC grade with purity levels higher than 98%. Two blank solutions, spiked with 10 ppm and 80 ppm nitrophenol mix standards, and treated as if they were samples, had recovery percentages of the spikes; the nitrophenol components, between 77.0% and 93.0 %.

The use of a GC-FID equipped with the HP-5 capillary column, used in the place of a GC-MS combined with a 5%–Phenyl-Arylene–95%-Dimethylpolysiloxane capillary column, which was unavailable during the time this study was carried out, did not give quantifiable results for 4-nitrophenol, 2-methyl-4-nitrophenol, and 3-methyl-4-nitrophenol, in the DEP samples that were taken.

Although no significant signals for 4-nitrophenol, 3-methyl-4-nitrophenol, and 2-methyl-4-nitrophenol could be detected in the DEP samples that were taken, and the nitrophenol components could not be identified, it cannot be said that these components are not present in DEP of the three types of diesel, from which was sampled. The reason these components could not be detected in the samples may be due to the GC instrument that was used, coupled with the FID and the column. It could be that the content of the nitrophenol components are far below the LoD of this method, and that the instrument with the detector and the column were not the right fit to do these analyses on.

The instrument combined with the HP-5 column and FID was semi-quantitative, as it was possible to detect the nitrophenol components in the standards quantitatively up to concentrations as low as 10 ppm.

In this study a HP-5 5% Phenyl Methyl Siloxane capillary column was used. Although this column is applicable for general purposes, and it should have low column bleeding, it was not the right column to determine the presence of nitrophenol components in DEP samples. In the chromatograms, column bleeding can be detected, which means that the stationary phase of the column was breaking down. This did have effects on the results, as the column bleeding occurred in the region where the nitrophenol components eluted from the column, and quantification of the signals from these components could more difficult.

The method using a GC-FID equipped with a HP-5 5% Phenyl Methyl Siloxane capillary column, could not determine the content of 4-nitrophenol (4NP), 2-methyl-4-nitrophenol (2M4NP), and 3-methyl-4-nitrophenol (3M4NP) in the DEP samples taken from locally produced diesel, imported diesel, and the blend diesel. As it was not possible to quantify the signals of the nitrophenol components in the samples, the concentration ratio between them could also not be determined, as well as determining which of the three nitrophenol components is the most abundant in the DEP samples that were taken.
Hence why no statement can be made regarding which type of diesel releases more 4NP, 3M4NP and 2M4NP to the atmosphere, after combustion.

RECOMMENDATIONS

- For following research regarding this topic, a recommendation would be to make the DEP samples more concentrated (e.g. twice as much DEP sampled in the same volume of NaOH sampling solvent). Sampling more DEP could mean that more nitrophenols will be present in the samples, and upon extraction and analysis will give quantifiable signals for the nitrophenol components.

- Furthermore, to determine the presence of the precursors, such as benzene and toluene, in the diesel that is sampled. If these components are not present or are present in low concentrations, the chances of nitrophenols being present in the exhaust that is sampled, would be lower.

- Another suggestion would be to use a different sampling method; for example the use of a constant-volume sampler system in which DEP will be collected on glass fiber filters, from which the nitrophenols can then be extracted.

- One other approach to improve the results on determining the nitrophenols content in DEP samples, could be to first derivatize the nitrophenols in the samples before analysis on the GC system. Nitrophenols are semi-volatile components, and derivatization of the nitrophenols into more volatile components right before analysis on a GC could result in better responses by the instrument and sharper signals, to be then quantified and to accurately determine the concentration of the components.

- The use of the recommended instrument: a gas chromatograph with a mass spectrometer system (GC-MS), equipped with a 5 % – Phenyl-Arylene – 95 % – Dimethylpolysiloxane capillary column, should also result in improvement of determining the concentration of nitrophenols in DEP samples in the range of ppb. Although other factors such as temperature programming, inlet systems, and detectors could also result in better results. With these changes, identification and determination of the exact content of 4-nitrophenol, 2-methyl-4-nitrophenol, and 3-methyl-4-nitrophenol in DEP samples could be improved.

REFERENCES

1. *Li C, Taneda S, Suzuki A, Furuta C, Watanabe G, Taya K. Anti-androgenic activity of 3-methyl-4-nitrophenol in diesel exhaust particles. European Journal of Pharmacology. 2006; 543:194–199.*
2. *Diesel Exhaust Particle Size [Internet]. Dieselnet.com. 2019 [cited 2 March 2019]. Available from: https://dieselnet.com/tech/dpm_size.php*
3. *Noya Y, Mikami Y, Taneda S, Mori Y, Suzuki A, Ohkura K et al. Improvement of an efficient separation method for chemicals in diesel exhaust particles: analysis for nitrophenols. Environmental Science and Pollution Research. 2008; 15:318-321.*
4. *Wichmann H. Diesel Exhaust Particles. Inhalation Toxicology. 2007; 19:241–244.*
5. *Sagai M, Saito H, Ichinose T, Kodama M, Mori Y. Biological Effects of Diesel Exhaust Particles. I .In Vitro Production of Superoxide and In Vivo Toxicity in Mouse. Free Radical Biology & Medicine. 1993; 14:37-47.*
6. *Tremp J, Mattrel P, Fingler S, Giger W. Phenol and nitrophenols as tropospheric pollutants: Emissions from automobile exhausts and phase transfer in the atmosphere. Water Air and Soil Pollution. 1993; 68(1):113-123.*
7. *Agency for Toxic Substances and Disease Registry U.S. Public Health Service. Toxicological Profile for Nitrophenols: 2-Nitrophenol and 4-Nitrophenol. Agency for Toxic Substances and Disease Registry; 1992; 1 - 82.*
8. *Guo X, Wang Z, Zhou S. The separation and determination of nitrophenol isomers by high-performance capillary zone electrophoresis. Elsevier. 2004; 64:135 - 139.*
9. *Yuan B, Liggio J, Wentzell J, Li S, Stark H, Roberts J et al. Secondary formation of nitrated phenols: insights from observations during the Uintah Basin Winter Ozone Study (UBWOS) 2014. Atmospheric Chemistry and Physics. 2016; 16:2139–2153.*
10. *Harrison M, Barrab S, Borghesib D, Vioneb D, Arsenec C, Olariuc R. Nitrated phenols in the atmosphere: a review. Atmospheric Environment. 2005; 39:231–248.*
11. *Mori Y, Kamata K, Toda N, Hayashi H, Seki K, Taneda S et al. Isolation of Nitrophenols from Diesel Exhaust Particles (DEP) as Vasodilatation Compounds. Biological and Pharmaceutical Bulletin. 2003; 26:394-395.*
12. *Lüttke J, Scheer V, Levsen K, Wünsch G, Cape J.N, Hargreaves K.J et al. Occurrence and formation of nitrated phenols in and out of cloud. Atmospheric Environment. 1997; 31(16):2637-2648.*
13. *Taneda S, Mori Y, Kamata K, Hayashi H, Furuta C, Li C, Seki K, Sakushima A, Yoshino S, Yamaki K, Watanabe G, Taya K, Suzuki AK (2004b) Estrogenic and anti-androgenic activity of nitrophenols in diesel exhaust particles (DEP). Biological and Pharmaceutical Bulletin. 2004; 27:835–837.*
14. *OSHA Annotated PELs | Occupational Safety and Health Administration [Internet]. Osha.gov. 2019 [cited 9 August 2019]. Available from: https://www.osha.gov/dsg/annotated-pels/index.html*
15. *Shellie R. Gas Chromatography. Encyclopedia of Forensic Sciences. 2013; 2:579-585.*
16. *Thet K, Woo N. Gas Chromatography [Internet]. Chemistry LibreTexts. 2019 [cited 6 July 2020]. Available from: https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Instrumental_Analysis/Chromatography/Gas_Chromatography*
17. *de Zeeuw J. Injection Techniques used in GC: Names and What are we actually doing with each Technique? « ChromaBLOGraphy: Restek's Chromatography Blog [Internet]. Blog.restek.com. 2014 [cited 5 December 2020]. Available from: https://blog.restek.com/injection-techniques-used-in-gc-names-and-what-are-we-actually-doing-with-each-technique/*
18. *Harris D. Quantitative Chemical Analysis. 8th ed. New York: W. H. Freeman and Company; 2010.*

19. *HP-5 GC column | Agilent [Internet]. Agilent.com. [cited 25 July 2020]. Available from: https://www.agilent.com/en/product/gc-columns/standard-polysiloxane-gc-columns/hp-5-columns#productdetails*
20. *Raynie D. The Vital Role of Blanks in Sample Preparation [Internet]. Chromatography Online. 2018 [cited 5 December 2020]. Available from: https://www.chromatographyonline.com/view/vital-role-blanks-sample-preparation*
21. *Guidance for the validation of analytical methodology and calibration of equipment used for testing of illicit drugs in seized materials and biological specimens. New York: United Nations; 2009.*
22. *Herterich R, Herrmann R. Comparing the distribution of nitrated phenols in the atmosphere of two German hill sites. Environmental Technology. 1990; 11:10, 961-972.*
23. *Leuenberger C, Czuczwa J and Giger W. Nitrated phenols in rain; atmospheric occurrence of phytotoxic pollutants. Chemosphere. 1988; 17:511-515.*
24. *Wang Y, Hu M, Wang Y, Zheng J, Shang D, Yang Y et al. The formation of nitro-aromatic compounds under high NOx and anthropogenic VOC conditions in urban Beijing, China. Atmospheric Chemistry and Physics. 2019;19(11):7649-7665.*
25. *Valkenburg C, Munslow W, Butler LC. Evaluation of modifications of extraction procedures used in analysis of environmental samples from superfund sites. J Assoc Off Anal Chem. 1989; 72:602-608.*
26. *Nojima K, Kawaguchi A, Ohya T, Kanno S and Hirobe M. Studies on photochemical reaction of air pollutants. X. Identification of nitrophenols in suspended particulates. Chem. Pharm. Bull. 1983; 31:1047-1051.*
27. *Taneda S, Kamata K, Hayashi H, Tada N, Seki K, Sakushima A, Yoshino S, Yamaki K, Sakata M, Mori Y et al. (2004a) Investigation of vasodilatory substances in diesel exhaust particles (DEP): isolation and identification of nitrophenol derivatives. Journal of Health Science. 2004; 50:133–141.*
28. *McHale W. A Better Approach to Calculating Limits of Detection and Limits of Quantitation [Internet]. Eraqc.com. 2012 [cited 27 October 2020]. Available from: http://www.eraqc.com/Portals/0/PDF%20documents/ERA_LOD-LOQ_White_Paper_R2.pdf*
29. *Thode J. How to determine the LOD using the calibration curve? [Internet]. Mpl.loesungsfabrik.de. 2019 [cited 28 October 2020]. Available from: http://www.mpl.loesungsfabrik.de/en/english-blog/method-validation/calibration-line-procedure*
30. *Marathe S. Excelling With Excel: Analytical Method Validation Using LOD and LOQ [Internet]. Bitesize Bio. 2020 [cited 27 October 2020]. Available from: https://bitesizebio.com/48459/excel-lod-loq/*
31. *Norbert F, Pfundstein B, Frei E, Ruchirawat M. International Training on Detection of Environmental Pollutants and Monitoring of Health Effect. Lecture presented at; 2018; Bangkok, Thailand.*
32. *European Commission. Commission Decision of 12 August 2002 Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. 2002*
33. *Dichloromethane, Reagent (ACS) | GFS Chemicals [Internet]. Gfschemicals.com. 2020 [cited 16 November 2020]. Available from: https://www.gfschemicals.com/Product/dichloromethane-reagent-acs-cas-75-09-2-item-909-sku-33502*

APPENDIX I

Equipment and instrument used for sampling and analysis

Figure 20. Glass Impinger

Figure 21. Flow Meter

Figure 22. Vacuum air pump

Figure 23. Setup for DEP sampling

A glass funnel placed at the end of the exhaust pipe, from which DEP was sampled. The glass funnel is connected to the impinger containing 1N NaOH solution, and placed in a cooling box with ice, through which the DEP was bubbled. At the other end the impinger is connected to a flow meter, from which the amount of exhaust air sampled, was read (the flow was set at 3 L/min), and at the end a vacuum air pump, to suck the DEP through the impinger solution.

Figure 24. Hewlett Packard HP 6890Series Gas Chromatography instrument, with a Flame Ionization Detector, at the Polytechnic College (PTC).
This GC instrument was used to carry out the analysis of all the standards and the samples of this study.

Calculations for the preparation of the solutions and standards

Preparation of 1N sodium hydroxide solution (NaOH) and 10N sulfuric acid (H_2SO_4)
A solution of 1N sodium hydroxide (NaOH) was prepared by dissolving 20 g; 0.5001 moles in 500 mL of distilled water.

- Sodium hydroxide pellets (20 g) were weighed on an analytical balance
- The pellets were then dissolved into ~200 mL distilled water
- The NaOH solution was transferred into a 500 mL volumetric flask and the solution was diluted to the mark with distilled water

The N stands for Normality; which is 1 gram-equivalent weight of solute per litre of solution.

$$\textit{Equation 1: } N = \frac{gram\ molecular\ weight}{valence}$$

$$\textit{Equation 2: } Weight\ (in\ g) = desired\ N * volume\ (in\ L) * \frac{GMW}{Valence}$$

or

$$W = N * V * \frac{GMW}{Valence}$$

1N NaOH solution:
N = 1
GMW = 39.99 g/mol
Valence = 1
V = 0.500 L

$$\textit{Equation 3: } W = 1 * 0.500L * 39.99\frac{g}{mol} = 19.995\ g = 20\ g$$

Preparation of 10N sulfuric acid (H_2SO_4)

- In a 100 mL graduated cylinder, measure 55.40 mL concentrated (96.2%) H_2SO_4
- Slowly add this solution into a 250 mL beaker, that already contains ~100 mL distilled water
- Slowly add the solution from the beaker into a 200 mL volumetric flask and dilute to the mark with distilled water

10N H_2SO_4 solution:
N = 10
GMW = 98.08 g/mol
Valence = 2
V = 0.200 L
Concentration = 96.2 %
Density = 1.84 g/mL

$$W = N * V * \frac{GMW}{Valence}$$

$$\textit{Equation 4: } W = 10 * 0.200L * \frac{98.08\frac{g}{mol}}{2} = 98.08\ g$$

$$\textit{Equation 5: } Volume, H2SO4 = \frac{mass,H2SO4}{(conc,H2SO4)*density,H2SO4}$$

$$\textit{Equation 6: } Volume, H2SO4 = \frac{98.08\ g}{0.962*1.84\frac{g}{mL}} = 55.40\ mL$$

Preparation of the calibration standards

a) Preparation of 500 ppm 4-nitrophenol stock solution:
   - Solid 4-nitrophenol (0.0252 g; $1.81 * 10^{-4}$ mol) was dissolved in ~25 mL dichloromethane (DCM)
   - The solution was transferred into a 50 mL volumetric flask, and further diluted to the mark with DCM

b) Preparation of 500 ppm 2-methyl-4-nitrophenol stock solution:
   - Solid 2-methyl-4-nitrophenol (0.0251 g; $1.64 * 10^{-4}$ mol) was dissolved in ~25 mL dichloromethane (DCM)
   - The solution was transferred into a 50 mL volumetric flask, and further diluted to the mark with DCM

c) Preparation of 500 ppm 3-methyl-4-nitrophenol stock solution:
- Solid 3-methyl-4-nitrophenol (0.0251 g; $1.64 * 10^{-4}$ mol) was dissolved in ~25 mL dichloromethane (DCM)
- The solution was transferred into a 50 mL volumetric flask, and further diluted to the mark with DCM

d) Individual nitrophenol standards were prepared in concentrations of 10 ppm and 100 ppm

