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Validation of a Method for the Analysis of Volatile Organic Compounds in Water

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Erasmus Mundus Master in Quality in Analytical Laboratories

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Amresh Karmacharya

Abstract

Many of the volatile organic compounds (VOCs) which can be harmful to humans have their origin in petroleum products. The VOCs have been found in water sources and leakage from storage tank and accidental spills have been regarded as the main causes of contamination from VOCs. The main objective of this study was to validate detection method of some 15 VOCs by solid-phase microextraction – gas chromatography – mass spectrometry. SPME-GC-MS has been a widely accepted method for analysis of VOCs.

The compounds analyzed in this study are; MTBE, 3-ethyltoluene, 4-ethyltoluene, 2-ethyltoluene, 1,2,4-trimethylbenzene, 4-isopropyltoluene, 1,3-diethylbenzene, Indane, 1,4-diethylbenzne, 1,3-dimethyl-5-ethylbenzene, 1,2-diethylbenzene, 1,4-dimethyl-2-ethylbenzenene, 1,3-dimethyl-4-ethylbenzene, 1,2-dimethyl-4-ethylbenzene, 1,2-dimethyl-3-ethylbenzene and hexachlorobutadiene. After separation by the gas chromatograph the compounds were detected in full scan mode and later further studies were carried out in selected ion monitoring (SIM) mode of mass spectrometer. Method validation parameters for the detection of these compounds included selectivity, linear working range, limit of detection (LOD), limit of quantification (LOQ) precision, accuracy and measurement uncertainty. Various statistical tools like regression analysis, residual analysis, Mandel's test of linearity, RIKILT, and normalized area test were applied to derive and ascertain the results and arrive at a conclusion.

The retention time and representative mass fragments were identified for each compound. A linear curve (regression analysis) in the working range was also identified for each of these compounds after suitable dilution of the pure compounds. Working range was between less than 0.1 µg/L and 0.5 µg/L (the minimum and maximum calibration standards) for all the compounds except for MTBE and indane. Linearity was confirmed by residual analysis and Mandel's test for linearity. Two of the compounds 1,4-diethylbenzene and 1,3-dimethyl-5-ethylbenzene coelute and appear as a single peak in the chromatogram and therefore, their quantity is expressed as the combined quantity of

the two. LODs are well above the baseline and LOQs are either equal to or lower than the lowest calibration standards. LOD and LOQ were also quantified from precision data. Precision was studied by determining repeatability and intermediate precision and was expressed as relative standard deviation (RSD %). Council Directive 98/83/EC has prescribed a limit value of 25% for precision. None of the values of repeatability and intermediate precision exceeded the limit of 25%. Accuracy was determined by recovery study of three types of spiked water matrices; tap water, river water and groundwater. Recovery was expressed by comparing the spiked results with the theoretical value (a value provided by the commercial supplier) of a compound in terms of percentage of recovery. Also 10 replicate analysis of the spiked sample gave its precision. Most of the recovery results have been found between 90 and 115%. All the recovery values meet the criterion of 25% recovery set by the Council Directive 98/83/EC.

ISO 17025 requires that the laboratories express the results accompanied by the estimated uncertainty. Expanded uncertainty of the method was determined for each compound by combining the component uncertainty of precision, calibration standards and regression interpolation and then multiplying the combined uncertainty by a coverage factor of two for a 95% confidence level. Uncertainty values ranged from 8.2% to 23%. It has been found that for the same compound the uncertainty values for the three different matrices are similar. VOCs targeted in this study can be used as possible indicators of petroleum product contamination of water sources. Each compound has its own retention time and mass spectra which can be used for its detection. Linearity of the working range has been confirmed by various statistical tests. LOD, LOQ and recovery results meet European regulation requirement and this indicates validity of the method and can be applied to detect the compounds in water. HS-SPME sampling is solvent free and less time consuming and therefore is preferable. There has been only limited research in method validation of many target VOCs. So this study contributes to methods of analysis used to detect the target VOCs in different water matrices.

Key words: VOCs, Petroleum, method validation, SPME, GC, calibration, LOD, LOQ, precision, accuracy.

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A list of abbreviations

CAR - Carboxen

EPAL - Empresa Portuguesa das Águas Livres

GC-MS – Gas chromatography – mass spectrometry

HS – Head space

MTBE – Methyl-tert-butyl-ether

EU – European Union

SPME – Solid phase microextraction

VOCs –Volatile organic compounds

LLE – Liquid liquid extraction

SPE – Solid phase extraction

EI – Electron ionization

LOD – Limit of detection

LOQ – Limit of quantification

DVB – Divinylbenzene

PDMS – Polydimethylsiloxane

PS – Primary solution

RSD – Relative standard deviation

FS – Full scan

SIM – Selected ion monitoring

ISO – International standards organization

WHO – World Health Organization

EM – Electron multiplier

PAT – Purge and tap

SOP – Standard operating procedure

A list of symbols

K_D – Distribution constant

t_R – Retention time

t_R'- Adjusted retention time

t_M – Hold-up time

k' – Retention factor

α – Selectivity factor

R – Chromatographic resolution

N – Number of theoretical plates

$W_{1/2}$ – Peak width at half height

H – Height of a plate

L – Length of column

r – Correlation coefficient

r^2 – Coefficient of determination

a – y-intercept

b – Slope of the calibration curve

y – Instrument response

x – Concentration of unknown

$S_{y/x}$ – Residual standard deviation

S_{xo} – Standard deviation of the method

m/z – Mass to charge ratio

$\overline{x}$ - mean of calibration standards

$\overline{y}$ - mean of instrument response

y_i – Experimental response

$\hat{y}_I$ – Predicted response

$y_{i\ res}$ – Regression residual

I. INTRODUCTION

1.1 Background

The topic of environmental pollution and adverse health impact it can cause, directly or indirectly, is no longer a topic of debate and, in a sense it has been an undesired part of our daily lives. Many categories of contaminants like heavy metals, pesticides, fertilizers, toxic organic compounds and greenhouse gases are released on land, into water bodies and atmosphere throughout the world. Huge quantities of fossil fuel (petrol or gasoline, diesel, kerosene, jet fuel, coal and natural gas) are consumed to meet the world's ever increasing energy demand. Burning of fossil fuel inevitably emits greenhouse gases like carbon dioxide and methane, the biggest contributors to global warming. The other byproduct of fuel burning is the release of numerous health hazardous organic compounds. Toxic organic compounds are also released from other many synthetic commodities that people commonly use.

Many of the volatile organic compounds (VOCs) detected in soil and groundwater are toxic and mainly come from petroleum products like gasoline, diesel and jet fuel. VOCs like benzene, toluene, xylene, ethylbenzene (commonly called BTEX), hexachlorobutadiene, methyl-tert-butyl-ether (MTBE) and many others originate from fuel burning. The second category of VOCs are chlorinated solvents used in various activities like dry cleaning, refrigeration, painting, pesticides, plastics and pharmaceuticals.[1-3] Examples of chlorinated solvents are trichloroethylene, trichloroethane, carbon tetrachloride, methylene chloride, vinyl chloride among others.[4]

There are many more other VOCs that are emitted during combustion of petroleum. VOCs are hydrocarbons which are released from fossil fuel after incomplete combustion. The petroleum products are also used in plastics, fertilizers, paints, pesticides, refrigerants, cleaning fluids, detergents, antifreeze and synthetic fibers.[1] Gasoline mainly contains hydrocarbons, which have carbon atom C4-12, while diesel is composed of heavier fraction of C7-24. The hydrocarbons include alkanes, cycloalkanes, benzene, benzene derivatives and other monocyclic and polycyclic aromatic compounds.[5] After

their release in the environment VOCs undergo transformation through physical, chemical and biological processes. Most transformation in the environment especially in groundwater is caused by microorganisms.[6]

Subsurface spills of petroleum compounds may be the most frequently stated cause of groundwater contamination.[7] Leaking underground and above ground tank and accidental spills are major routes of soil and groundwater contamination and underground tanks being the most common cause.[8] The leaking underground storage tanks containing petroleum products have contaminated groundwater and drinking water across the United States.[9] After the spill or release, because of their volatility some VOCs evaporate away. The left over ones may be carried deep into the groundwater table by rain, water, or snow melt.[10] Therefore, VOC concentrations found in groundwater may be many more times higher than that found in surface water[11].

VOCs can react with sunlight and nitrogen oxides to produce ground level ozone which can cause lung and tissue damage [5]. Groundwater is a major source of drinking water, and groundwater contaminated with VOCs has been associated with human-health concern. Toxic effect of VOCs can vary which can range from being benign in its effect to being highly toxic. Benzene and formaldehyde are known human carcinogens. The health effect also depends upon nature and length of exposure. Long term exposure to VOCs can adversely affect liver, kidneys and central nervous system. Short term exposure to VOCs can cause eye and respiratory tract irritation, headaches and dizziness.[12-15]

The main objective of this study was to detect and identify mainly monocyclic aromatic VOCs by head space solid-phase microextraction followed by gas chromatography and mass spectrometry and validation of the analytical method. Most of these compounds have origin in petroleum products. Detection of these compounds in drinking water, surface water, wastewater or groundwater can be used as an indicator of petroleum contamination. A couple of the compounds have been regulated for drinking water or surface water use and have stipulated water quality standards within the European Union.

Following is a list of compounds (Table 1) that have been the focus of this study.

Table 1: A list of target compounds.