Table 1. Individual nitrophenol standards

Concentration of individual nitrophenol standard [ppm]	**Volume of 500 ppm nitrophenol stock solution added [mL]**	**Final volume [mL]**
10	0.10	5
100	1.0	5

The nitrophenol mix calibration standards contained: 4-nitrophenol (4NP), 2-methyl-4-nitrophenol (2M4NP) and 3-methyl-4-nitrophenol (3M4NP)

e) Preparation of 100 ppm nitrophenol mix working solution
- To prepare this solution, 10 mL 500 ppm 4NP, 10 mL 500 ppm 2M4NP and 10 mL 500 ppm 3M4NP were transferred into a 50 mL volumetric flask, using a volumetric pipette
- The solution was then diluted to the mark with DCM

To prepare the following calibration standards, volumetric flasks of 5 mL were used, and the solutions were diluted to the mark with DCM

Table 2. Nitrophenol mix calibration standards

Concentration of nitrophenol mix standard [ppm]	**Volume of 100 ppm nitrophenol mix working solution added [mL]**	**Final volume [mL]**
1	0.05	5
10	0.5	5
20	1.0	5
40	2.0	5
60	3.0	5
80	4.0	5

The calibration standards, including a blank (DCM) were then analyzed on the GC-FID instrument

Preparation of nitrocyclopentane (internal standard) solutions

Preparation of 500 ppm nitrocyclopentane (internal standard) stock solution:

- Liquefied nitrocyclopentane (NCP) (4.6 μL; 0.0050 g; $4.34 * 10^{-5}$ mol) was transferred into a 10 mL volumetric flask, using a micropipette
- DCM was added to the flask, until the mark, to dilute the solution to 500 ppm

Preparation of 100 ppm nitrocyclopentane (internal standard) working solution:

- From the 500 ppm NCP stock solution, 1 mL was pipetted into a 5 mL volumetric flask
- The solution was then diluted to the mark with DCM

APPENDIX II

Chromatograms of first set of nitrophenol mix calibration standards

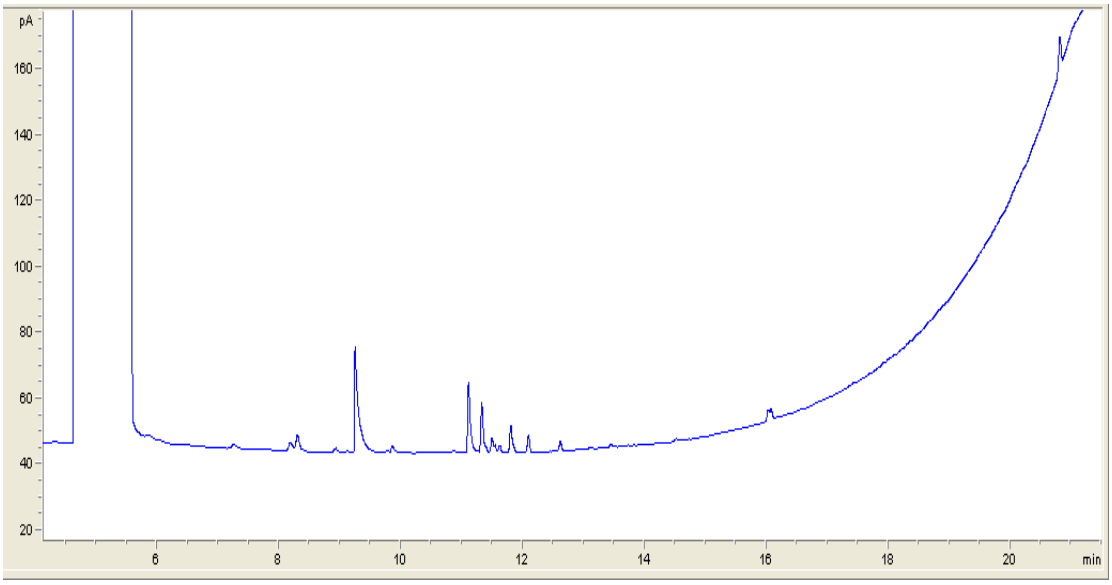

Figure 1A. Chromatogram of DCM (Blank)

The first analysis was of the blank; the solvent in which the nitrophenols would be solved. The solvent used was dichloromethane (DCM). The signal of the DCM on the GC-FID used, came at 4.7 minutes; that is the large signal response seen in the graph.

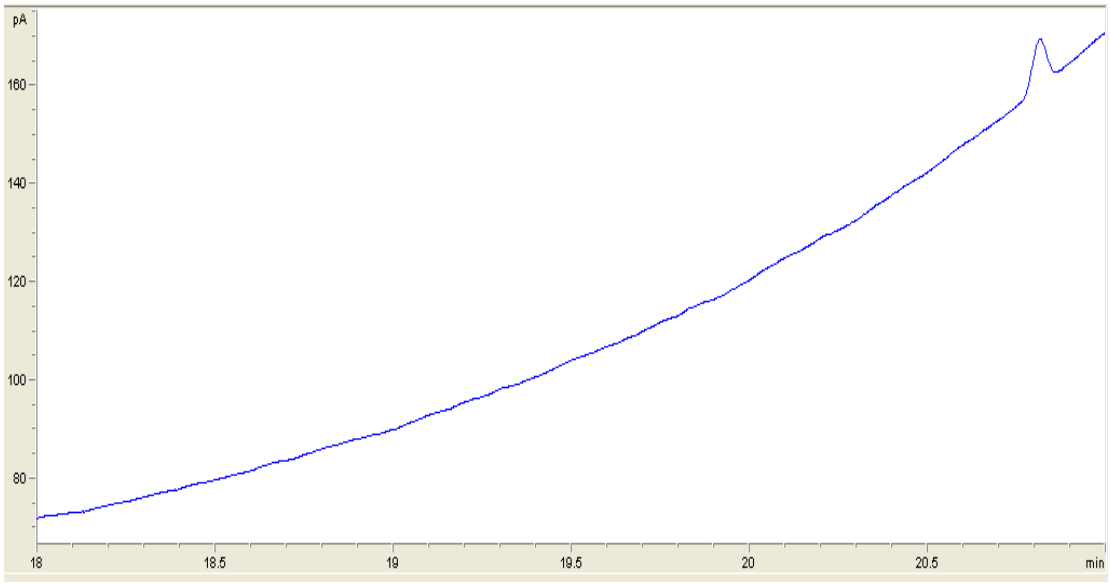

Figure 1B. Chromatogram of DCM (Blank), zoomed in on the region 18 min – 21 min

This is the same graph as above, only zoomed in on the region 18 min – 21 min, where the signal responses of the nitrophenol standards was expected.

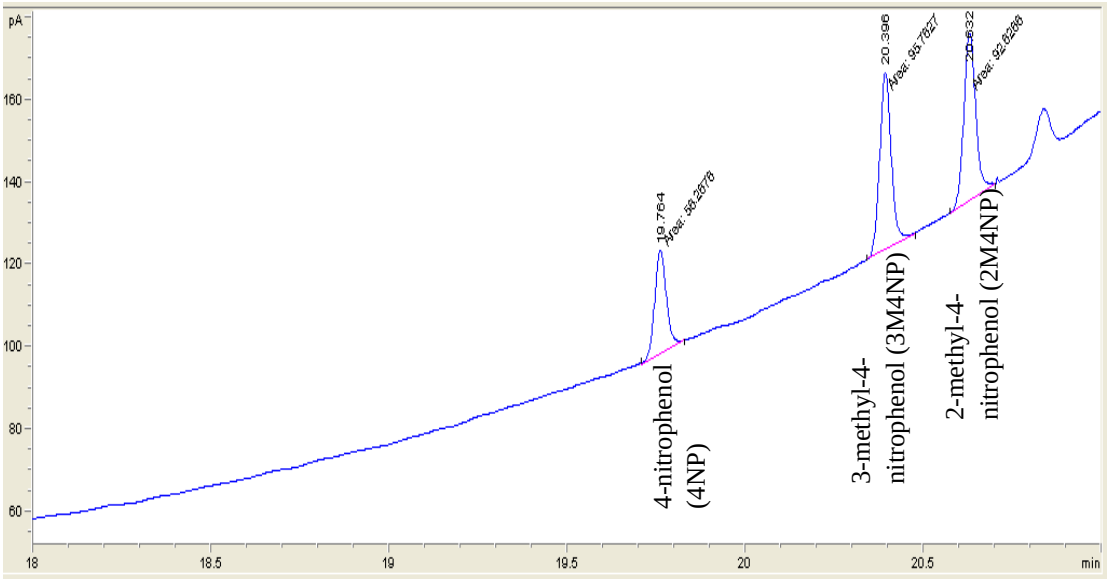

Figure 2. Chromatogram of 10 ppm mix standard

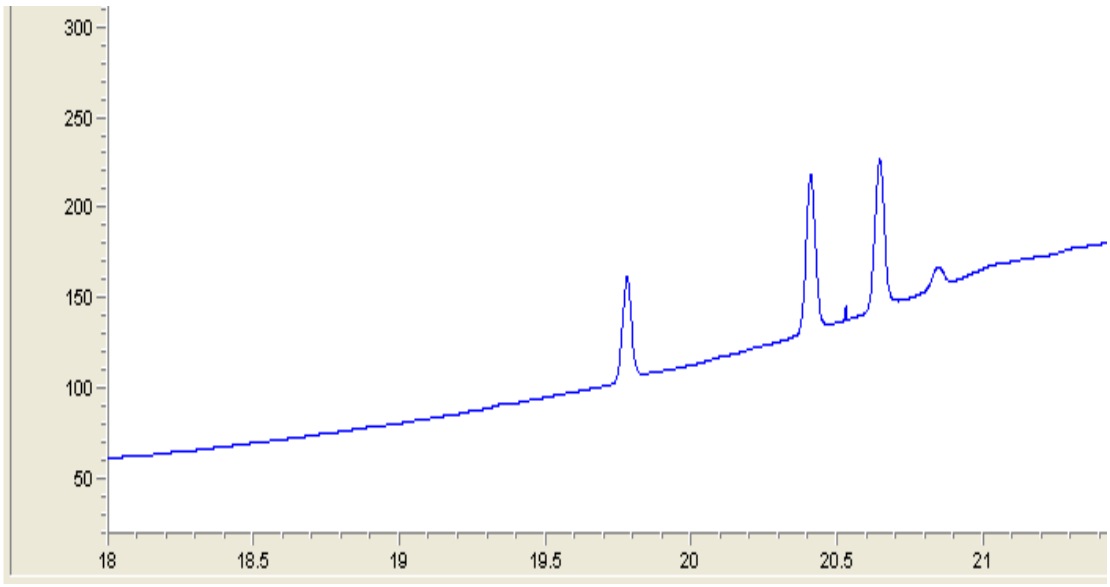

Figure 3. Chromatogram of 20 ppm mix standard

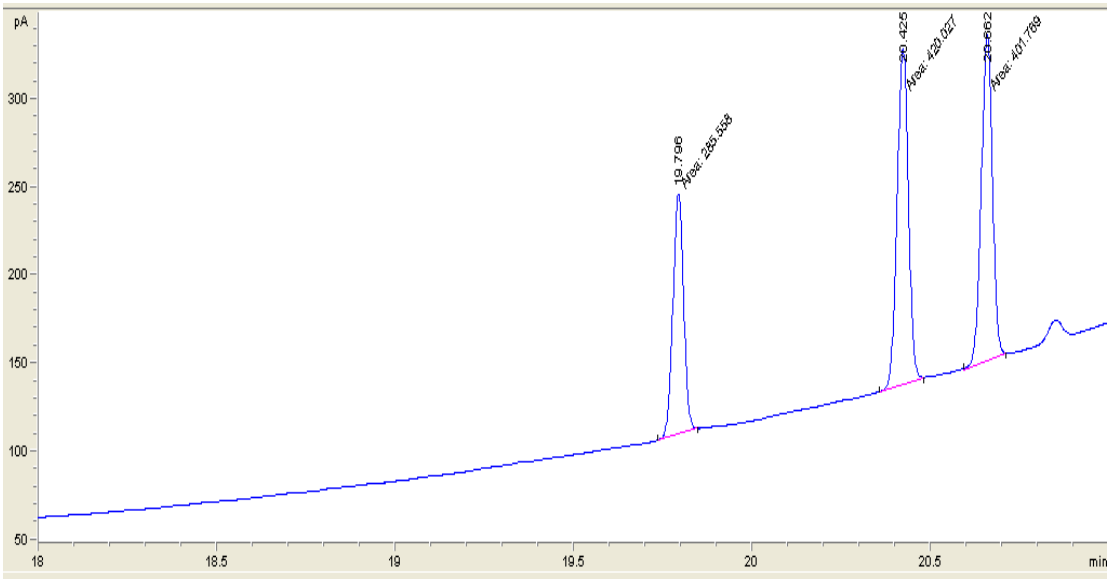

Figure 4. Chromatogram of 40 ppm mix standard

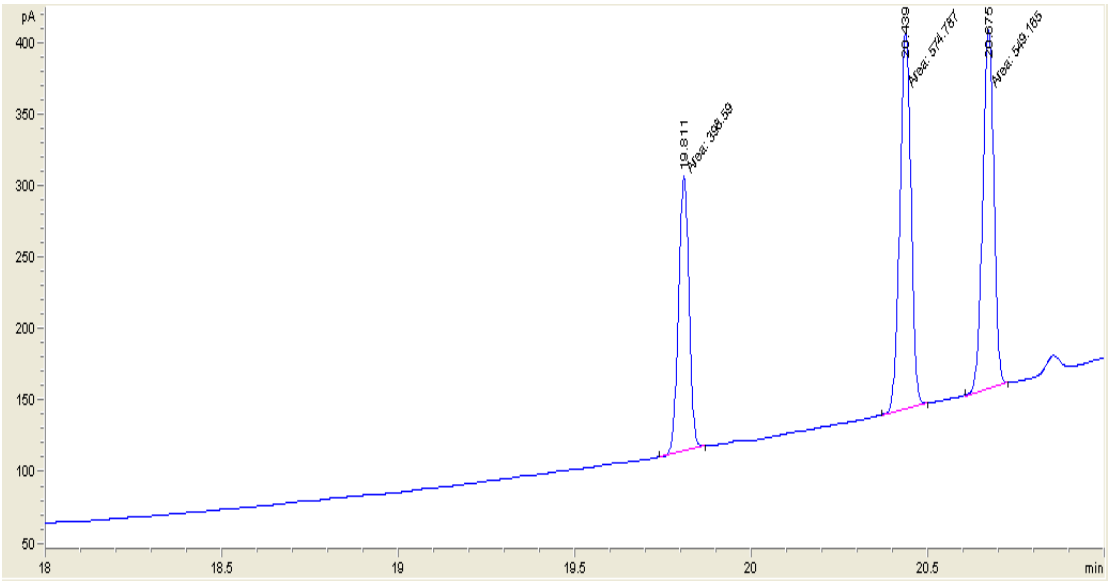

Figure 5. Chromatogram of 60 ppm mix standard

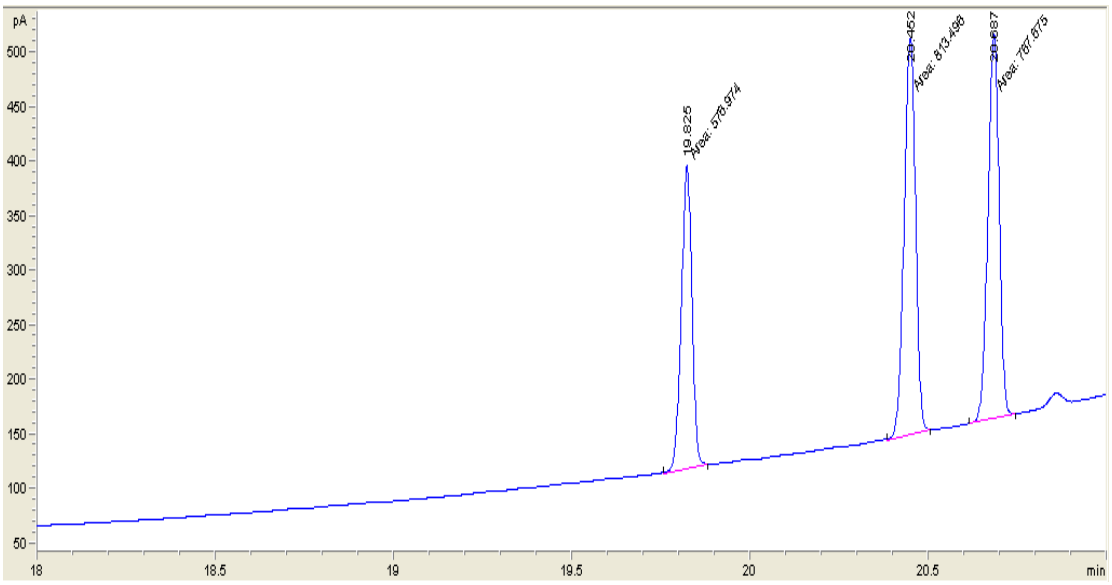

Figure 6. Chromatogram of 80 ppm mix standard

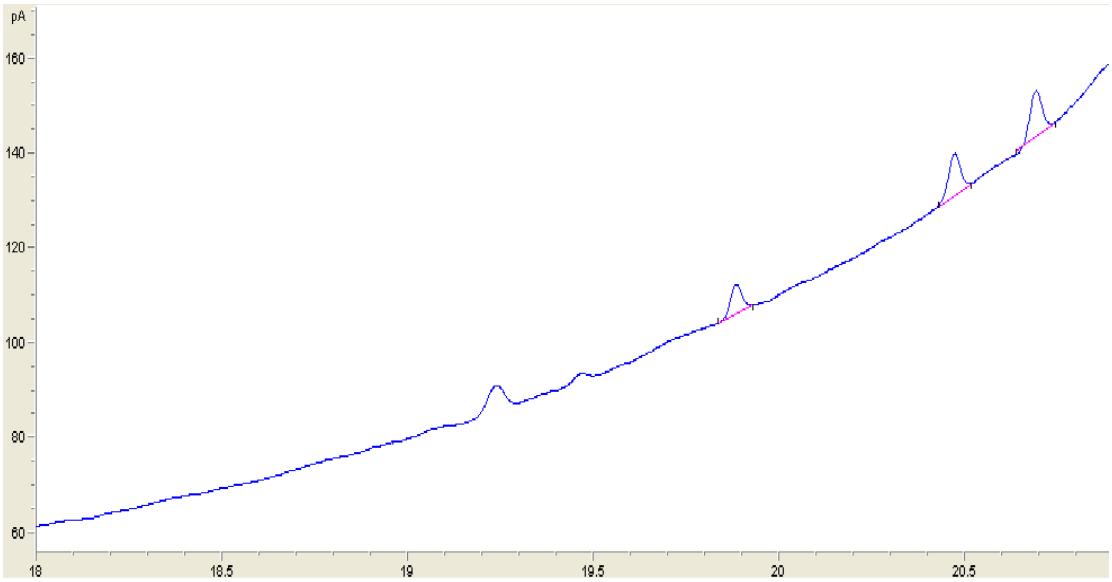

Figure 7A. Chromatogram of 2 ppm mix standard (LoD check)

The following two chromatograms are the same as figure 7A, only zoomed in on the region 19.6 min – 20.2 min and 20.3 min – 20.8 min, respectively. These were zoomed in to get a better view of the signal responses of the 2 ppm 4-nitrophenol, 2 ppm 3-methyl-4-nitrophenol and 2 ppm 2-methyl-4-nitrophenol.

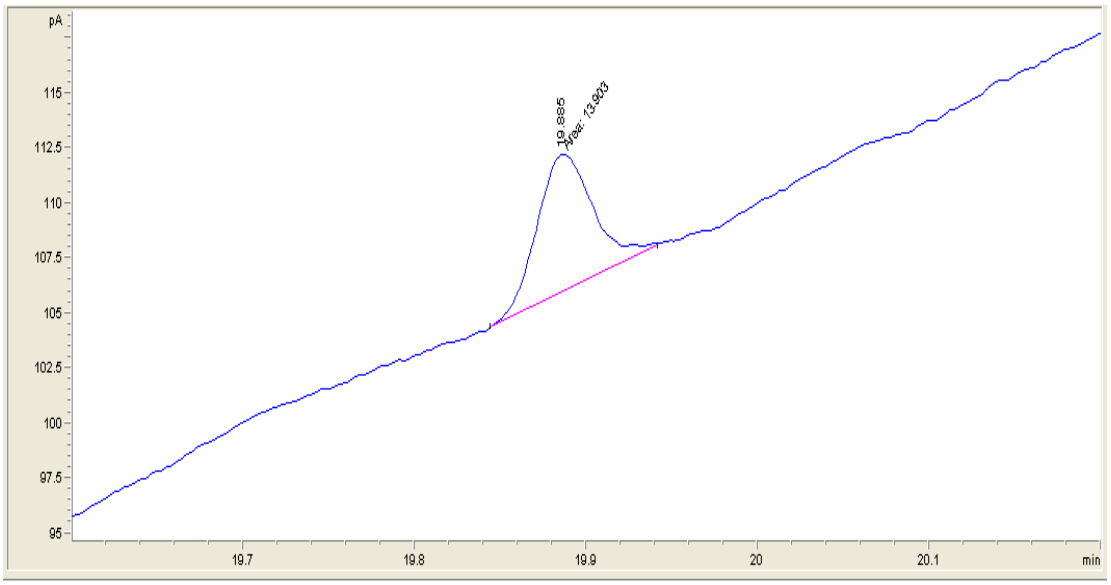

Figure 7B. Chromatogram of 2 ppm 4-nitrophenol (LoD check), zoomed in the region 19.6 min – 20.2 min

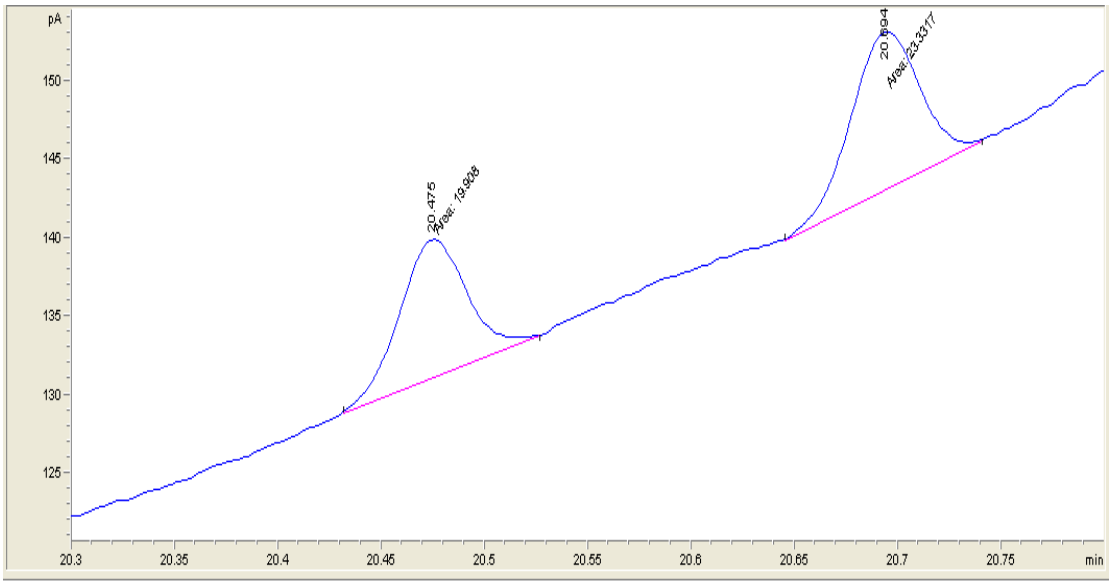

Figure 7C. Chromatogram of 2 ppm 2-methyl-4-nitrophenol and 3-methyl-4-nitrophenol (LoD check), zoomed in the region 20.3 min – 20.8 min

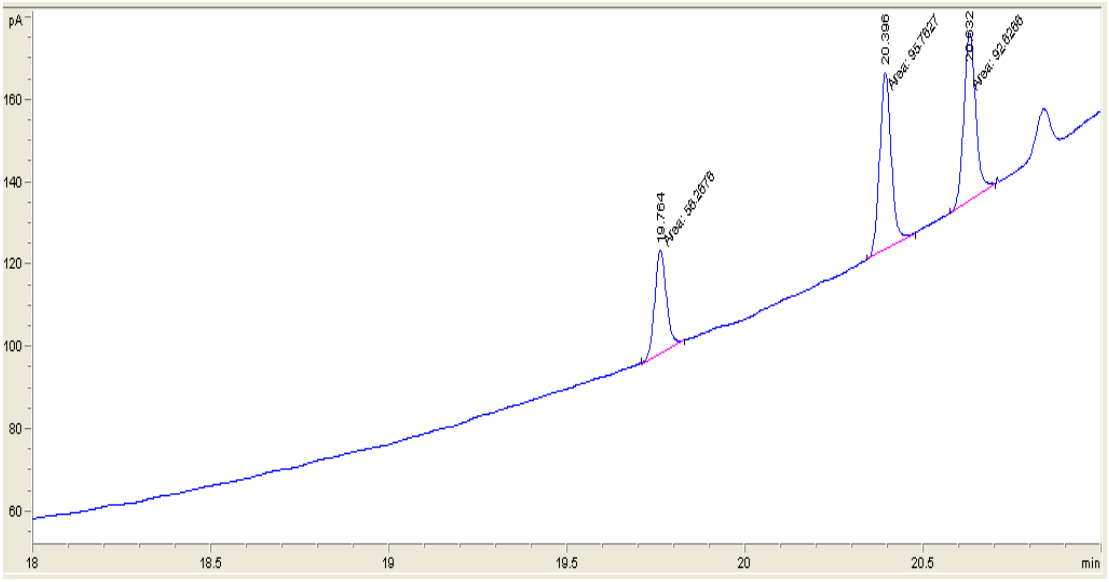

Figure 8A. Chromatogram of 10 ppm mix standard (LoQ check)

The two following chromatograms are the same as figure 8A, only zoomed in on the region 19.5 min – 20.0 min and 20.3 min – 20.8 min, respectively, to get a better view of the signal responses of the 10 ppm 4-nitrophenol, 10 ppm 3-methyl-4-nitrophenol and 10 ppm 2-methyl-4-nitrophenol.

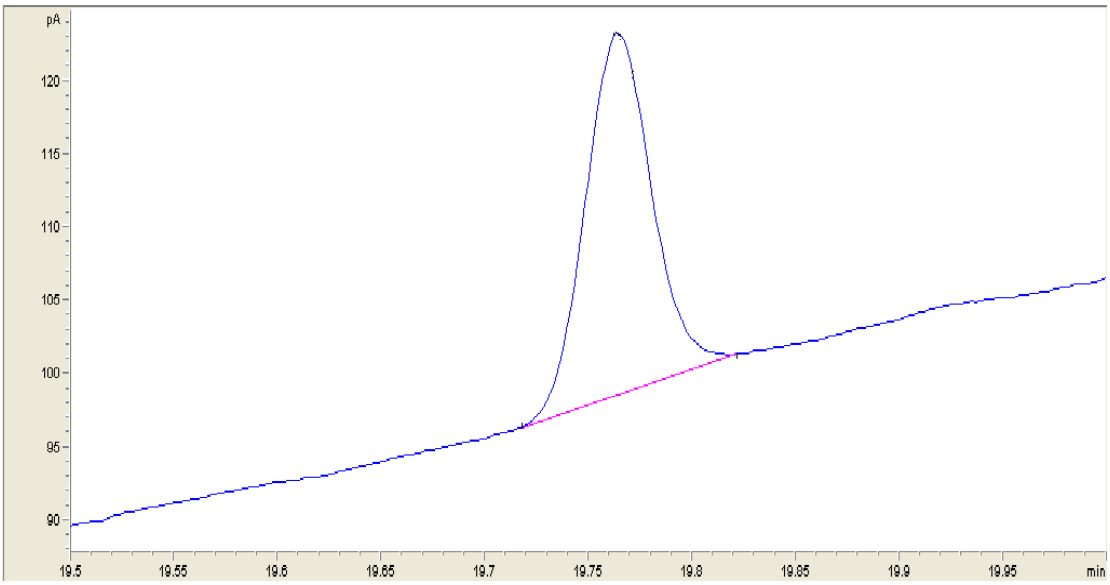

Figure 8B. Chromatogram of 10 ppm 4-nitrophenol (LoQ check), in the region 19.5 min – 20.0 min

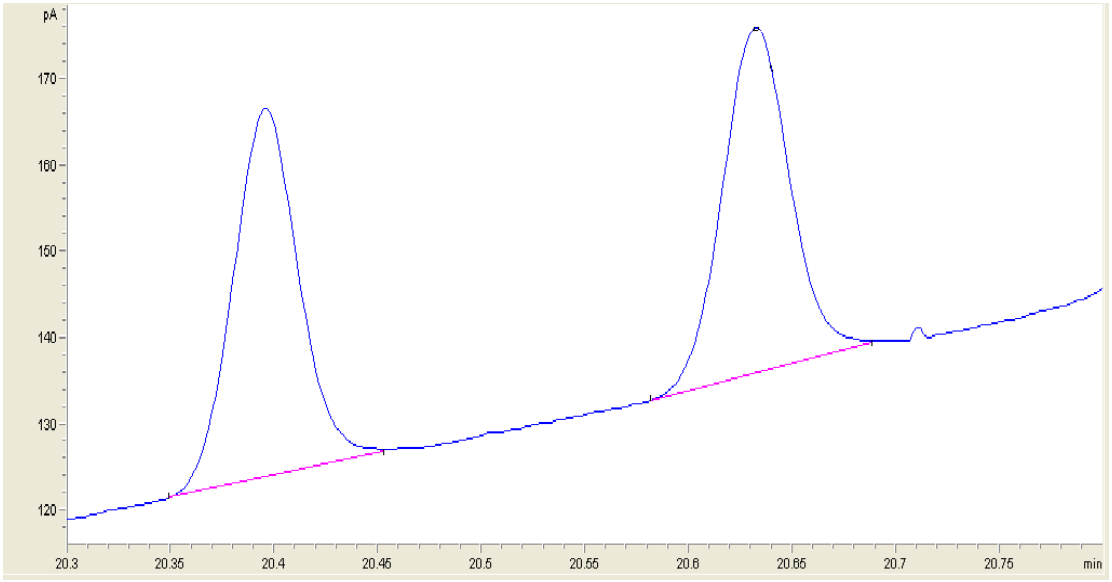

Figure 8C. Chromatogram of 10 ppm 2-methyl-4-nitrophenol and 3-methyl-4-nitrophenol (LoQ check), in the region 20.3 min – 20.8 min