S.N.	Compound name
1	MTBE
2	3-ethyltoluene
3	4-ethyltoluene
4	2-ethyltoluene
5	1,2,4-trimethylbenzene
6	4-isopropyltoluene
7	1,3-diethylbenzene
8	Indane
9	1,4-diethylbenzene
10	1,3-dimethyl-5-ethylbenzene
11	1,2-diethylbenzene
12	1,4-dimethyl-2-ethylbenzene
13	1,3-dimethyl-4-ethylbenzene
14	1,2-dimethyl-4-ethylbenzene
15	1,2-dimethyl-3-ethylbenzene
16	Hexachlorobutadiene

1.2 Introduction of target compounds

Hexachlorobutadiene

Hexachlorobutadiene is a clear colorless liquid. It is insoluble in water but soluble in ethanol (Figure 1). It is used in chlorine gas production and in manufacture of rubber compounds, lubricants and pesticide. In studies with oral introduction of hexabutadiene, kidney tumors were observed in rats. European Union has set hexachlorobutadiene maximum allowable concentration 0.6 μg/L in inland surface waters and WHO guideline value of drinking water is also 0.6 μg/L.[16-18]

Methyl-tert-butyl-ether (MTBE)

MTBE is a clear colorless liquid. It has a strong characteristic odour. There is a low risk of contamination in surface water due to its volatility. Spills and leakage of gasoline storage tanks can cause more serious groundwater contamination with MTBE where it is

more persistent. The major use of MTBE is as a gasoline additive to raise octane number. WHO mentioned a threshold value for odour of 15 μg/L. At high levels of exposure MTBE can cause cancer and non-cancer effects in laboratory animals.[17,19]

4-isopropyltoluene

4-isopropyltoluene is a flammable, colorless water insoluble liquid and has a characteristic odour. It is used in the manufacture of paint and furniture. It can cause irritation of eyes and skin and the substance may be toxic to central nervous system and repeated or prolonged exposure can produce target organ damage. New York State human health fact sheet has established a threshold value for 4-isopropyltoluene as 5 μg/L in ambient water.[20-23]

Indane (Indan)

Indane is a clear colorless liquid. It is a type of hydrocarbon found in petroleum products. Indane is used as fuel for supersonic military aircraft. Environment Protection Agency of Ireland has categorized it as a hazardous substance.[24-25]

1,2,4-trimethylbenzene

1,2,4-trimethylbenzene is a clear colorless liquid with a distinctive odor. It is a byproduct of petroleum refining process. It is also used as a solvent in coatings, cleaners, pesticides and inks. Exposure to 1,2,4-trimethylbenzene can occur through inhalation, ingestion or contact with skin or eye. It can cause irritation of eyes, skin and respiratory system. It can adversely affect eyes, skin, respiratory system, central nervous system and blood. Based on human health criteria a concentration of 72 μg/L has been proposed for ambient water quality. [26-27]

Diethylbenzene isomers

Diethylbenzene isomers include 1,2-diethylbenzene, 1,3-diethylbenzene and 1,4-diethylbenzene. They are clear colorless liquid which are insoluble in water. Diethylbenzene isomers are components of petroleum products and are released to the environment as a byproduct of combustion in engines. The substance may enter body

through inhalation or ingestion. Short term exposure to diethylbenzene isomers can be irritating to eyes, skin and nervous system. The isomer 1,4-diethylbenzene has been suspected of inflicting adverse effect on kidney and liver.[28-29]

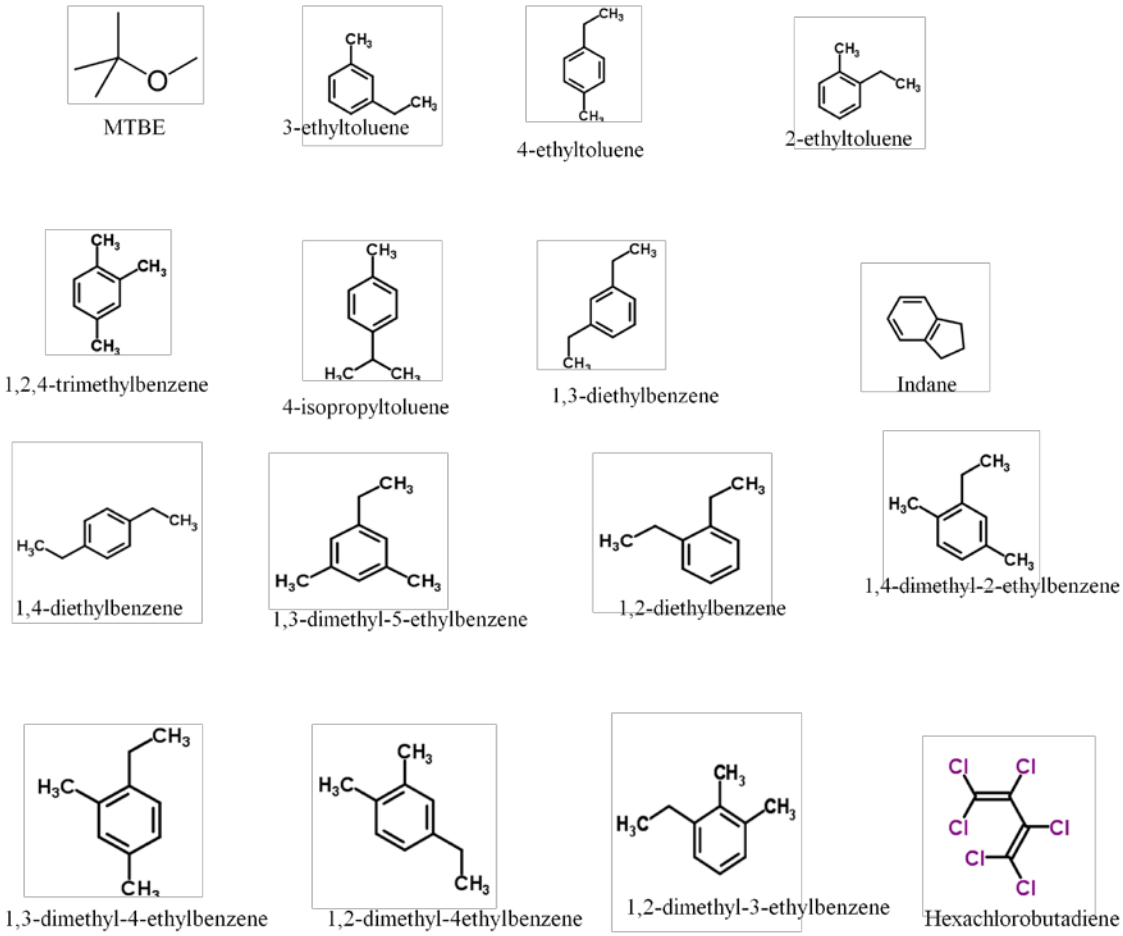


Figure 1: Skeletal diagram of the target VOCs

Ethyltoluene isomers

Ethyltoluene isomers are clear colorless liquid. They are flammable and volatile and water insoluble. Ethyltoluene is added to petrol to increase its performance. It can be released from petroleum refineries, petrol stations and in vehicle exhaust fumes. It can help to form ground level ozone. Ethyltoluene is an irritant of mucous membrane and upper respiratory tract. It may cause central nervous system effects. In laboratory animals it has been found to cause kidney, liver and reproductive system effects.[30-31]

Dimethylethylbenzene isomers

Isomers of dimethylethylbenzene that were included in this study are 1,3-dimethyl-5-ethylbenzene, 1,2-dimethyl-4-ethylbenzene, 1,3-dimethyl-4-ethylbenzene, 1,2-dimethyl-3-ethylbenzene and 1,4-dimethyl-2-ethylbenzene. These isomers also originate from petroleum products and therefore can be used as indicators or petroleum contamination of water sources. Information available on toxicity of these compounds are rather limited.

1.3 European Union policy related to water quality

Since 1975 a number of EU Directives and Regulations have been promulgated which have specified the quality of waters required for different uses. The legislation is aimed primarily at the safeguarding of human health by protecting water resources in general and for particularly human consumption.[32] Following are some of the Directives that are directly related to water quality issues.

Surface Water Directive 75/440/EEC[33]

This Directive was promulgated in 1975. Its main objective was to address the concern of the quality of surface water that is intended to be abstracted for human consumption after treatment. Groundwater was not subject to this Directive. It has set the threshold values of the surface water quality. Phenols and PAH are the VOCs mentioned in the standard. This directive has been repealed by the Directive 2000/60/EC.

Bathing Water Directive 76/160/EEC[34]

This Directive was also promulgated in 1975. It addresses the protection of waters used for bathing but it does not include swimming pools. Member states are required to set the threshold values of bathing waters. Bathing Water Directive has laid down quality requirement for all freshwater and sea waters defined as bathing waters. Among the VOCs, threshold for phenol was included.

Groundwater Directive 2006/118/EC[35]

This was promulgated in 2006 and addresses the issue of protection of groundwater. Member states have to establish threshold values for the parameters given in the Directive. Trichloroethylene and Tetrachloroethylene are the VOCs that the member states should monitor.

Drinking Water Directive 80/778/EEC[36]

This Directive was related to the quality of water intended for human consumption. For the parameters given the member states should fix the threshold less than or same as the values Maximum admissible concentration. The organic parameters covered were PAHs, PCBs and organochlorine, for example. This directive has been repealed by the Council Directive 98/83/EC.

Council Directive 98/83/EC[37]

The objective of the Directive is to protect human health from adverse effects of any contamination of water intended for human consumption. Human consumption includes drinking, cooking and other domestic purposes. Regular monitoring of water quality is required and analytical methods have been listed in Annex III. Commission Decision 2002/657/EC[38] has given performance criteria and validation parameters for the analytical methods for testing water quality. Council Directive 98/83/EC has laid down standards for many water quality parameters.

Following are the quality standards of VOCs.

Benzene	1.0 μg/L
Benzo(a)pyrene	0.01 μg/L
1,2-dichloroethane	3.0 μg/L
PAHs	0.10 μg/L
Tetrachloroethene and trichloroethene	10 μg/L
Trihalomethanes	100 μg/L
Vinyl chloride	0.50 μg/L

Similarly, Directive 2008/105/EC[39] is on environmental quality standards in the field of water policy. It has given an extended list of standards of priority substances for surface waters. Among the VOCs it includes benzene, carbon tetrachloride, 1,2-dichloroethane, dichloromethane, DEHP, hexachlorobutadiene, naphthalene, pentachlorobenzene, pentachlorophenol, PAH, benzo(a)pyrene, tetrachloroethylene, trichloroethylene, trichlorobenzene and trichloromethane, for example.

1.4 Solid-phase microextraction (SPME)

Sample extraction is an important step of sample preparation meant for subsequent separation and detection of the compounds by gas chromatography or liquid chromatography. Physico-chemical characteristics like molecular weight, boiling point and polarity of an organic compound can indicate the solubility of that compound in water. Based on some of the above mentioned characteristics different types of compounds may be extracted by different extraction techniques.[40] In general organic compounds with smaller molecules are more volatile and less polar.

Several sample extraction techniques have been applied for analysis of organic compounds in different matrices. Liquid-liquid extraction (LLE), solid phase extraction (SPE), purge and trap (PAT or dynamic head space), static head space, immersion solid-phase microextraction and head space solid phase microextraction (HS-SPME) are some of the most commonly used techniques for extraction of organic compounds from water. LLE [41-43] and SPE [44-46] are mostly used for polar and water soluble compounds. Direct SPME has been used for both polar compounds and VOCs.[45-48] Present study has employed head space solid-phase microextraction (HS-SPME) as the sample extraction technique to extract VOCs from water.

In LLE and SPE solvent wastes are generated, multiple operation steps are needed and interfering compounds are more likely to be extracted.[49] Many investigators have considered SPME a viable technique for overcoming matrix effects in samples.[49-51] It is a quick and handy method which integrates different steps of sample preparation like sampling, extraction, concentration and sample introduction in one single step. This saves

time, reduces cross-contamination and loss of analytes and does not generate solvent waste.[52-54]

In SPME analytes are extracted from the matrix by fused silica fiber coated with a polymeric stationary phase. SPME can be performed in two ways, immersion or direct sampling and head space sampling (HS-SPME). In direct sampling the silica fiber is immersed in the sample itself whereas in headspace sampling the fiber is exposed in the headspace of a sealed container.[55] Headspace techniques are more suitable for extracting VOCs. Headspace techniques may be static, dynamic (purge and trap) or SPME. Many studies have found HS-SPME useful in detecting VOCs.[48,54,56-57] although purge and trap is also considered a good extraction technique.[58-60] Headspace techniques are generally coupled to gas chromatograph and HS-SPME coupled with GC-MS has been extensively used in the analysis of VOCs.[43,49,61-64] Because SPME is so simple yet effective it has been accepted even by the official methods and standards: ASTMD6520, ASTMD6889, EPA Method 8272 and ISO 27108.[65-68] Normally, direct SPME is coupled to liquid chromatography for detection of the compounds that are soluble in liquid phase, weakly volatile and thermally labile.[69-71]

1.5 Gas chromatography and mass spectrometry

Gas chromatography and mass spectrometry have been used for separation, identification and quantification of the volatile organic compounds for a long time. The technique is very efficient and widely practiced. A gas chromatograph separates the compounds according to the retention time and mass spectrometer detects the compounds eluted from GC and quantifies them. Because of ever increasing demand for analysis of a variety of harmful compounds its use has become more and more important and with time the technology has made a lot of advancement. GC-MS technique has been used for analytical purposes in a variety of materials like food and beverage products, pharmaceutical products, and environmental media. Many studies have focused on the compounds released from petroleum products and other chemicals used in refrigeration, dry cleaning, painting, degreasing etc. Benzene, toluene, ethylbenzene and xylene (BTEX) and MTBE have been studied for many years by many investigators in drinking

water and natural waters.[72-76] Similarly, analysis of compounds of biological origin have also been a common practice.[77-80]

1.5.1 Gas chromatography

Gas chromatography is a technique that is used to separate a mixture into its individual components and identify and quantify the unknown compounds. Like for other chromatography, GC requires a mobile phase which is an inert gas (hydrogen, helium) and a stationary phase which makes the separation possible. The stationary phase is a solid or liquid coated on a solid support. Because, the different analytes interact differently with the stationary phase they are carried out by the mobile phase in different rates. Some are carried sooner than the others. The individual compounds separated by the gas chromatograph are detected by a detector and translated into a chromatogram. The compounds are represented by peaks in the chromatogram. Each peak corresponds to one compound and each peak appears from the column after specific time period. In the chromatogram (Figure 2) detector response (y-axis) is plotted against the elution time or retention time (x-axis). The position of the peaks in the retention time axis serves to identify a compound and the area of the peak provides the quantity (concentration) of the compound in the mixture.

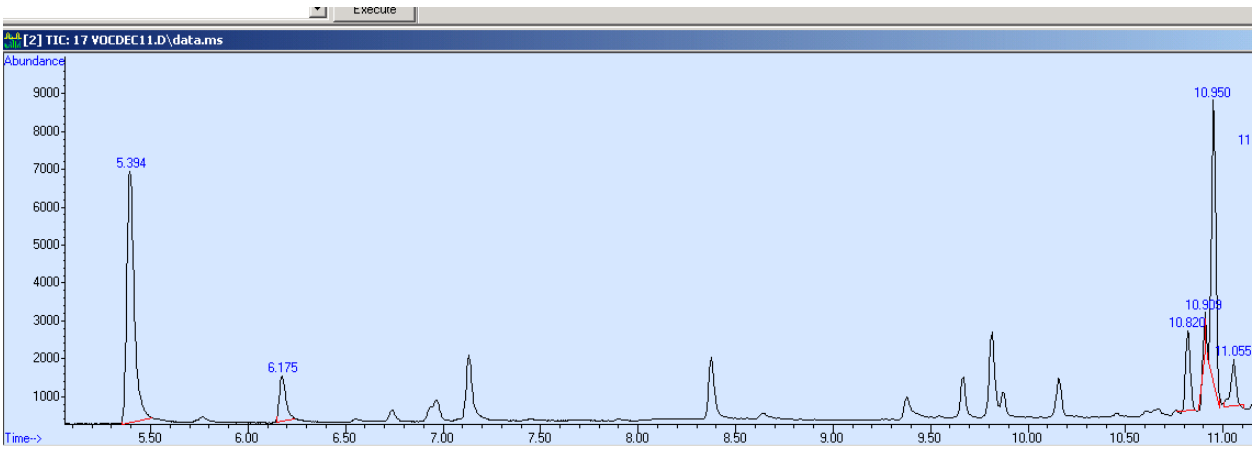


Figure 2: A chromatogram with different peaks.

Theory of separation

When a sample is injected into a gas chromatograph the molecules of the compound interacts with the stationary phase as the analyte molecules are carried by the mobile phase. Selective partitioning of the analyte between the mobile phase and the stationary phase depends on the analyte molecule's nature of interaction with the stationary phase. The compounds that interact strongly are retained longer in the stationary phase and are released late. Therefore their elution takes longer time. This time taken for a compound to get eluted from the column is called retention time. The most common interactions between the compounds and the stationary phase that play major role in giving relative retention times to different types of compounds are the non-covalent interactions, namely, dispersion, dipole and hydrogen bonding. The type of interaction depends on polarity of the compounds and the stationary phase. Polar compounds are retained for a longer time in a polar stationary phase and non-polar compound in a non-polar stationary phase.

Chromatographic parameters

Followings are a brief description of some of the important chromatographic parameters.

Distribution constant[81]

It was mentioned earlier that the separation of the different compounds are based on the selective partitioning of the compound between the mobile and the stationary phase. An analyte is in equilibrium between the two phases represented by the following equation.

$$A_{mobile} \xleftarrow{K_D} A_{stationary} \qquad \text{Distribution constant } K_D = C_s/C_m$$

A_{mobile}: Analyte quantity in mobile phase

$A_{stationary}$: Analyte quantity in stationary phase

C_s : concentration of analyte in the stationary phase

C_m : concentration of analyte in the mobile phase

The equilibrium constant for this reaction is called distribution constant, or partition coefficient or partition ratio K_D which is the ratio of the compound in the stationary phase and in the mobile phase. Higher K_D values indicate that the compound is more adsorbed in stationary phase than it is in the mobile phase.

Retention time (t_R)

The time taken for a compound to travel between the sample injection and detection by a detector after elution is called retention time. Therefore, it is the total time the compound spends in mobile and stationary phase. Usually each analyte in a sample will have a different retention time. Sometimes more than one compound interacts with the stationary phase in the same manner and they may have the same retention time. This results in a single peak that represents more than one compound. Such a condition is called coelution. In this study also two of the 17 compounds studied coelute and have the same retention time. The separation of coeluted compounds may be achieved by using a stationary phase with a different polarity or by the signature mass fractions of the compounds given by the mass spectrometric detection.

Dead time (hold-up time) t_M and adjusted retention time t_R'

Dead time is the time taken to travel for a compound in the absence of retention in the stationary phase. Adjusted retention time is the peak's retention time minus the dead time.