Chromatograms of second set of nitrophenol mix calibration standards

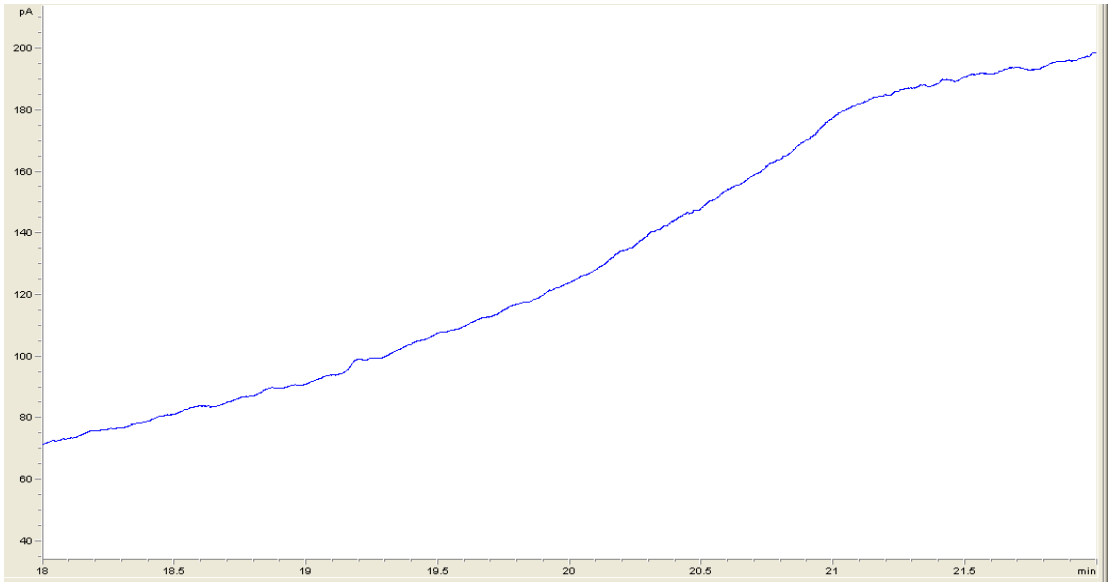

Figure 9. Chromatogram of the blank (DCM)

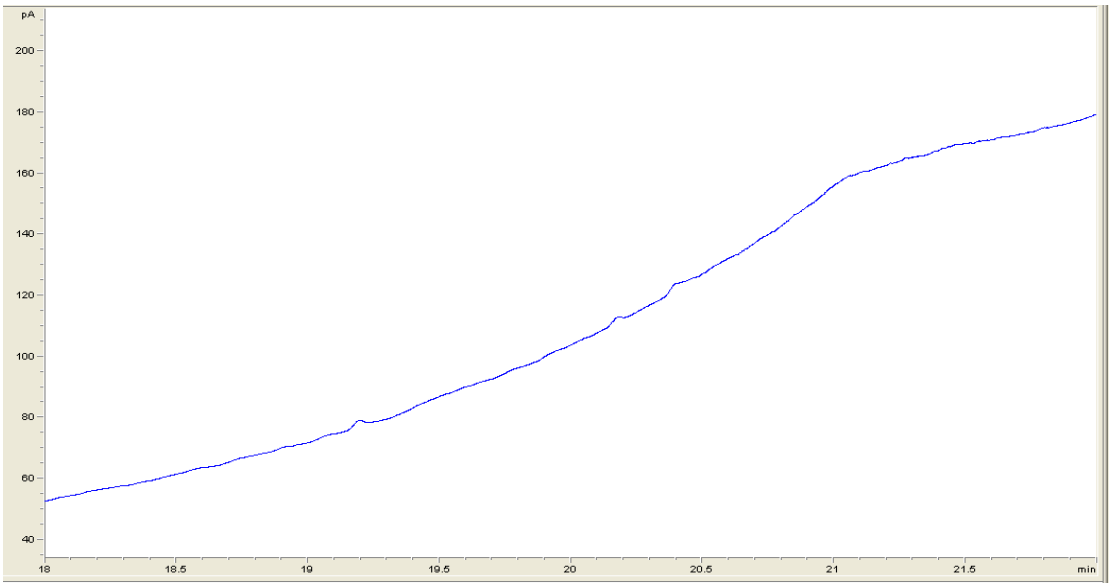

Figure 10. Chromatogram of 2 ppm mix standard

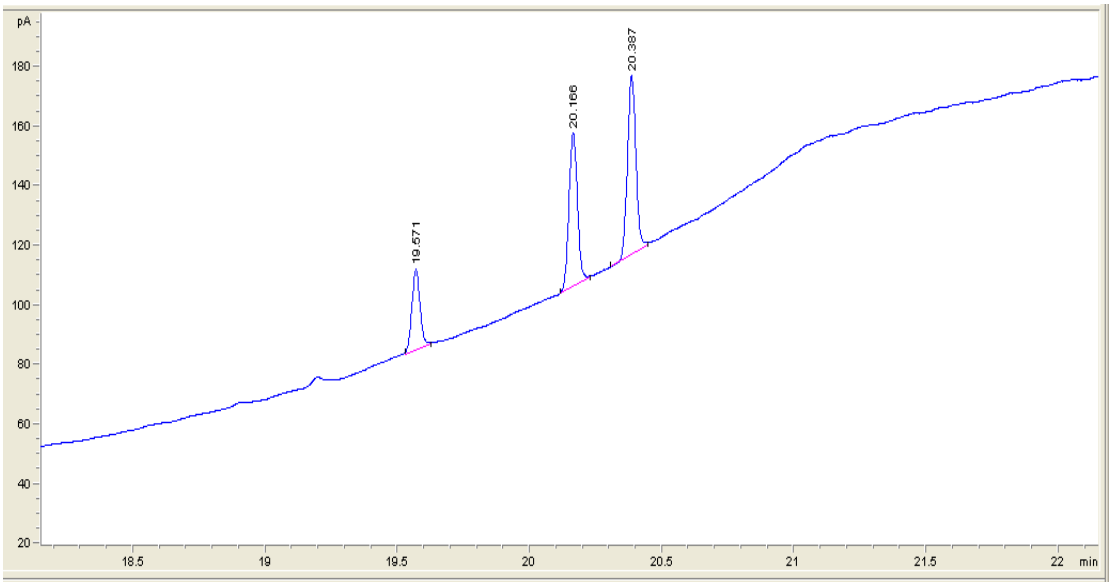

Figure 11. Chromatogram of 10 ppm mix standard

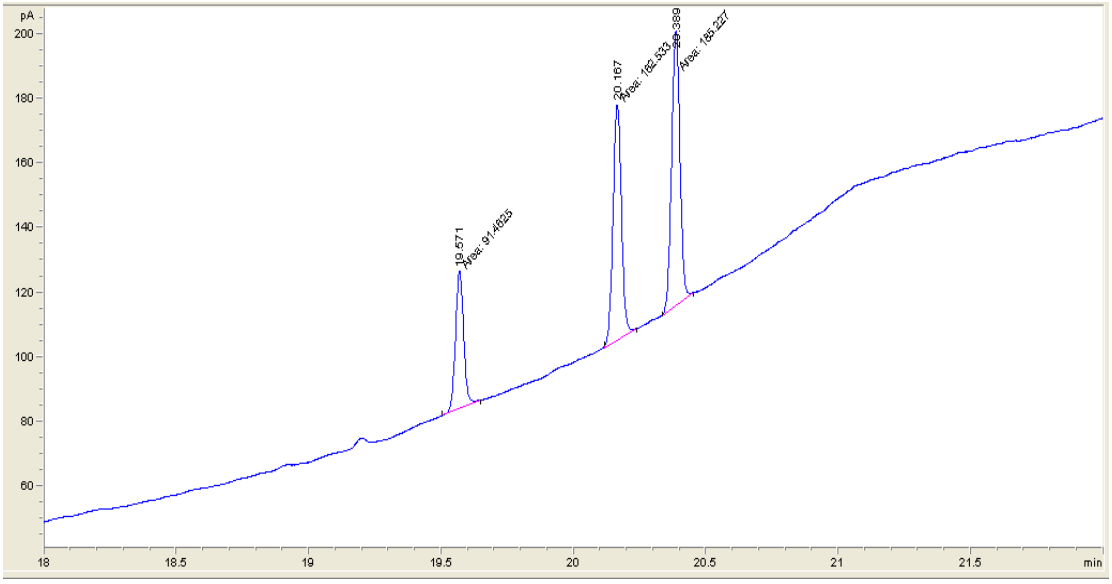

Figure 12. Chromatogram of 20 ppm mix standard

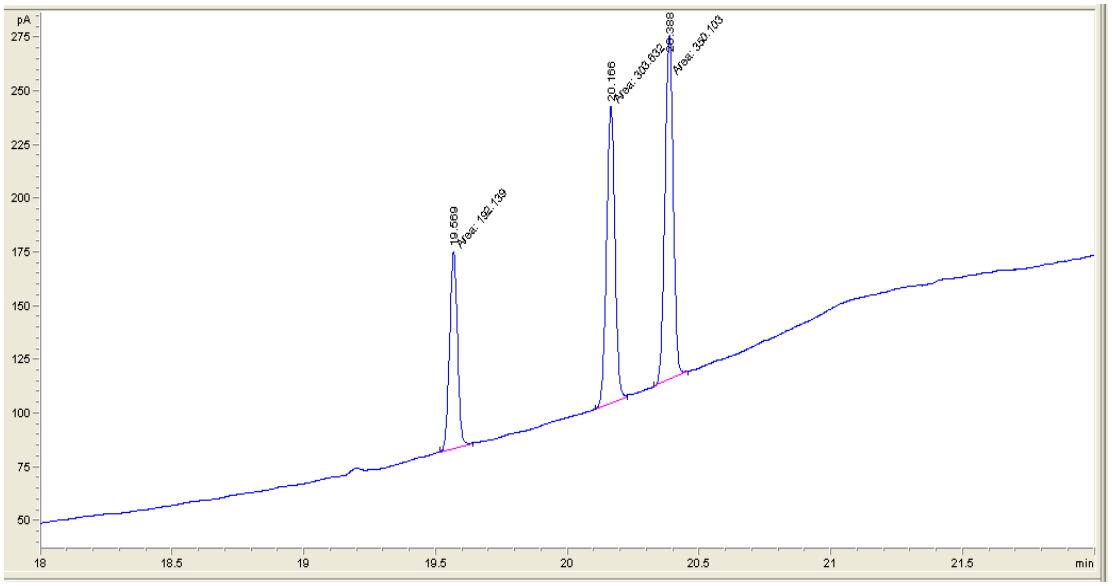

Figure 13. Chromatogram of 40 ppm mix standard

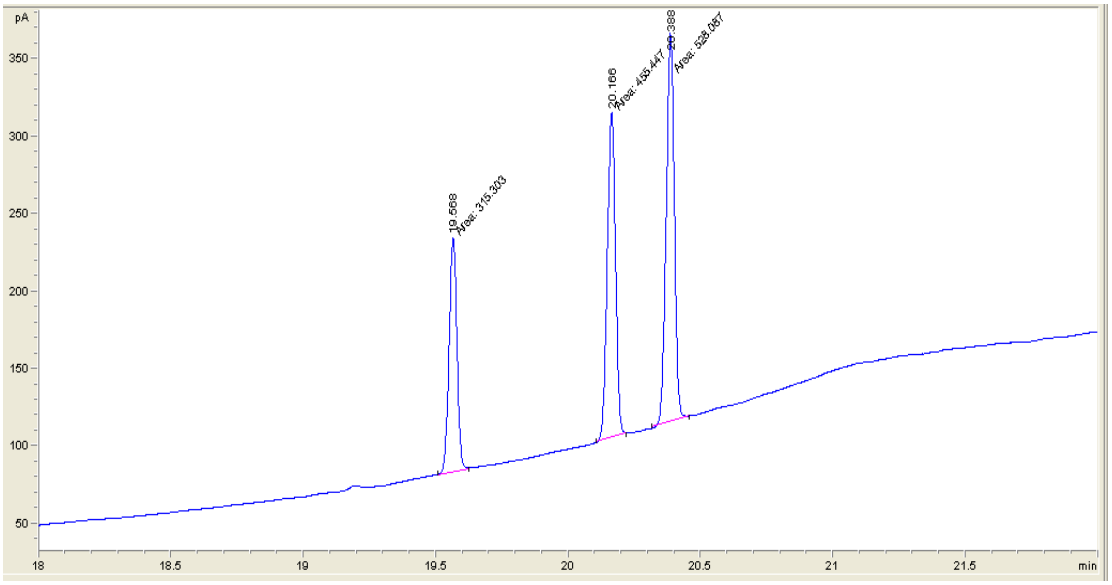

Figure 14. Chromatogram of 60 ppm mix standard

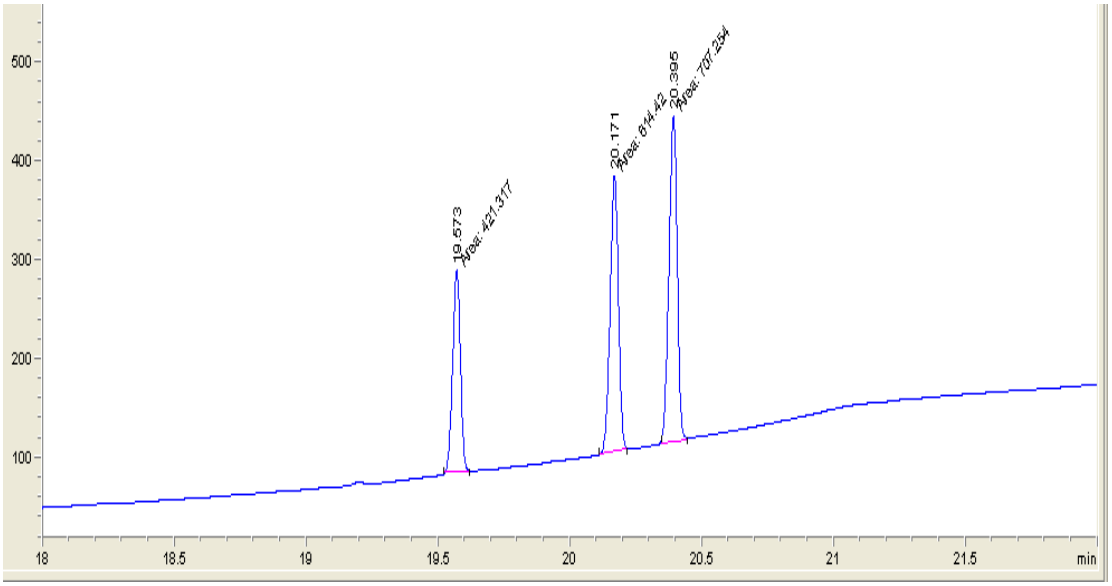

Figure 15. Chromatogram of 80 ppm mix standard

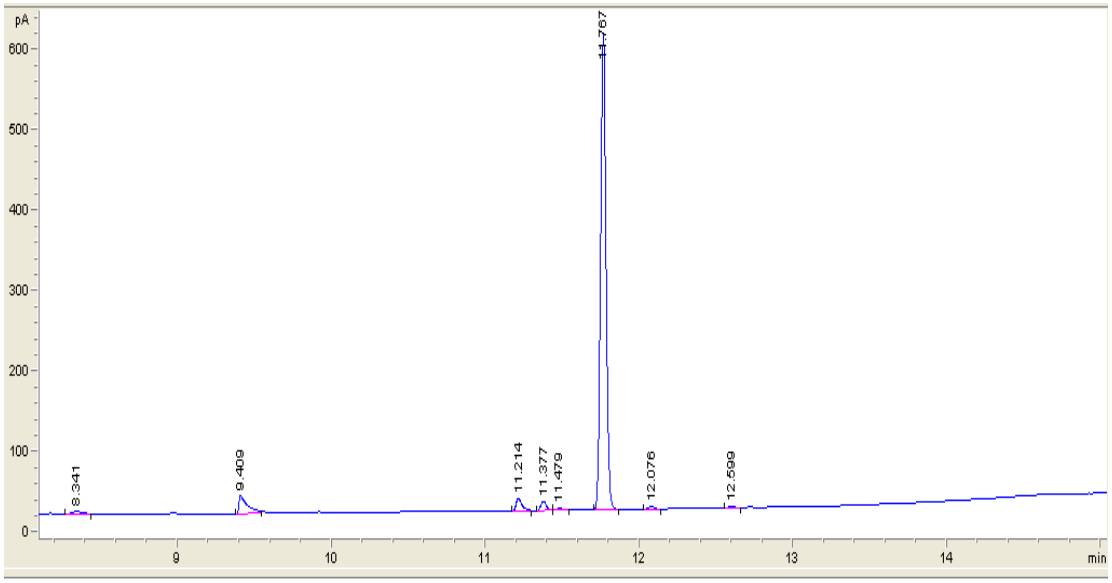

Figure 16. Chromatogram of 100 ppm nitrocyclopentane (internal standard)