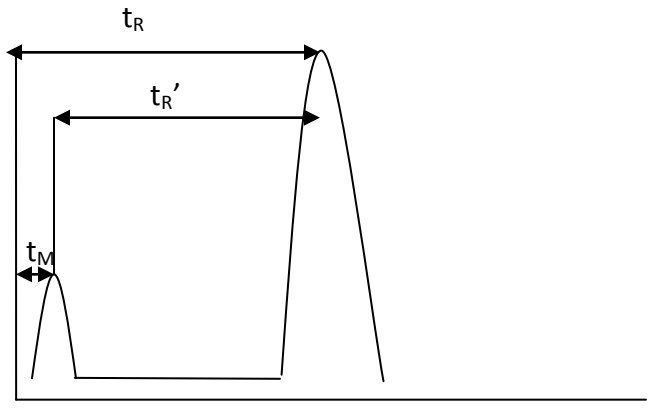


Figure 3: Dead time and adjusted retention time

Retention factor k' (capacity factor)

Retention factor is often used to describe migration rate of an analyte on a chromatographic column. Mathematically, it is a ratio of the adjusted retention time and the hold-up time.

$$k' = t_R'/ t_M = (t_R - t_M)/t_M$$

When retention factor is much less than one, elution occurs rapidly, and when it is much larger elution time becomes much longer. It is a measure of the time the sample component resides in the stationary phase relative to the time it resides in the mobile phase. Retention factor is defined as the quantity of solute in the stationary phase (s) divided by the quantity in the mobile phase. The quantity of solute in each phase is equal to its concentration (C_s or C_m) times the volume of the phase (V_s or V_m). Retention factor is a measure of solute velocity through a chromatographic column compared to the mobile phase.

$$k' = (C_s . V_s)/(C_m . V_m)$$

$C_s/C_m = K_D$, the Distribution constant

Chromatographic resolution (R)

Resolution is a measure which tells us how well two species have been separated. It takes the width of the chromatographic peaks into account. Separation and enough resolution is the goal of chromatography. Mathematically, resolution R between two compound A and B is given by;

$$R = 2(t_{RB} - t_{RA})/(W_A + W_B)$$

t_{RB} = retention time of the second peak

t_{RA} = retention time of the first peak

W_A = width of the first peak

W_B = width of the second peak

An R value of 1.5 gives the separation at the baseline of the chromatogram. The resolution for a given stationary phase can be improved by increasing column length which increases the number of theoretical plates. A high resolution chromatographic column separates peaks down to the baseline of the chromatogram.

Column selectivity

Selectivity of a column depends on relative migration rates of the compounds being separated. The relative migration rates of A and B is given by the ratio of the distribution constants of A and B. This is called selectivity factor α. Selectivity factor is a measure of the amount of peak separation.

$$\alpha = K_{DB}/K_{DA}$$

The relationship between the selectivity factor and retention factor is given by;

$$\alpha = k'_B/k'_A = (t_{RB} - t_M)/(t_{RA} - t_M)$$

When calculating the selectivity factor, species A elutes faster than species B. The selectivity factor is always greater than 1. If $\alpha = 1$ then the peaks have same retention time and thus they coelute.

Column efficiency and band broadening

Band broadening is the increase in width of a peak and this happens because of increase in retention time due to more interaction of a compound with the stationary phase. All the peaks in a chromatogram do not have equal widths, some are narrower and others are wider. A chromatogram with narrower peaks can accommodate more numerous peaks with less overlapping, if any. In other words more compounds can be separated if the peaks are narrow. Column efficiency is the ability of a column to separate compounds from a mixture. Greater the column efficiency, the higher the number of compounds that can be separated. Band (peak) broadening causes the column to be less efficient.

Quantitatively, column efficiency may be expressed as the number of theoretical plates (N) and plate height (H) described by the theoretical plate model. A column with lower N

has more overlapping peaks and with higher N has thinner peaks. The plate model supposes that the chromatographic column contains a larger of separate layers called theoretical plates. Improving the efficiency of a column would be to increase the number of plates and decrease the plate height. The theoretical plates are imaginary sections and the number of theoretical plates is related to retention time and width of the peak of a compound.

$$N = 5.45 (t_R/W_{1/2})$$

N = number of theoretical plates

t_R = retention time

$W_{1/2}$ = peak width at half height

N varies depending on compound as well as packing material. N also varies with the flow rate and the column length. Efficiency can also be measured by the height of a plate (H). The column efficiency increases as the plate height becomes smaller.

$$H = L/N$$

H = height of a plate

N = total number of theoretical plates

L = column length

Rate theory of chromatography

This theory takes into account time taken for the solute to equilibrate between the stationary and the mobile phase; the plate model does not take time into account. The resulting band shape of a peak is affected by rate of elution (flow rate of mobile phase). It is also affected by the different paths available to solute molecules as they travel between particles of stationary phase. If we consider the various mechanisms which contribute to band broadening, we arrive at the VanDeemter equation for plate height.

$$HETP (H) = A + B/u + Cu = A + B/u + (C_S + C_M)u$$

u = average velocity of the mobile phase

A is Eddy diffusion: The mobile phase moves through the column which is packed with stationary phase. Solute molecules will take different paths through the stationary phase

at random. This will cause broadening of the solute band, because different paths are of different lengths.

B is Longitudinal diffusion: The concentration of an analyte is less at the edges of the band than at the center. Analyte diffuses out from the center to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column which decreases the effects of longitudinal diffusion.

C is Resistance to mass transfer: The analyte takes a certain amount of time to equilibrate between the stationary and mobile phases. If the velocity of the mobile phase is high, and the analyte has a strong affinity for the stationary phase then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the broadening becomes.

Peak capacity n_c

It is the maximum number of peaks that can be fitted in a chromatogram or in other words the number of solutes that can be separated. Quantitatively, peak capacity n_c is given by the following equation;

$$n_c = 1+ (\sqrt{(N)})/4\ln(V_{max}/V_{min})$$

N = number of theoretical plates

V_{max} = largest volume of the mobile phase in which we can elute and detect a solute

V_{min} = smallest volume of the mobile phase in which we can elute and detect a solute

Peak symmetry

It is assumed that solutes elute as a normal (Gaussian) peak. Peak tailing occurs when some sites on the stationary phase retain the solute more strongly than other sites. Peak fronting is most often the result of overloading the column with sample. Peak symmetry is determined by bisecting the peak through the apex. The width at 10% height is measured for each half. The width of the back half is divided by the width of the front

half. A perfectly symmetrical peak has a symmetry factor of 1.00 and larger deviations from 1.00 may be an indicator of peak tailing or peak fronting.

1.5.1.1 Gas chromatography instrumentation

It has already been mentioned that GC separates compounds by passing the vaporized mixture through a tube containing a material that non-covalently interacts with the solutes in the mixture. The type and degree of interaction is different for different compounds and therefore they are retained in the column for different periods of time. Gas chromatograph is used to perform separation of the compounds. Gas chromatograph consists of a source of gas as mobile phase, an injection port, chromatographic column, a detector and the data display system.

1.5.1.1.1 Carrier gas

Carrier gas acts as the mobile phase which carries the samples through the instrument. The most common gas used is helium although sometimes hydrogen or nitrogen is also used. The carrier gas should be of high purity (99%). Gas purifiers may be used to produce high purity gas. For the present study helium was used as the carrier gas. Helium is suitable for the stationary phase of the gas chromatograph used in this study.

1.5.1.1.2 Injection mode

Chromatographic process begins when a sample is introduced into the instrument at the injection port. The sample is injected with the help of a syringe manually or automatically by using a robotic system. In the present study, an automatic sample injector was used. Injector port has a heated glass liner in which liquid sample is vaporized to be carried by the carrier gas or if sample is a vapor then it will not get condensed. The SPME fiber, used in this study desorbs its adsorbed solutes upon heating in the injector.

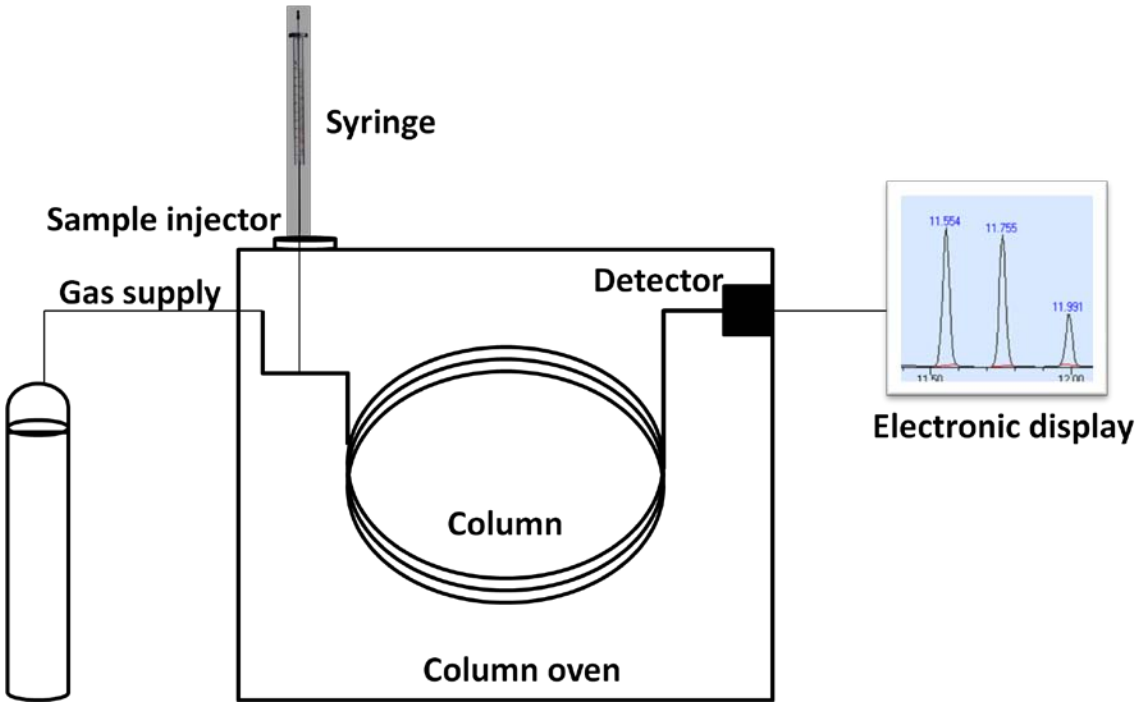


Figure 4: A Schematic diagram of a gas chromatograph

Injection can be split or splitless. Sample injection in gas chromatography depends on the nature of the sample. Sample volume should be kept to a minimum for best column efficiency. The concentrations of many samples can exceed the capacities of a column being used. Therefore, quantity of excess sample has to be reduced before it reaches the column. Split injector divides the total sample into two parts and allows only the smaller part to enter into the column. The large portion is vented away. However, when sample contains sufficiently lower concentrations (trace level) of an analyte, splitless injector is used. Splitless injector sends all the injected sample into the column. Most injectors can act both in split and splitless modes. The present study used splitless mode since analytes were injected at trace level.

1.5.1.1.3 Chromatographic column

Column is considered the heart of a gas chromatograph because the main activity of chromatography, the separation of the solute, takes place inside the column. Usually the

separation is temperature dependent and the desired temperature is maintained by placing the column in an oven. The columns are coated with stationary phase which allows different compounds to elute in different times. There are two types of columns; packed column and capillary columns. In packed column the stationary phase is coated onto packed solid adsorbent and the sample and the mobile phase pass through the packed solid adsorbent. Capillary column is an open tube in which stationary phase is coated on the inner wall of the tube on fused silica support. Since capillary column provides better resolution, capillary columns are more commonly used than packed column. Packed column are more used for gas analysis. GC used in the present study is equipped with a low polarity capillary column.

1.5.1.1.4 Stationary phase

Stationary phase is a thin layer of coating of polymers on the inner wall of the capillary column. Stationary phase should withstand high temperatures, and be inert to the mobile phase and the compounds being analyzed. The chemical nature of stationary phase influences separation of a mixture and column dimensions mainly affects peak resolution. The most common type of stationary phase is made up of back bone of polymer polysiloxanes which has alternating silicon and oxygen atoms connected by covalent bonds. To each silicon atom 2 functional groups are attached and it is the variety in these functional groups that distinguish each type of stationary phase. The most common functional groups are methyl, phenyl, cyanopropyl and trifluoropropyl. These groups are used in various proportions that give specific characteristic of separation to a stationary phase. Stationary phase polarity is directly related to the amount and polarity of each functional group.

1.5.1.1.5 Detectors

The vapor phase solutes that elute from the chromatographic column are detected by a detector. Detection of a compound generates an electrical signal whose size is related to the amount of the corresponding compound. The electrical signal is then sent to a recording device which in turn depicts it as a chromatogram. Different types of detectors

are used based on the nature of the analytes and fit for purpose of the analysis. For the present study a mass spectrometer was used as the detector.

1.5.2 Mass spectrometry

It was already mentioned that gas chromatograph was coupled with the mass spectrometer detector in the present study. A mass spectrometer determines the mass of a molecule by measuring the mass to charge (m/z) ratio. The mass to charge ratio can be used to identify a compound. In mass spectrometry ions of a target compound are generated by the loss or a gain of a charge from a neutral species. Thus formed ions are electrostatically directed towards a mass analyzer where they get separated based on their mass to charge ratio and are detected by a detector. The whole mass spectrometric analysis is performed in a vacuum system. A mass spectrometer has four basic parts: a sample inlet, an ionization source, a mass analyzer and an ion detector. The mass spectrometer that was used in the present study is equipped with an electron ionization source, a quadrupole mass analyzer and an electron multiplier detector.

Most stable organic compounds have an even number of total electrons. During ionization in the ion source of a mass spectrometer, a neutral ion loses an electron to give a molecular ion (parent ion) with an odd number of total electrons. Such a molecular ion with the odd number of electrons is called a radical cation. The molecular ion in mass spectrometry is always a radical cation. Molecular ion ($M^{\cdot +}$) undergoes fragmentation to give two parts; and it can produce an ion with an even number of electrons plus a radical or a molecule plus a new radical cation.

$$M + e^- \text{ -----} \rightarrow M^{\cdot +} + 2e^-$$

$$M^{\cdot +} \text{ --------} \rightarrow EE^+ + R^{\cdot} \text{ or } OE^{\cdot +} + N$$

(even ion plus radical or odd ion plus molecule)

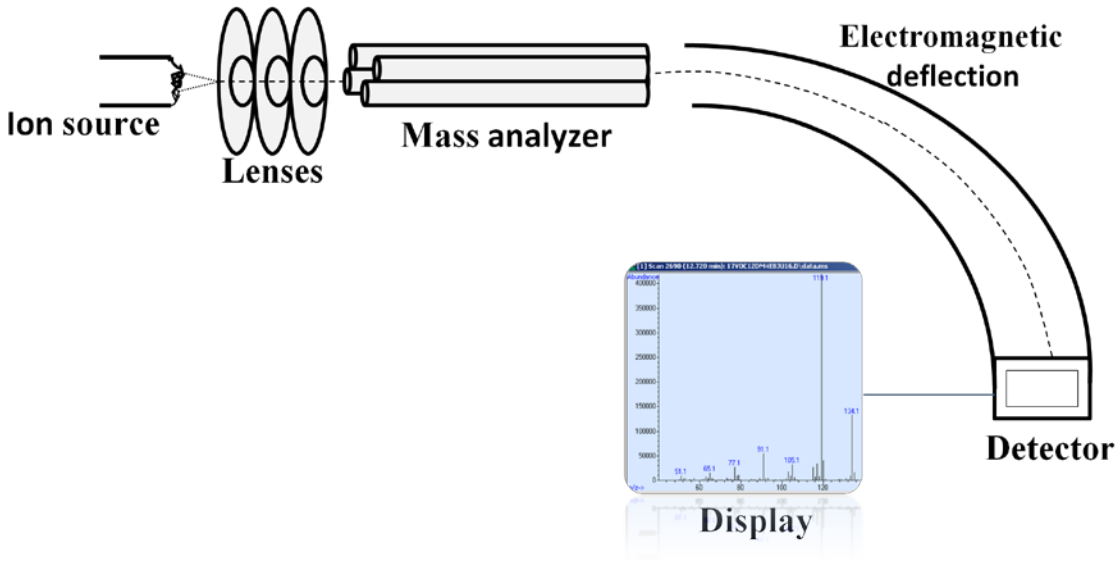


Figure 5: A schematic diagram of a mass spectrometer

1.5.2.1 Ionization source

The capillary column from the gas chromatograph directly introduces the vapor phase sample into the ionization source without compromising the vacuum condition in the source. A vacuum interlock allows sample to be introduced into the vacuum. In the ionization source the analyte molecules can be ionized by any combination of mechanisms like protonation, deprotonation, cationization, electron ejection and electron capture. Some ionization techniques are very energetic and cause extensive fragmentation of the molecule while others produce ions of low fragmentation. Some of the common ionization techniques used are electron ionization, chemical ionization, electrospray ionization, atmospheric pressure chemical ionization, atmospheric pressure photoionization and matrix assisted laser desorption ionization. The mass spectrometer employed in this study is fitted with an electron ionization (EI) device. Electron ionization is one of the most important ionization techniques and is routinely used for analysis of hydrophobic and thermally stable molecules. EI is a hard ionization source because it generates extensive fragmentation of the molecules. The ions source consists

of a heated filament which ejects electrons. The electrons are accelerated towards an anode and collide with gaseous molecules of the analyzed sample injected into the source. The current of 70ev produces high energy electrons which produce energy fluctuation around neutral molecules and induce ionization and fragmentation. The electron ejected from the heated filament form a continuous electron beam through which the sample molecules pass to be ionized and fragmented.

1.5.2.2 Mass analyzer

Various types of mass analyzers have been applied based on the nature of the analytes and the objective of the analysis. Some of the commonly used analyzers are quadrupole, ion trap, and time –of-flight. Mass analyzers use electrical and magnetic field to separate different mass to charge ratio. The performance of a mass analyzer is measured by: accuracy, analysis speed, mass range limit, resolution and transmission. A quadrupole mass analyzer is fitted with 4 parallel electrodes, two of them are positively charged and two are negatively. An electrical field accelerates ions out of the source region and into the quadrupole analyzer. The quadrupole uses electrical field to separate ions according to their mass to charge m/z ratio. There is a particular ratio of u/v (direct current voltage/radio frequency voltage) for a particular m/z and therefore, by manipulating u/v one can select a m/z of ions that travel at any moment. Only ion m/z corresponding to u/v applied move along the parallel electrodes. A quadrupole can be operated in two modes. In full scan all the m/z ions in a specified range is determined. In selected ion monitoring (SIM) mode only few m/z ions are monitored.

1.5.2.3 Detector

The ions coming out of the mass analyzer are received by a detector which transforms them into a usable signal. The detector generates electric current from the incident ions and the electric current is proportional to the abundance of detected ions. The most common method of detection is the use of an electron multiplier (EM). An electron multiplier is made up of a series of aluminum oxide (Al_2O_3) dynodes. The dynodes have ever increasing potential. The ions striking the first dynode emit electrons. These electrons are then attracted to the next higher dynode and more secondary electrons are

generated. Ultimately a numerous dynodes are involved and a cascade of electrons is ormed that produces an electric current.