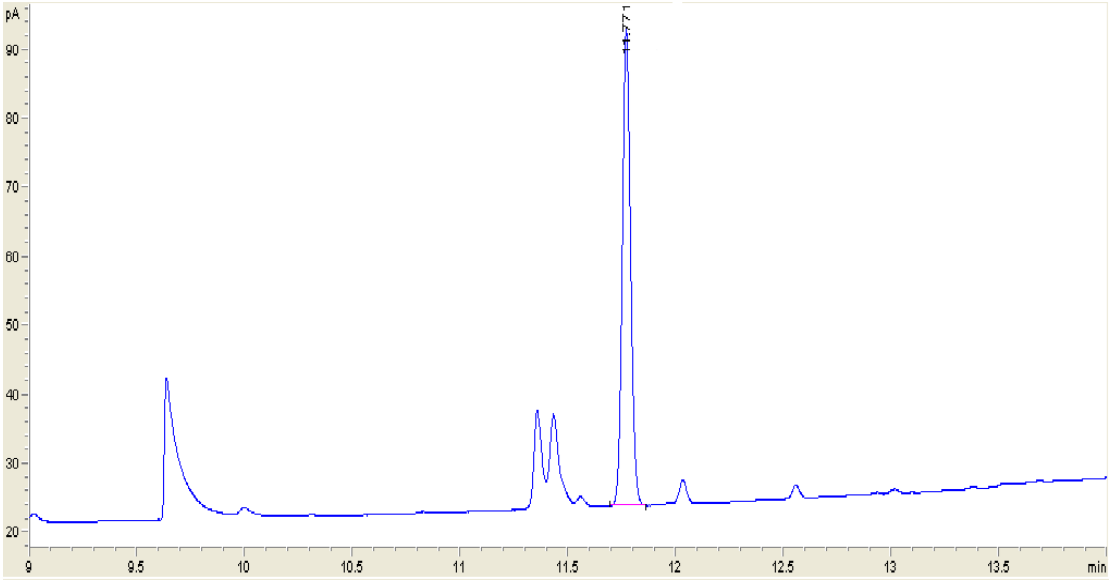

Figure 17. Chromatogram of 10 ppm nitrocyclopentane (internal standard) I

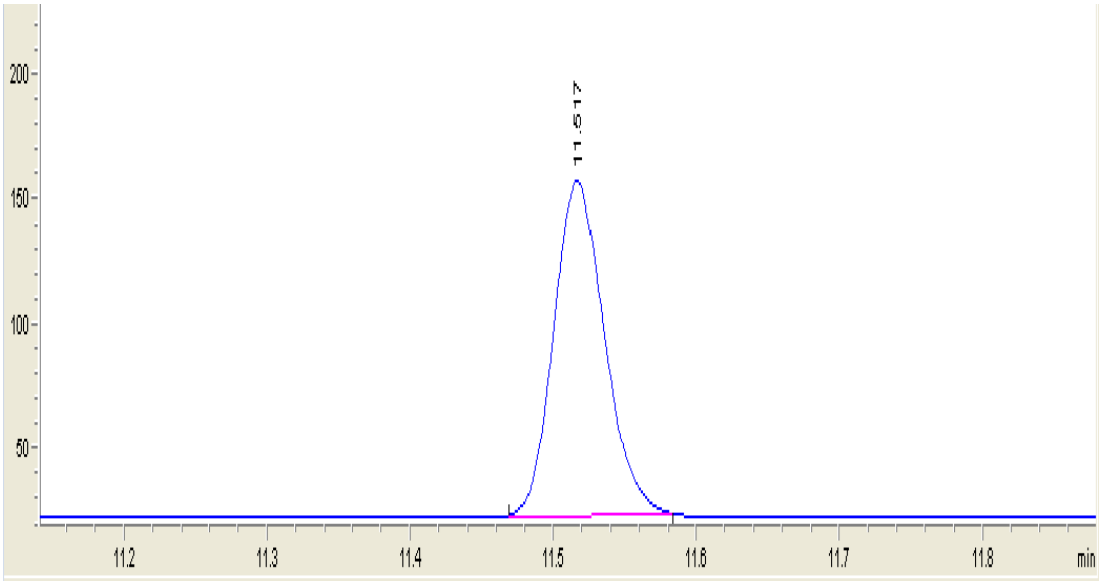

Figure 18. Chromatogram of 10 ppm nitrocyclopentane (internal standard) II

APPENDIX III

Chromatograms of the samples

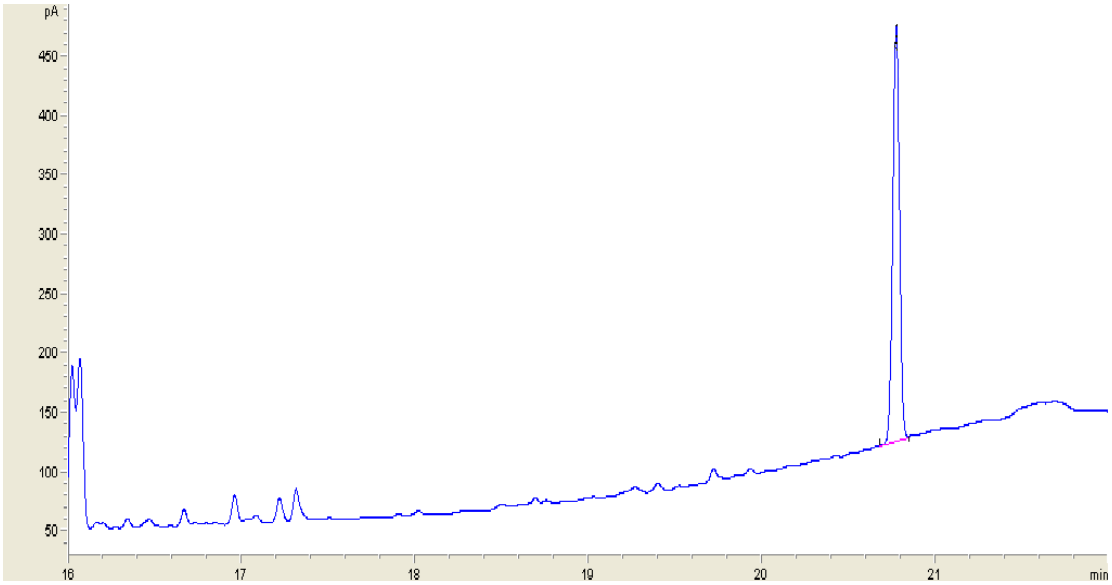

Figure 19. Chromatogram of Fieldblank 2

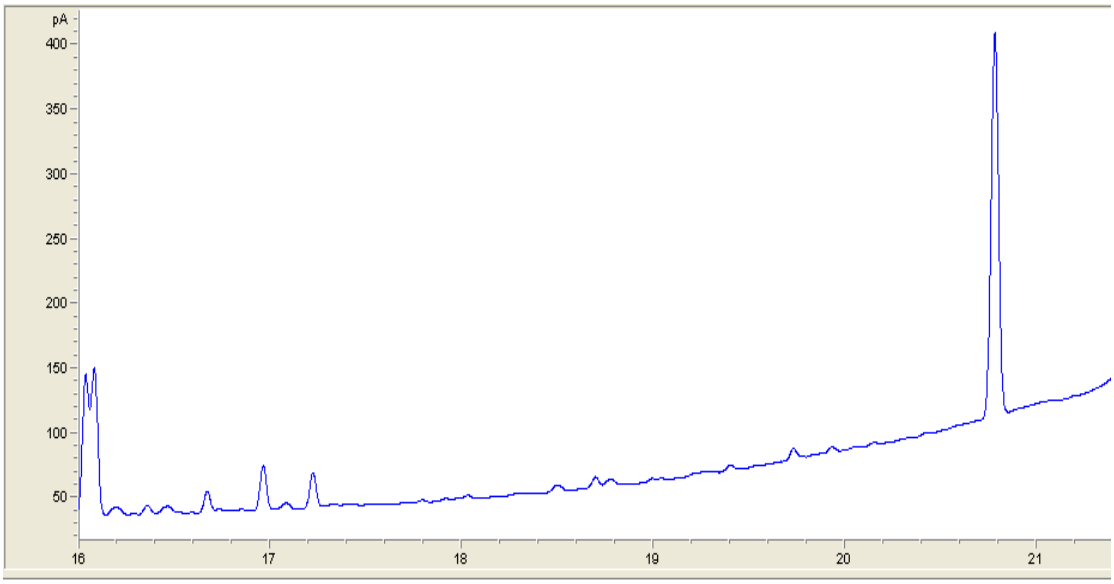

Figure 20. Chromatogram of Sample 3A

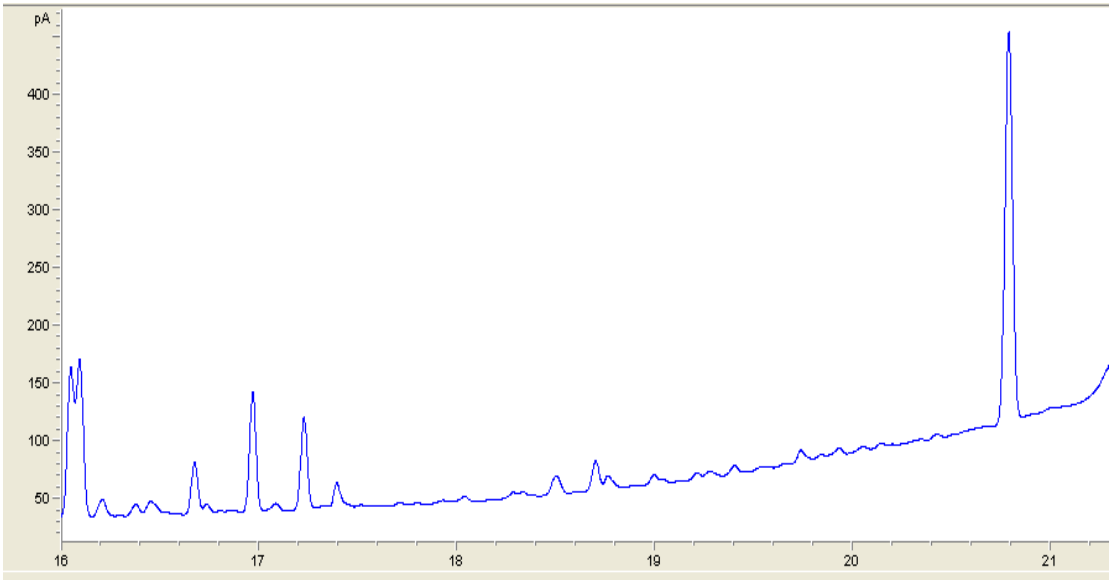

Figure 21. Chromatogram of Sample 3B

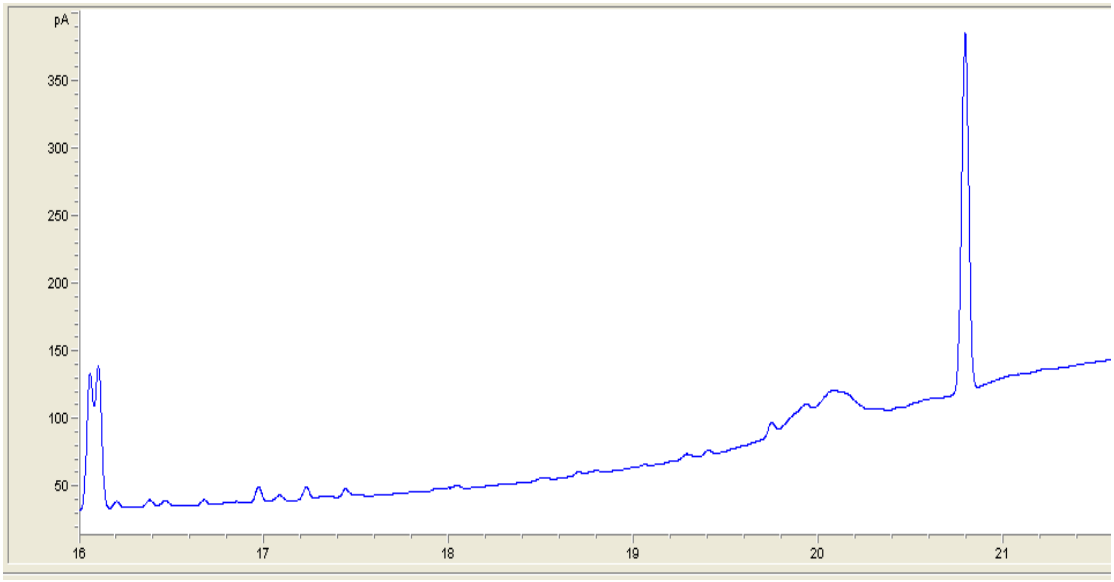

Figure 22. Chromatogram of Sample 4A