1.6 SPME principle, apparatus and operation

SPME uses different types of stationary phases to provide selectivity, thermal stability and different polarity[82]. The extraction is due to adsorption of volatile molecules on the polymeric coating. All polar, non-polar and semipolar stationary phase are commercially available. The most common polymer coatings are non-polar polydimethyl siloxane (PDMS), semipolar divinyl benzene (DVB) and polar polyacrylate and carbowax. Present study used DVB/PDMS/Carboxen (gray color coded syringe) polymers for VOC extraction in the SPME fiber. In HS-SPME distribution of analytes are equilibrated in all three media; sample, headspace and the fiber. When the fiber is exposed to a head space of the sealed heated sample container, many volatile compounds get adsorbed to the polymer coating. The amount of analyte adsorbed by the coating at equilibrium is directly related to its concentration in the sample and it is given by the following equation.[82]

$$N = K_{fs} * V_f * C_o * V_s / K_{fs} * V_f + V_s$$

N = mass of analyte adsorbed by coating
V_f = volume of coating
V_s = volume of sample
C_o = Initial concentration of analyte in sample
K_{fs} = partition coefficient of analyte between coating and sample

SPME is provided with 1 cm long fused silica fiber coated with a polymeric phase (Figure 6). The fused silica fiber is connected to a stainless steel tubing to provide mechanical support to the fiber. The fiber and the steel tubing is fitted in a syringe in order to facilitate insertion of the fused silica fiber into sample vial and the gas chromatograph injector. In the injector the analytes adsorbed on the polymeric phase during heating of the sample vial get desorbed due to high heat and the analyte is carried to the chromatograph column by the carrier gas for separation.

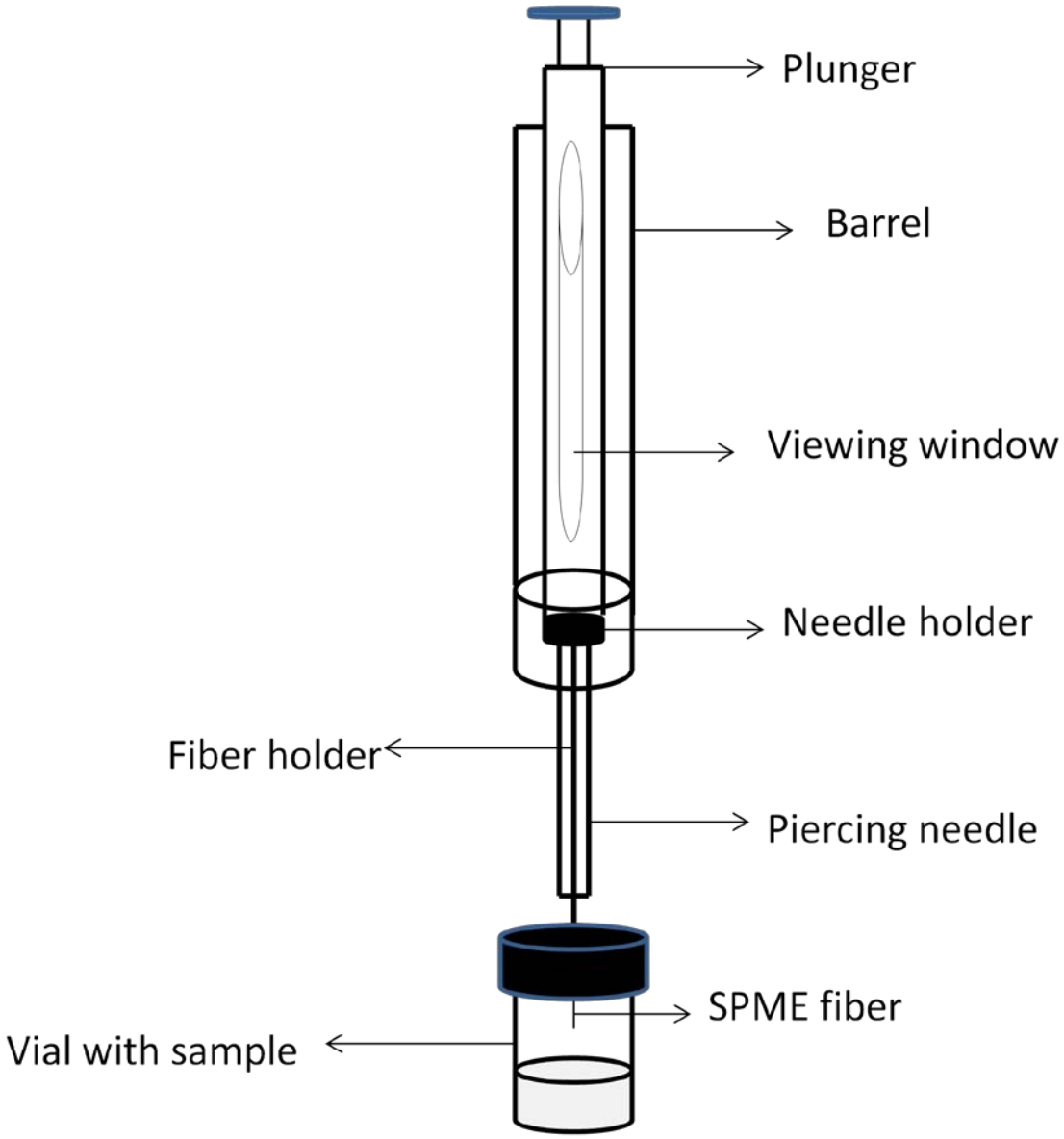


Figure 6: Solid-phase microextraction assembly

An in-house condition was used for all SPME, GC and MS analytical conditions.

1.7 Statistical tools for data analysis

Followings are short descriptions of the statistical tools used for processing, analyzing and interpreting of the data obtained in the present study.

1.7.1 Linearity and working range of calibration

In analytical chemistry laboratory, use of a calibration curve or a graph is a very common practice. Calibration curve method is used to determine concentration of an unknown analyte in a particular matrix sample by comparing it with known concentration of a standard. The calibration curve is constructed by plotting instrument response of a series of calibration standards against the concentration of the standards. The concentration of the unknown samples is derived from interpolation of the curve.

Since the response of the instrument against the concentrations (correlation) is not ideal, least square regression analysis is performed to prepare a calibration curve. Mostly linear regression analysis is applied although non-linear analysis may be performed depending upon the relationship between the two variables. In linear regression the two variables are best fit in such a way that their relationship is expressed by a common straight line curve. The best fitting line is the one which yields the minimum of the sum of squares of the distance between modeled line and the experimental points. Mathematically, a linear regression model or equation is derived from which concentration of the unknown is determined and the model is[83-84]

$$y = a + bx$$

a = y-intercept of the calibration straight line

b = slope of the calibration line

y = instrument response

x = concentration of unknown

Therefore concentration of the unknown sample is given by,

$X = (y-a)/b$

The slope of the line $b = \sum^{N}_{i=1} [(x-\bar{x})(y-\bar{y})]/\sum^{N}_{i=1}(x-\bar{x})^2$

N = number of calibration standards

$\bar{x}$ = mean of calibration standards

$\bar{y}$ = mean of instrument response

1.7.2 Errors of regression equation

There is always some errors associated with measurement. Likewise, the regression equation also has some errors (residuals) associated with it. Regression residual ($y_{i\ res}$) is the difference between the experimental response values y_i and the response predicted by the regression equation $\hat{y}_i$.

$y_{i\ res} = (y_i - \hat{y}_i)$

The distribution of residuals is random if the calibration data is linear. The method of least square tries to minimize these residuals (errors). Because of the residuals there is an error associated with the slope and the intercept. The greater the regression residual, the greater the uncertainty where the true regression line actually lies. The error is expressed by the residual standard deviation (standard error) of the regression line.

$$S_{y/x} = \sqrt{\sum^{N}_{i=1} (y_i - \hat{y}_i)^2/N-2}$$

$S_{y/x}$ = Residual standard deviation

N = Number of calibration points

$\hat{y}_I$ = predicted response of the instrument

y_i = measured response of instrument

N-2 = degrees of freedom (two parameters; slope and intercept)

$S_{xo} = S_{y/x}/b$

S_{xo} is the standard deviation of the method (standard deviation of the calibration procedure)

Standard error of the slope $S_b = S_{y/x}/\sqrt{\sum^{N}_{i=1} (x_i - \bar{x})}$

Confidence interval of the slope for N-2 degrees of freedom is,

$$b = \pm t_{n-2} * S_b$$

Standard error of the intercept $S_a = S_{y/x} \sqrt{\sum^{N}_{i=1} x_i^2/N\sum^{N}_{i=1} (x_i - \bar{x})^2}$

Confidence interval of intercept = $a \pm t_{n-2} * S_a$

1.7.3 Correlation coefficient (r)

Correlation coefficient simply tells us how strongly the two variables, concentration and instrument responses are related. It does not however provide quantitative information about the size of change brought in instrument response (dependent variable) as a result of change in concentration (independent variable) which regression analysis does. Coefficient of determination (r^2) is just the square of the correlation coefficient r.

$r = N(\sum xy) - (\sum x)(\sum y)/\sqrt{[N(\sum x^2) - (\sum x)^2]}\sqrt{[N(\sum y^2) - (\sum y)^2]}$

1.7.4 Working range

Usually, in analytical methods, only a certain range of concentrations shows linearity and outside this range the relationship is not linear and linear regression model cannot be used.

1.7.5 Tests for linearity Mandel's test

Curves with a correlation coefficient $r \geq 0.995$ are usually considered to be linear. However, investigators have stated that correlation coefficient alone may not ascertain linearity because sometimes non-linear relationship can have correlation coefficient value close to one. Mandel's fitting test can further verify where the chosen regression model adequately fits the data.[85-87]. In Mandel's fitting test residual variance of linear regression is compared with residual variance of a non-linear regression model. If the two variances are different then the linearity of regression does not hold true and non-linear regression model should be used. If the variances are not different then linear regression should be used.

The difference in the variance DS^2 is calculated from the following equation (ISO 8466-1:1990E).

$$DS^2 = (N-2)S_{y1}^2 - (N-3)S_{y2}^2$$

N= number of measurement points (calibration points)

S_{y1}^2 = linear residual variance

S_{y2}^2 = non-linear residual variance

DS^2 and the variance of the non-linear calibration function are submitted to F-test in order to examine for significant differences. The test value is calculated by;

$$VT = DS^2 / S_{y2}^2$$

F tabulated or critical value is obtained from 1 and (N-3) degrees of freedom at 95% confidence level ($\alpha = 0.05$). The hypothesis (H_o) is that there is no significant difference between the linear and non-linear residual variances.

If VT<F: non-linear calibration function does not lead to significantly better adjustment, i.e. the calibration function is linear

If VT>F: non-linear calibration function should be used

Non-linear calibration model is given by the following equation.

$$Y = a + bx + cx^2$$

Y = instrument response

X= unknown concentration

a, b and c are coefficients

1.7.6 RIKILT Test

In chromatography, analysis of samples and standards can take significantly long time period. Therefore, it would be advisable to seek a single factor to determine sample concentration instead of using complete calibration curve which uses 5 or more calibration standards. RIKILT test tells us whether a response factor can be used instead of a calibration curve for quantitative determination of a sample analyte.[88] For the test to perform concentration, area, ratio of area and concentration and percentage of the ratio should be determined from the calibration curve. The test has defined that the percentage value should fall between 90 and 110%. If so the response factor is valid and can be used. If any of the calibration standard falls outside the limit a calibration curve must be prepared and response factor cannot be applied. The following equation gives the percentage of the ratio mentioned above.

$Y_i/x_{i\,\%} = \underline{y_i/x_i} * 100$

y_i = peak area

x_i = concentration

M = mean of all peak y_i/x_i

1.7.7 Standardized area test

The regression equation tells that what exact response areas should be for any concentration. However, most of the time not all the points fall exactly on the regression line. They are spread around the line. The objective of the standardized areas test is to compare the experimental values with the predicted values of the equation. For each point in the calibration curve, the ratio between the experimental area and the area predicted by the curve is obtained. The concentration at which the ratio between the two areas is closest to 1 is considered as the concentration with best correlation. Then the ratio of the ratio of concentration of best correlation and area to concentration and area of each of the other points are calculated.

$$\text{Standardized area} = \frac{(Ai/C_i) * (100 * C/100)}{A_{100}}$$

Where:

A_i = peak area of a calibration point

C_i = concentration of Ai

A_{100} = peak area of experimental point with the best correlation

C_{100} = concentration of experimental point with the best correlation

The standardized area values were plotted against the concentrations. The test defines an acceptable range of 85% to 115% within which the standardized areas should fall. Any value outside the range is excluded and the test is again applied until the requirement is met.

1.7.8 Grubb's test for outlier

Sometimes it is important to remove outliers in a set of data before the data is processed. An outlier is one or more data points which clearly stand out from the rest of the data. Grubb's test has been widely used to detect an outlier. Grubb's test detects an outlier one at a time.[89] The test compares minimum or maximum values with the mean. The difference between the mean and the minimum or maximum is statistically tested to reach at conclusion. Grubb's test is applied on normally distributed data so it is assumed that the data obtained for repeatability is approximately normally distributed. Grubb's test should not be used for a sample size of six or less. The Grubb's test is applied as follows. The data are first put in increasing order. Standard deviation and mean are calculated. For conducting test of hypothesis test statistic G_{exp} is determined which is compared with G_{crit} value found in the table. The test is usually conducted at 95% confidence level.

$Gexp = X_{mean} – X_{min}/S$ for minimum value

$Gexp = X_{max} – X_{mean}/S$ for maximum value

The null hypothesis is that there is no difference between the mean and the minimum or maximum value (i.e. there is no outlier). If $G_{exp} > G_{crit}$, then the value in question is an outlier and is discarded. If $G_{exp} <$ than G_{crit} then the null hypothesis is accepted i.e. there is no difference between mean and the suspect value and the value should be retained.

In this study LOD and LOQ are calculated in three different ways:

1. From calibration:

$LOD = S_{xo} * 3$ $S_{xo} = S_{y/x}/b$, $S_{y/x}$ = Residual standard deviation

b = slope of the calibration curve

$LOQ = S_{xo} * 10$

2. From repeatability study:

LOD = Repeatability standard deviation * 3

LOQ = Repeatability standard deviation * 10

3. From intermediate precision study:

LOD = Intermediate precision standard deviation * 3

LOQ = Intermediate precision standard deviation * 10

Response factor = concentration/area of the lowest calibration standard

Concentration of unknown = Response factor * area of unknown

1.8 Method validation

Millions of analytical measurements are made every day in thousands of laboratories around the world for a variety of uses such as in manufacturing industries, pharmaceuticals, cosmetics, foods and beverages, environmental health, ecological and pathological.[90] Analytical information can be used for a variety of purposes: to take decisions in manufacturing processes, to assess regulatory compliance, to take decisions in legal affairs, international trade, health problems and the environmental issues.[91] Therefore, generation of correct laboratory results cannot be compromised or costs involved can be enormous. In order to meet the expectations of all the above issues laboratories have to have a rigorous QA/QC system. It has been internationally recognized that the quality control system of an analytical laboratory should include accreditation by a competent institution, participation in proficiency testing, internal quality control, use of certified reference materials where possible, and use of validated assay methods.[92] Method validation alone cannot guarantee for accurate and reliable laboratory result but it should be a part of integrated quality assurance meant for analytical measurement.[93]

Validation may be 'in house' carried out by a single laboratory or it may be 'Full' which involves examination of characteristics of a method in an interlaboratory method performance study (also known as collaborative study or collaborative trial).[92] The present study is about method validation followed within a single laboratory that is, an 'in house'. An ISO definition of validation is "confirmation by examination and the provision of objective evidence that the particular requirements for a specified intended use are fulfilled.[94] The definition implies that validation should take into account the

requirement of specific application.[91] Most of the times customers are the source of information on the requirement.

"The validation of an analytical method demonstrates the scientific soundness of the measurement. The validation practice demonstrates that an analytical method measures the correct substance in the correct amount and in the appropriate range for the intended samples. It allows the analyst to understand the behavior of the method and to establish the performance limits of the method. In other words validation answers the question like which analyte can be determined, in which matrices, at what level of concentration, and with what level of precision and accuracy".[95] Validation should be carried out for non-standard procedures and standard procedures as well. Alteration in any number of factors during the transfer of the method and reapplication in a different laboratory may alter the performance characteristics.[96] Therefore, at least some level of verification should be performed even for the standard methods but full validation is always desirable. In order to perform method validation, the laboratory should follow a written standard operating procedure (SOP).[97]

Sometimes it is hard to draw a line between method development and method validation. Validation usually begins during method development. Many of the method performance parameters that are associated with method validation are in fact usually evaluated, at least, approximately, as part of method development to determine whether the method's capabilities are in line with the levels required.[90,98] Usually method validation evolves from method development and so the two activities are closely tied, with the validation study employing the techniques and steps in the analysis as defined by the method development.[99] Following is a brief introduction of method validation parameters.

1.8.1 Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte of interest in presence of other components in the sample. A sample may contain a variety of undesirable components which may interfere with identification of the target analyte. The interference may be due to isomers, metabolites, endogenous substances etc.

In the majority of the analytical methods it is necessary to ensure that the signal produced in the measurement is only due to the analyte of interest and not due to the presence of interferences in the sample. Hence it is necessary to test the selectivity of the analytical method. When a method responses to only one single analyte the method is said to be specific while selective method can detect more than one analyte. Presence of other components or matrix effect can enhance or suppress the signal. Many times foreign substances may be present but do not significantly interfere with the detection of the intended compound. In gas chromatography selectivity can also be obtained by bringing variation in temperature profile, stationary phase type or detector. Selectivity can be performed by more than one technique for confirmation. For example, in gas chromatography, columns of different polarity or representative ions from mass spectrometry can be used.

1.8.2 Linearity and working range

Linearity is established by measuring response at various concentrations by a regression plot. In the linear range of the calibration plot there is an equal increase in response for a unit increase in concentration. For most of the instruments linearity is usually observed within a range beyond which response becomes non-linear. A linear regression equation is used to determine concentration of an unknown from its measured response. It has been a general practice to use correlation coefficient (r) or coefficient of determination (r^2) value as the basis of accepting or rejecting the linearity of a calibration curve although it may need other supporting information. A high value of correlation coefficient and a random distribution of residual plot are normally enough to assess linearity. However, carrying out Mandel's test or goodness of fit test will further confirm linearity.

1.8.3 Limit of detection (LOD)

When measurements are made at low analyte levels (at trace level) it is important to know what is the lowest concentration that can be confidently detected by the method. There is a probability that there must be some distance between zero and the point of limit of quantification (LOQ) or in other words change from zero to LOQ must not just

have been abrupt. LOD is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. In chromatography, the detection limit is the injected amount that results in a peak with a height three times as high as the baseline noise level, that is signal to noise ratio of 3. So LOD can be reliably distinguished from baseline or zero. LOD is also determined from residual standard deviation of calibration, repeatability standard deviation and intermediate precision standard deviation (refer to the Experimental procedure).

1.8.4 Limit of quantification (LOQ)

LOQ is the performance characteristic that marks the ability of a chemical measurement to adequately quantify an analyte. LOQ is the minimum concentration level at which the analyte can be determined with acceptable accuracy and precision. LOQ can be determined in different ways. LOQ can be the level where the signal to noise ratio is 10. It can also be determined from residual standard deviation of calibration, repeatability standard deviation and intermediate precision standard deviation.

1.8.5 Precision

Precision is the closeness among the results of the repeated measurements of a sample. Precision is generally quantitatively expressed in standard deviation or relative standard deviation, RSD, also called coefficient of variation, CV. Different factors like analyst, environment, type of reagent, instrument, time of analysis and place of analysis can influence the repeated analysis results. Based on the influencing factors changed three types of precision are determined in method validation. They are repeatability, intermediate precision and reproducibility.