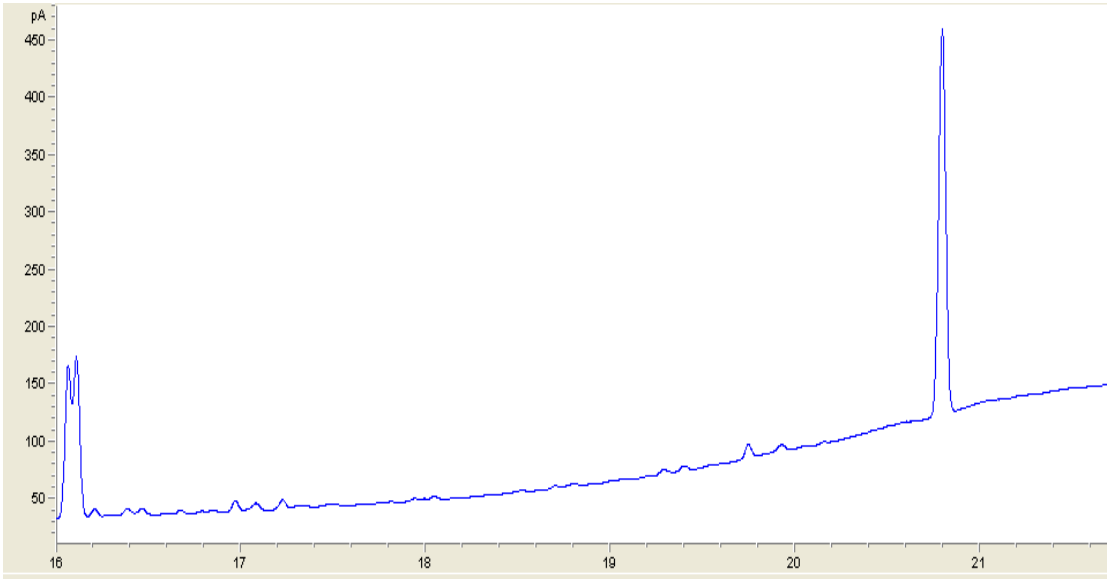

Figure 23. Chromatogram of Sample 4B

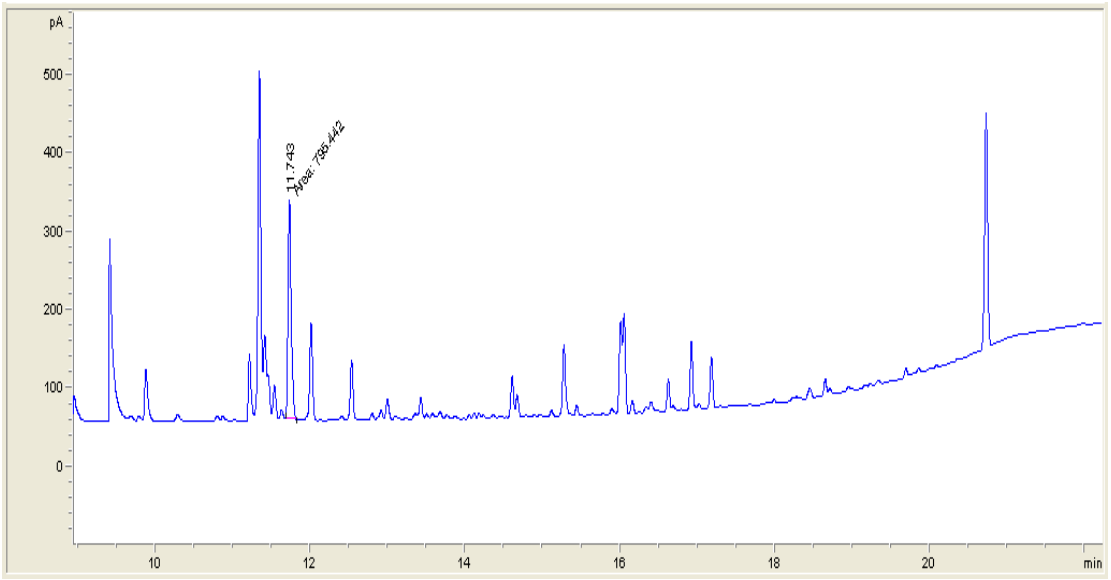

Figure 24. Chromatogram of Fieldblank 3

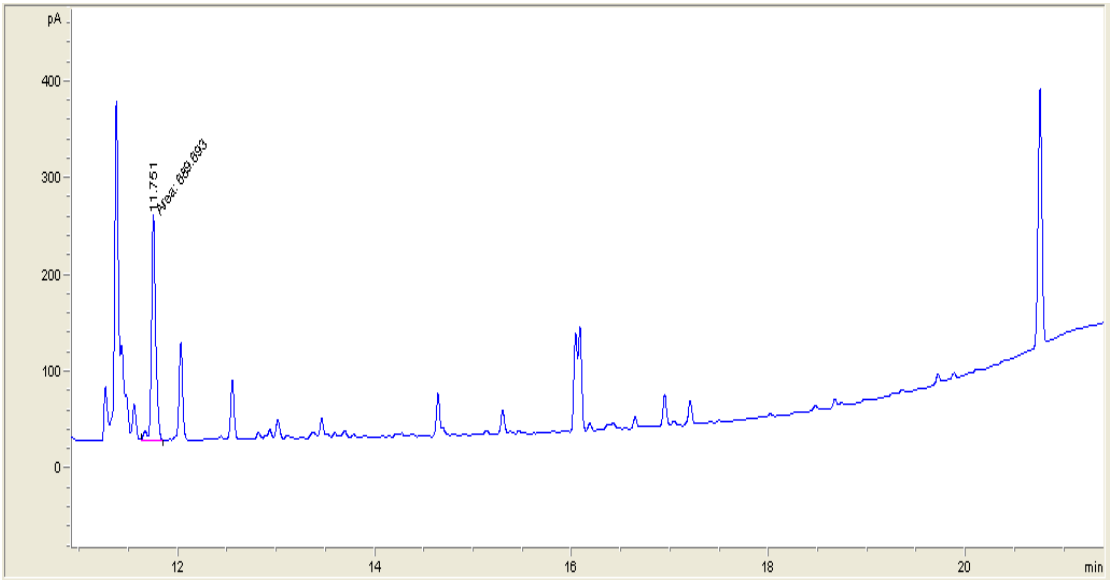

Figure 25. Chromatogram of Sample 5A

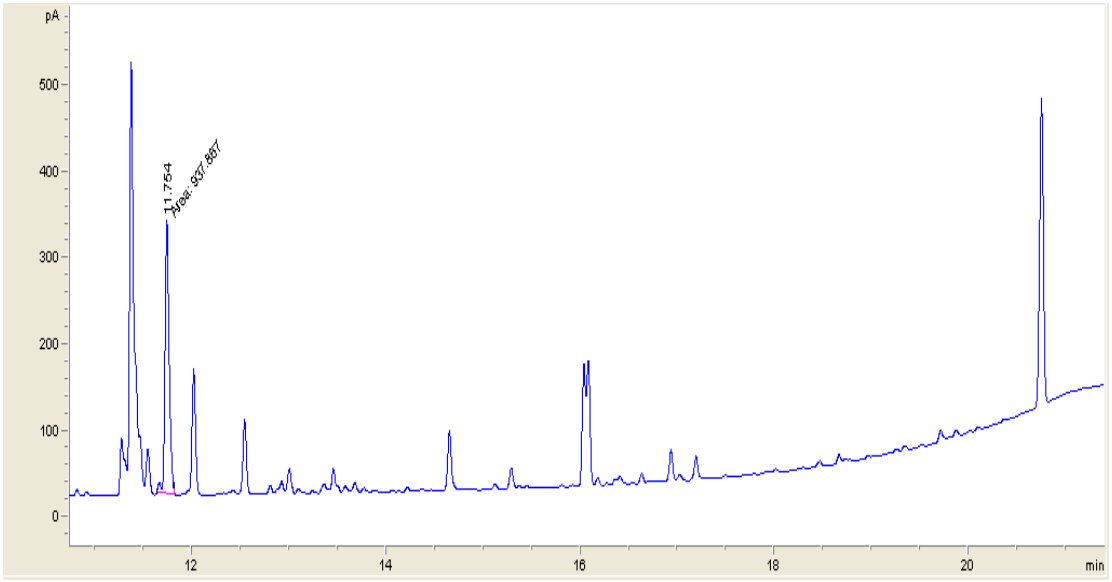

Figure 26. Chromatogram of Sample 5B

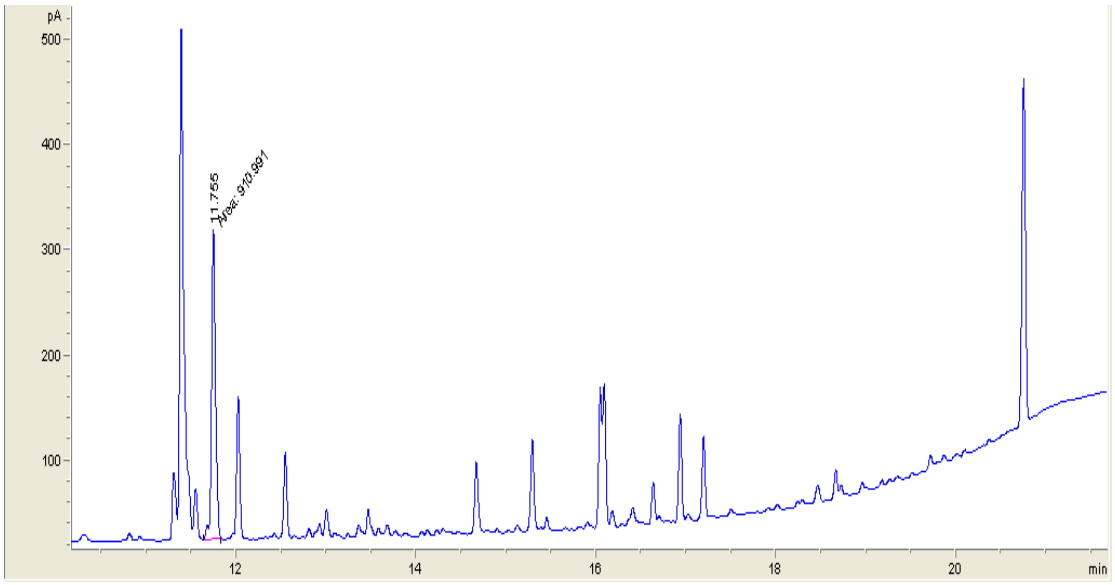

Figure 27. Chromatogram of Sample 6A

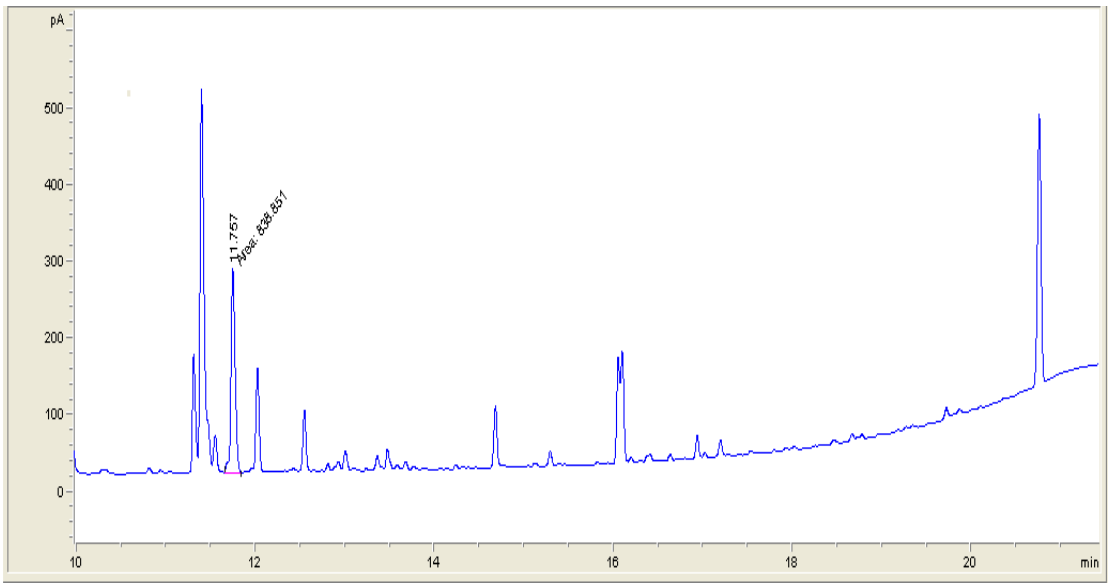

Figure 28. Chromatogram of Sample 6B

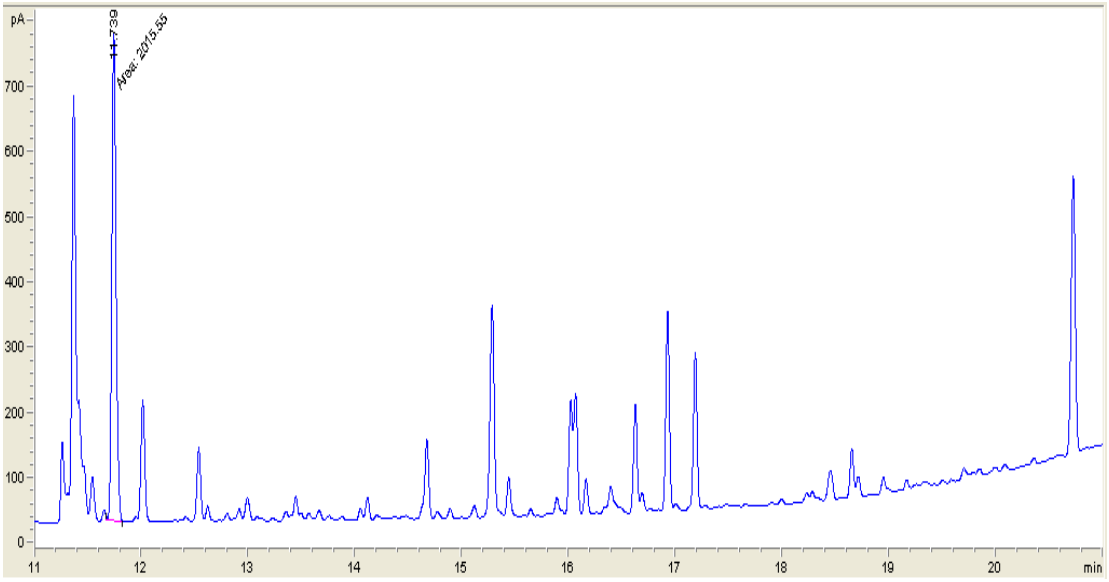

Figure 29. Chromatogram of Fieldblank 4

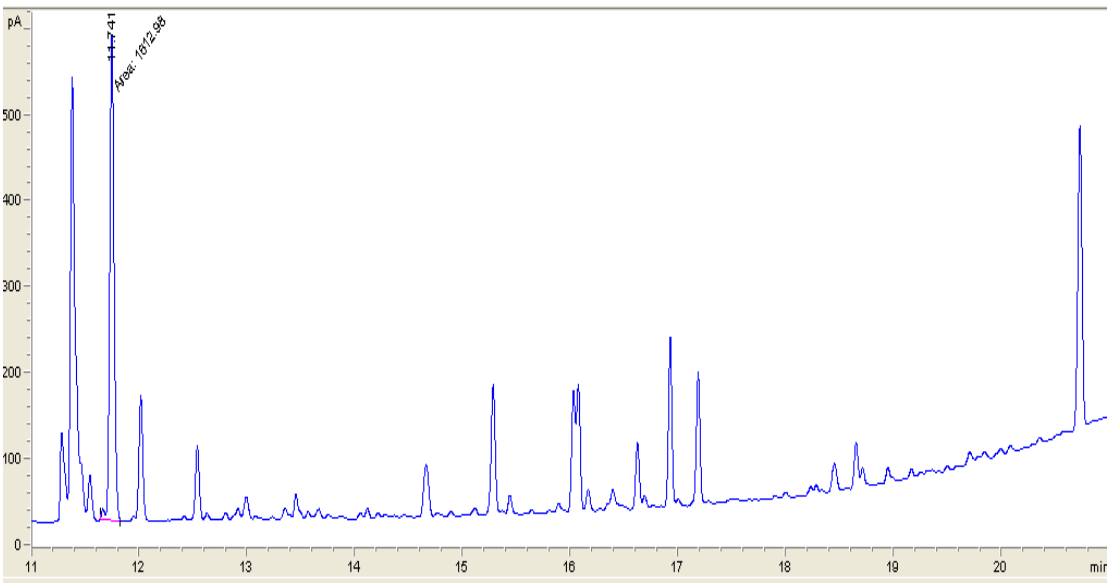

Figure 30. Chromatogram of Sample 7A

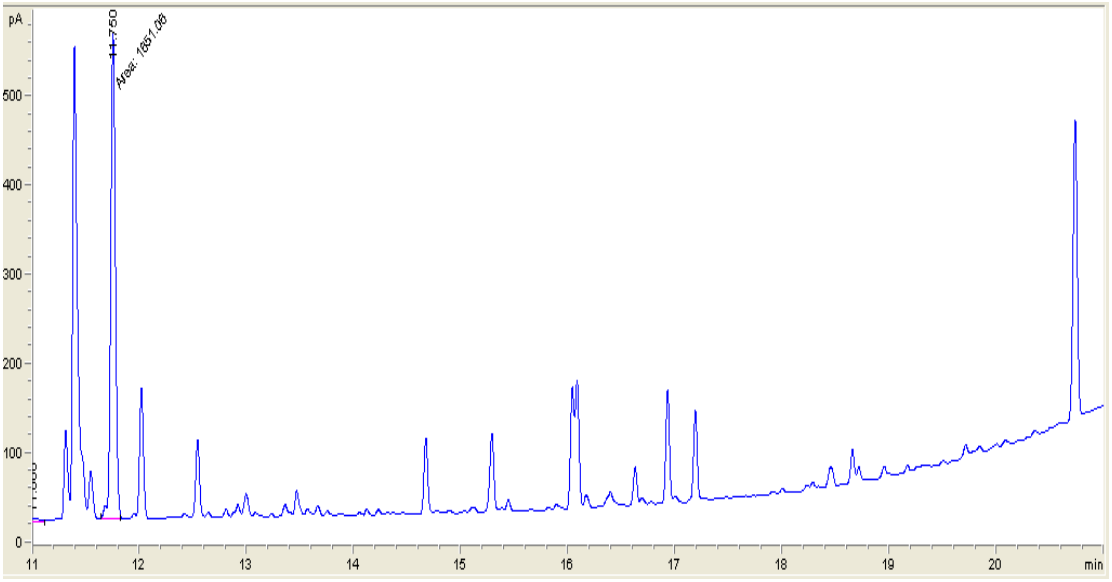

Figure 31. Chromatogram of Sample 7B

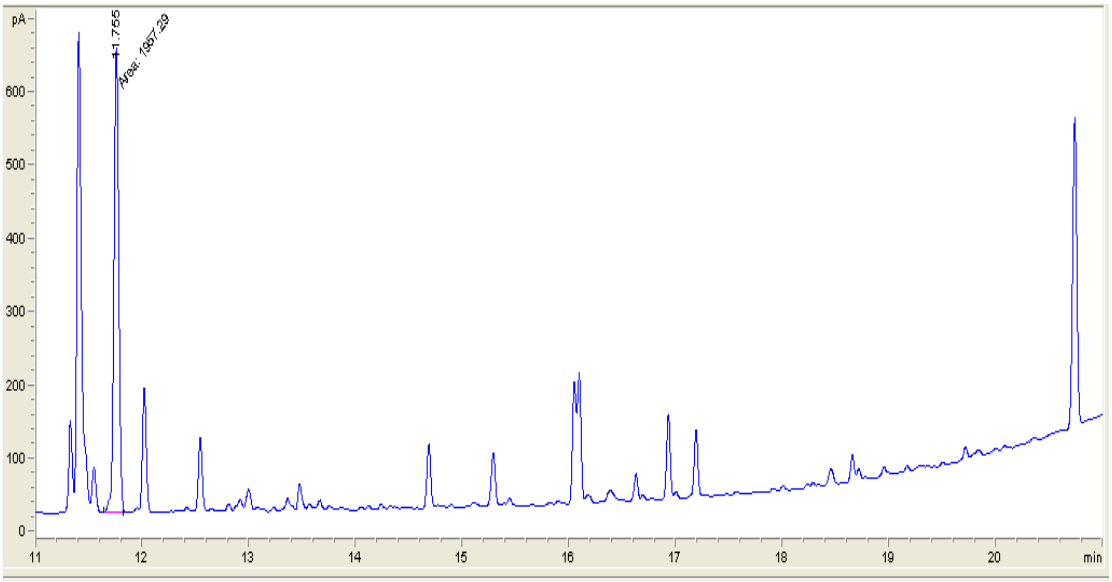

Figure 32. Chromatogram of Sample 8A

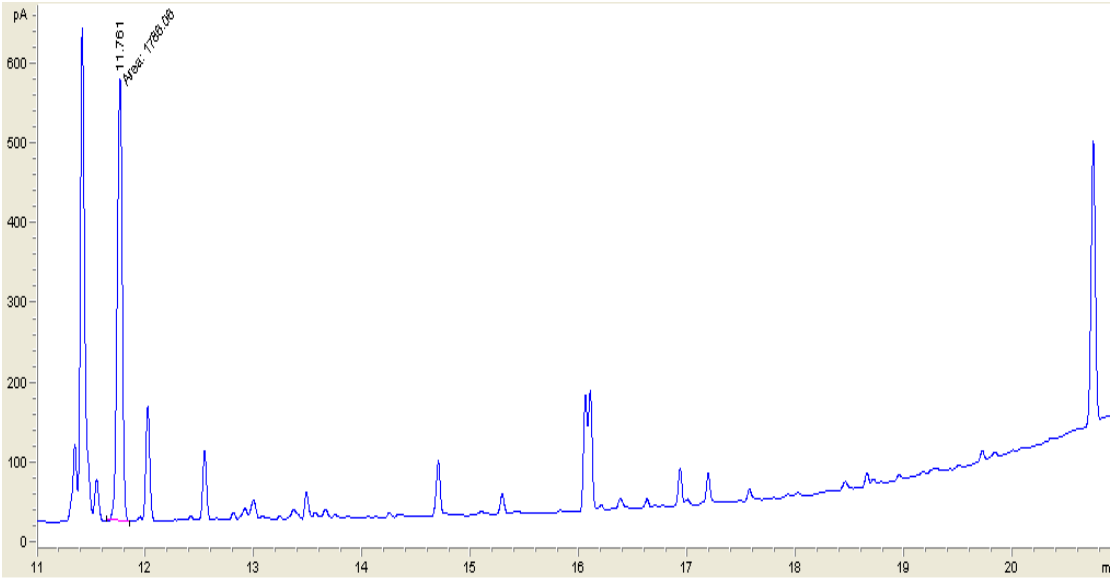

Figure 33. Chromatogram of Sample 8B

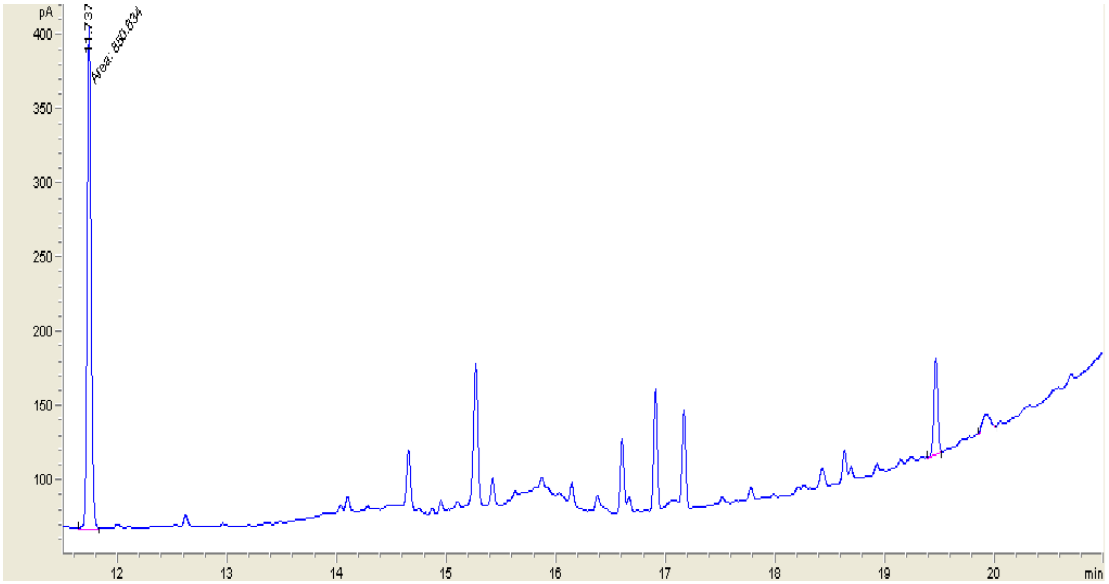

Figure 34. Chromatogram of Fieldblank 5

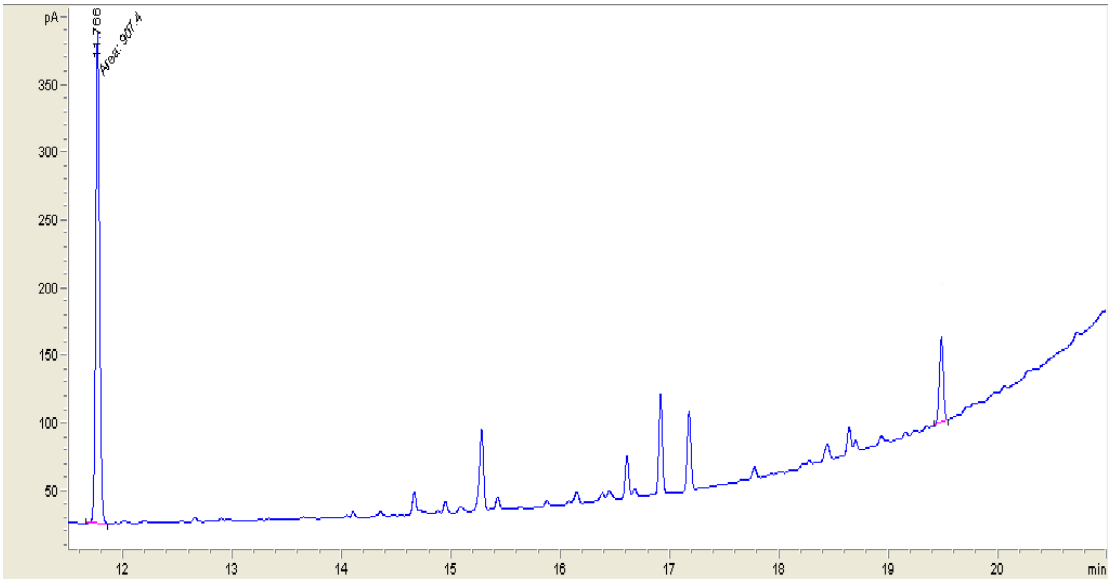

Figure 35. Chromatogram of Sample 9A

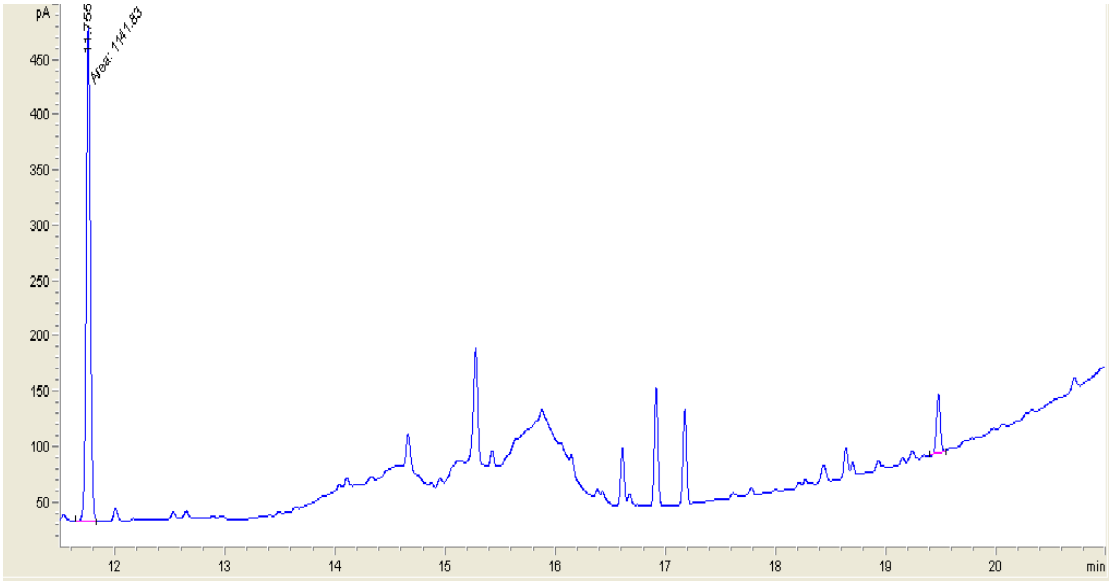

Figure 36. Chromatogram of Sample 9B

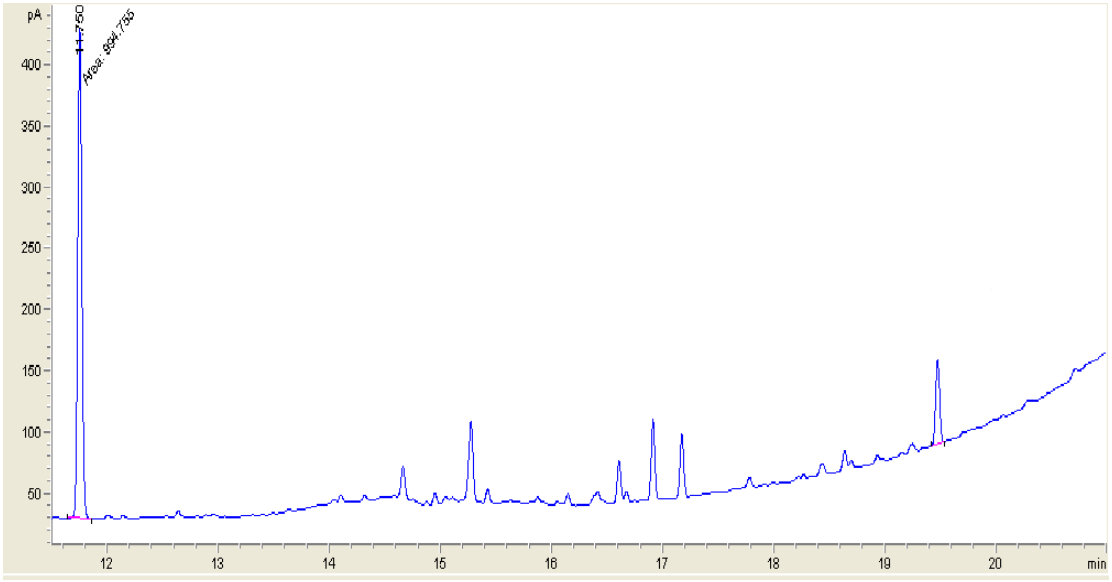

Figure 37. Chromatogram of Sample 10A

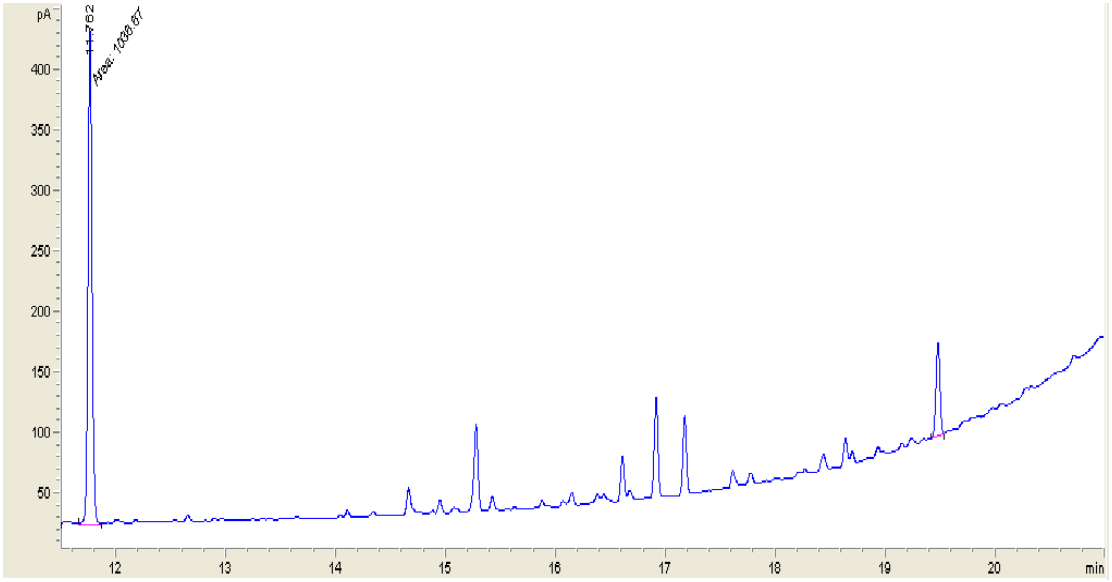

Figure 38. Chromatogram of Sample 10B

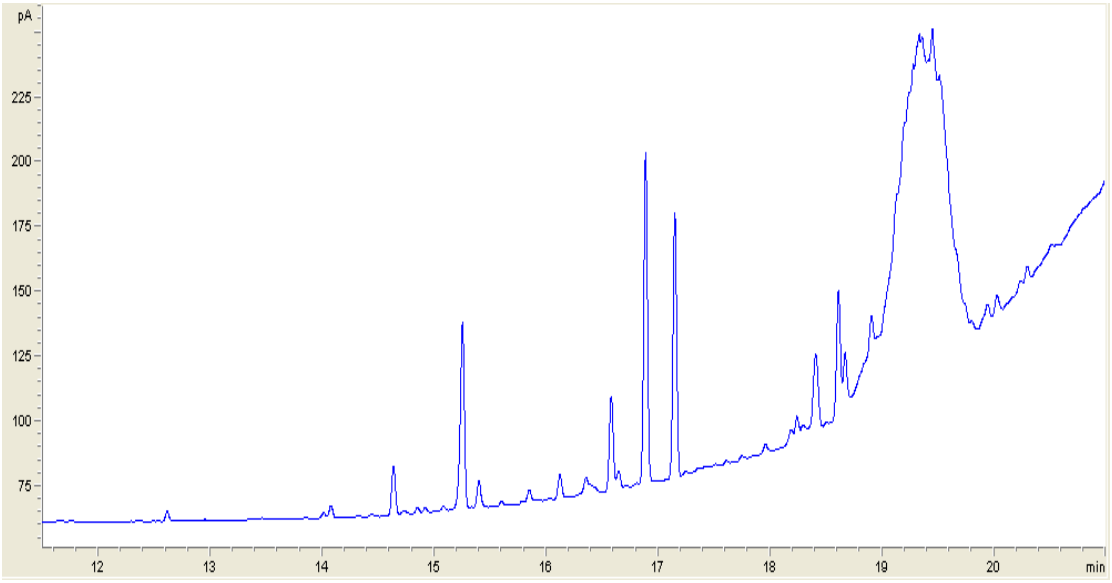

Figure 39. Chromatogram of Concentrated DCM (Blank)

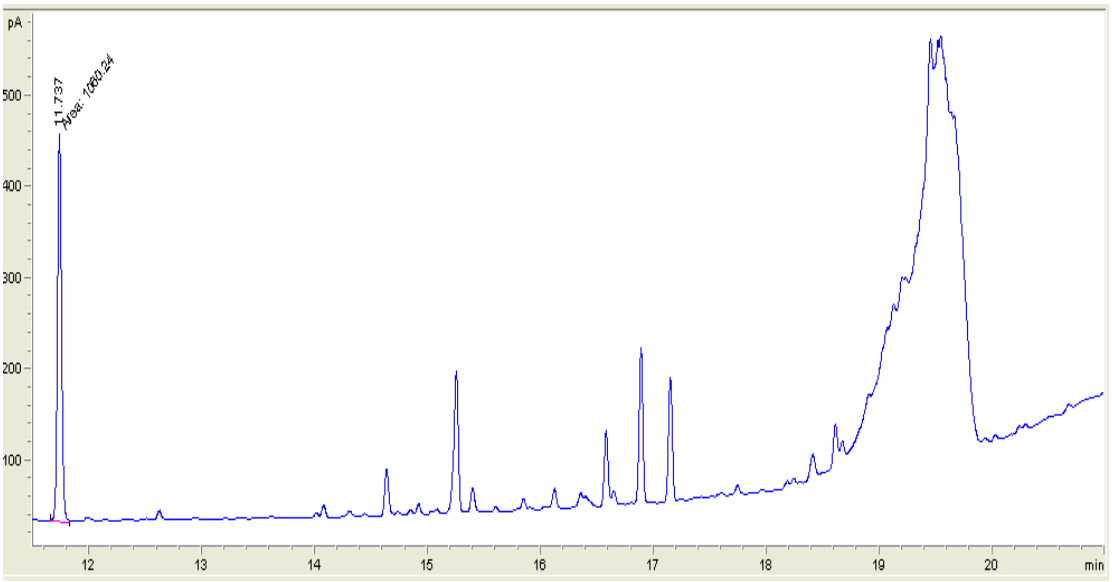

Figure 40. Chromatogram of Fieldblank 6

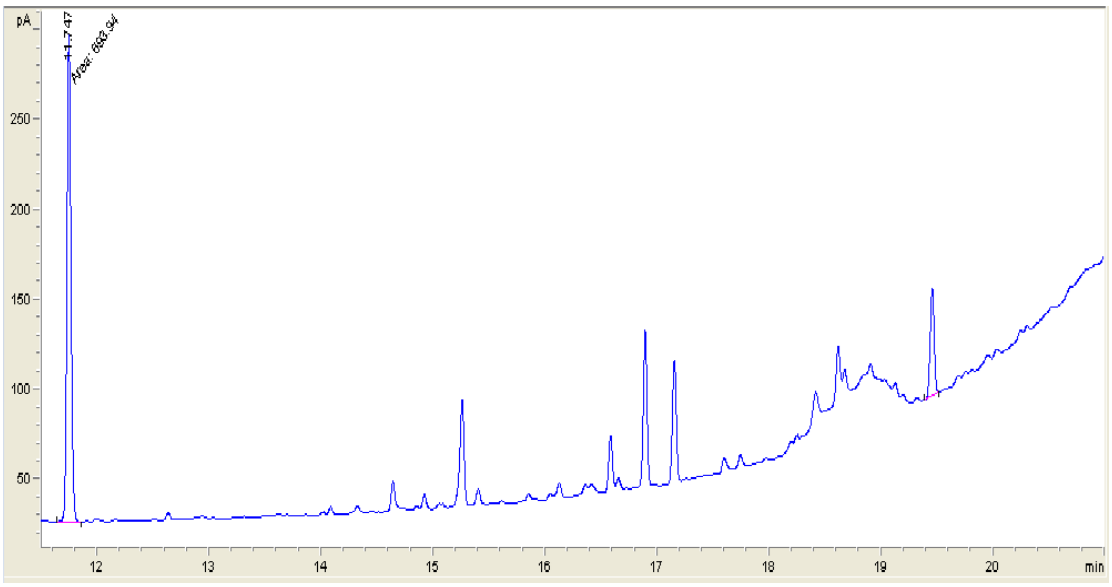

Figure 41. Chromatogram of Sample 11A

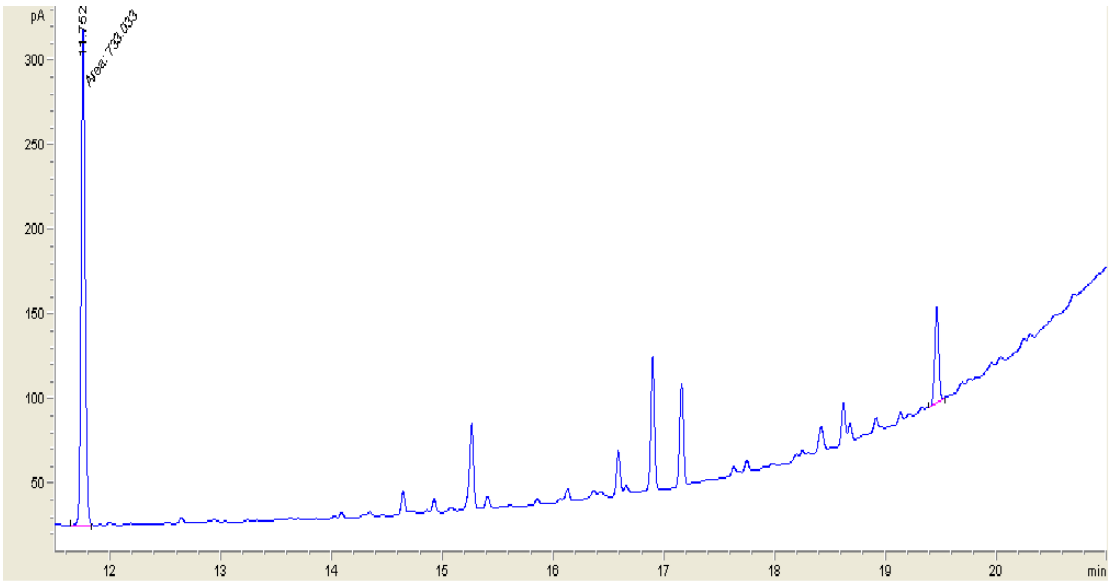

Figure 42. Chromatogram of Sample 11B

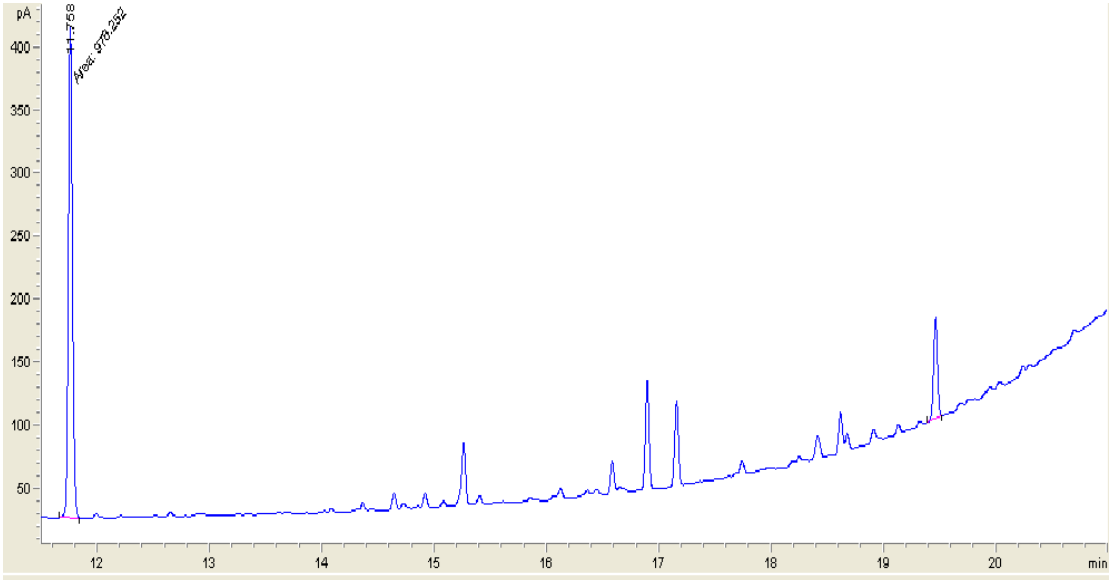

Figure 43. Chromatogram of Sample 12A

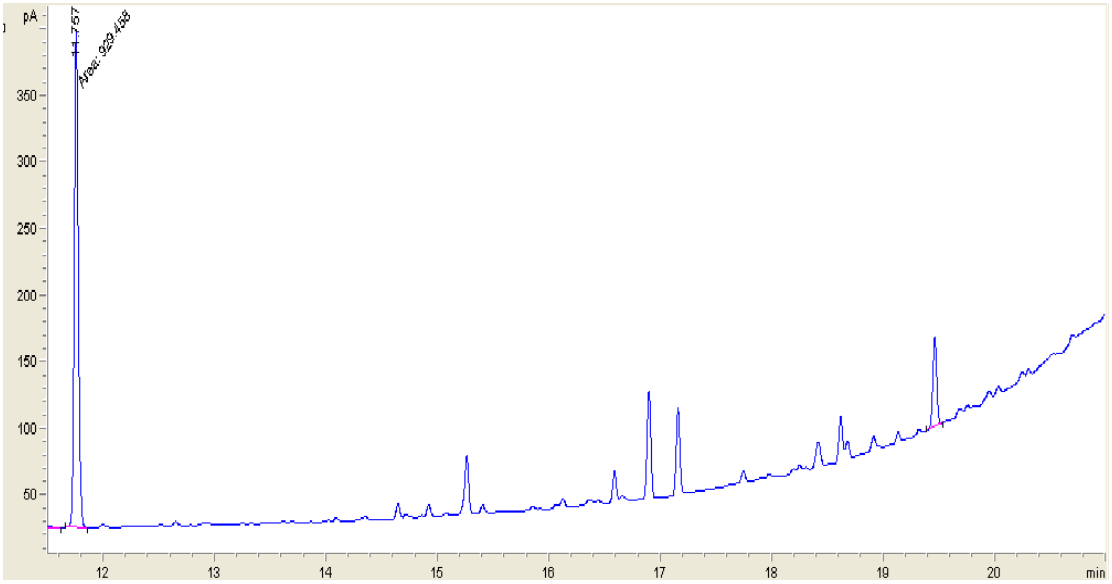

Figure 44. Chromatogram of Sample 12B

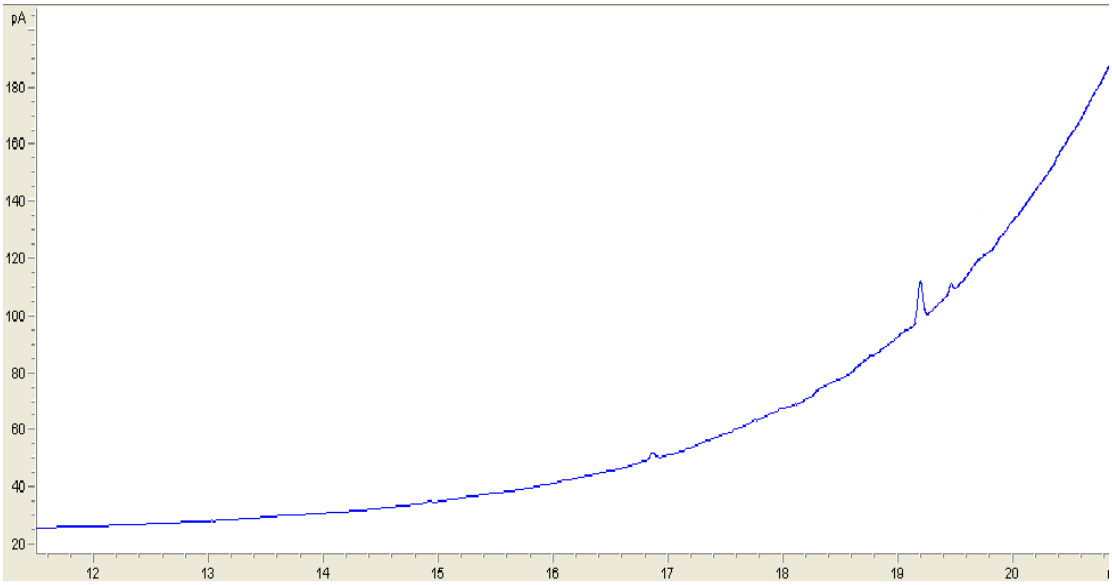

Figure 45. Chromatogram of DCM Blank