Repeatability is the closeness of results when the repeated analysis is performed within a short period of time, for example, repeated measurements carried out within the same day. Therefore, in repeatability same person, same equipment, same reagent and same environment are used on the same day for analysis. This is the kind of variability one expects from duplicate analysis. Reproducibility condition means precision under reproducibility conditions when test results are obtained with the same method on

identical test items in different laboratories with different operators using different equipment. Intermediate precision is an in between measure. Repeatability gives the smallest value of precision and reproducibility gives the largest value of precision. It is recommended that at least 7 measurements (preferably $n \geq 10$) should be carried out to obtain good precision estimates.

Intermediate precision relates to the variation in results when one or more factors such as time, operator, or equipment are varied within a laboratory. Different types of intermediate precision depending on factors changed between the measurements. For instance time-different intermediate precision is obtained when the test results are obtained on different days. If results are obtained in different days and by different operators, then we obtain (time+operator) – different intermediate precision, and so on. The objective of the determination of intermediate precision is to identify the various factors within a single laboratory that will contribute to the variability of the results and to find a mechanism to control them. For a laboratory, this is the most useful precision because it gives an idea of the sort of variability that a test may encounter in a laboratory.

1.8.6 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted as true value and the value experimentally observed. Accuracy can be assessed by analyzing a sample with known concentration, for example, a control sample or certified reference material and comparing the measured value of unknown with the true value of supplied material. If a control sample or a certified reference material are not available, a blank sample matrix of interest can be spiked with a known concentration. Recovery of an analyte of a sample can be determined by comparing response of the sample with the response of a reference material in a pure solvent. In this case, accuracy should be reported as percent recovery with the relative standard deviation of the 10 replicate spike measurement. Recovery tests may reveal a significant bias in the method used and may require a correction factor to be applied to the analytical results. Accuracy has both systematic error as shown by the relative error and random error as shown by relative standard deviation.

Accuracy can also be measured by interlaboratory comparison tests or proficiency tests (PT). A participating laboratory analyses the sample sent by the PT provider and send the results back to the PT provider. A ‘z’ score is a performance indicator of each participant. The PT provider calculates the z score (calculated statistically) for each participating laboratories based on the result obtained from a laboratory, true value of the sample distributed and the standard deviation of the all the results obtained. A z-score of less than two indicates a satisfactory result which means accuracy performance of the participating laboratory is satisfactory.

II. EXPERIMENTAL PROCEDURE

Experimental procedure includes a brief description of materials and reagents used, equipment and apparatus used, preparation of stock, intermediate and working solutions, instrument operation procedure, method of extraction, sampling and analysis and statistical techniques used in data analysis and method validation. All the activities of the research, both the laboratory work and thesis preparation were carried out in the Central Laboratory of EPAL in Lisbon. Method validation for detection of target VOCs was carried out according to the SOP followed in the EPAL laboratory.

2.1. Materials and reagents

Ultrapure water produced by Millipore Milli-Q system suited for volatile organic compounds (VOC-Pak, Polisher)
Hirschmann, 1 ml, 2 ml 5 ml, 10 ml and 25 ml capacity glass pipettes
Volumetric flasks of capacity 2 ml, 5 ml, 10 ml, 25 ml
Agilent Technology manual glass syringe of 10, 25, 50, 100 µl capacity
Glass vials of 20 ml capacity for SPME
Magnetic golden CRIMP caps for SPME 20 ml vials
GERSTEL Natural rubber orange/TEF septa for sealing the vials
Crimper for sealing the vials
SPME fiber and assembly is SUPELCO 50/30 µm DVB/CAR/PDMS Gray color

2.2. Equipment and apparatus

SPME sampler: Autosampler, GERSTEL Multipurpose MPS XL
Gas chromatograph: Agilent Technologies 6890N, Network GC systemSoftware: MSD Productivity ChemStation with the current version of MS Windows^{TM}NT
Injector: Splitless
Column: Low polar capillary column, Agilent J & W GC Column, DB-VRX 60 m length, 0.320 mm diameter and 1.80 µm film thickness, temperature limit -10^{o}C to 260^{o}C

2.3. Mass spectrometer

Agilent 5973 Network, Mass Selective Detector
Software: ChemStation MSD
Ion Source: Positive Electron Ionization (EI)
Analyzer: Single Hyperbolic Quadrupole Mass Filter
Detector: High energy Dynode Electron Multiplier

Analytical balance: Mettler AF 240 (0.0000g)
Vortex: VELP Scientific
Carrier gas: Helium C55 (He) 99.9995%, Gasin
Solvent: Methanol HPLC gradient grade, Fisher Chemical

2.4. Analytical standards

This study included 16 different compounds and the pure standards were procured from the two different vendors ULTRA SCIENTIFIC and Dr. Ehrenstorfer GmbH. As soon as they were received in the laboratory they were stored at -18^{o}C. A list of the compounds purchased has been given in the Table 2.

2.5. Preparation of intermediate and calibration solutions from the original standard solution, (standards supplied by the vendor)

All the dilutions for intermediate and working solutions were made in methanol. However, final dilution for injection was done in ultrapure water or in water samples brought from the field for recovery studies.

2.5.1. Solution for optimization of the peaks

For optimization studies the original standard solutions were appropriately diluted in methanol, in few steps, to bring down the concentrations to about 20 µg/L. This solution was then injected separately into GC in order to identify the chromatographic retention time and differentiate the target compounds in full scan acquisition mode of the mass spectrometer. Identification of the compounds was also done by comparing the

experimental mass spectra with the mass spectra listed in the NIST database. Retention time t_R and representative mass fragments were also obtained for each compound. Concentration/density of the original standard solution has been given in the Table 3.

Table 2: A list of original standards used in the study

S.N.	Original standard	Purity	Supplier
1	MTBE	98.0%	Dr. Ehrenstorfer GmbH, Germany
2	3-ethyltoluene	99.5%	Dr. Ehrenstorfer GmbH, Germany
3	4-ethyltoluene	99.0%	Dr. Ehrenstorfer GmbH, Germany
4	2-ethyltoluene	99.5%	Dr. Ehrenstorfer GmbH, Germany
5	1,2,4-trimethylbenzene	99.0%	Dr. Ehrenstorfer GmbH, Germany
6	4-isopropyltoluene	5000 µg/mL	ULTRA Scientific, USA
7	1,3-diethylbenzene	98.5%	Dr. Ehrenstorfer GmbH, Germany
8	Indane	99.0%	Dr. Ehrenstorfer GmbH, Germany
9	1,4-diethylbenzene	98.6%	Dr. Ehrenstorfer GmbH, Germany
10	1,3-dimethyl-5-ethylbenzene	98.0%	ULTRA Scientific, USA
11	1,2-diethylbenzene	96.6%	Dr. Ehrenstorfer GmbH, Germany
12	1,4-dimethyl-2-ethylbenzene	99.0%	ULTRA Scientific, USA
13	1,3-dimethyl-4-ethylbenzene	95 +%	ULTRA Scientific, USA
14	1,2-dimethyl-4-ethylbenzene	99.0%	ULTRA Scientific, USA
15	1,2-dimethyl-3-ethylbenzene	98.5%	ULTRA Scientific, USA
16	Hexachlorobutadiene	99.0%	Dr. Ehrenstorfer GmbH, Germany

Table 3: Physical properties of the compounds

S.N.	Name of compounds	Molecular Formula	Molar mass g/mol	Density g/ml	Melting point ^{0}C	Boiling point ^{0}C	CAS NO.
1	MTBE	C5H12O	88.15	0.7404	-109	55.2	1634-04-4
2	3-ethyltoluene	C9H12	120.19	0.865	-96	158	620-14-4
3	4-ethyltoluene	C9H12	120.19	0.861	-62	162	622-96-8
4	2-ethyltoluene	C9H12	120.19	0.887	-17	164	611-14-3
5	1,2,4-trimethylbenzene	C9H12	120.19	0.876	-43.78	170	95-63-6
6	4-isopropyltoluene	C10H14	134.22	0.86	-68.9	177.1	99-87-6
7	1,3-diethylbenzene	C10H14	134.22	0.864	-84.2	181.7	141-93-5
8	Indane	C9H10	118.18	0.965	-51.4	176.5	496-11-7
9	1,4-diethylbenzene	C10H14	134.22	0.862	-42.85	183.75	105-05-5
10	1,3-dimethyl-5-ethylbenzene	C10H14	134.22	0.87	-84	184	934-74-7
11	1,2-diethylbenzene	C10H14	134.22	0.88	-31	183	135-01-3
12	1,4-dimethyl-2-ethylbenzene	C10H14	134.22	0.88	-54	187	1758-88-9
13	1,3-dimethyl-4-ethylbenzene	C10H14	134.22	0.88		186	874-41-9
14	1,2-dimethyl-4-ethylbenzene	C10H14	134.22	0.88	-67	190	934-80-5
15	1,2-dimethyl-3-ethylbenzene	C10H14	134.22	0.89	-50	194	933-98-2
16	Hexachlorobutadiene	C4Cl6	260.76	1.665	-22	210	87-68-3

In order to have an approximate idea of instrument sensitivity the 20 µg/L solutions were further diluted until the minimum concentration for which the instrument would show measurable response. The diluted solutions were injected in selected ion monitoring (SIM) mode. Following (Table 4) are the approximate minimum concentrations at which level the compounds can be detected in the given GC-MS conditions. This in a sense concluded the sensitivity and selectivity study of the method for each compound.

Table 4: Approximate minimum concentration required for detection

S.N.	Compound	Concentration ug/L
1	MTBE	5.00
2	3-ethyltoluene	0.02
3	4-ethyltoluene	0.05
4	2-ethyltoluene	0.05
5	1,2,4-trimethylbenzene	0.05
6	4-isopropyltoluene	5.00
7	1,3-diethylbenzene	0.05
8	Indane	0.50
9	1,4-diethylbenzene	0.02
10	1,3-dimethyl-5-ethylbenzene	0.02
11	1,2-diethylbenzene	0.05
12	1,4-dimethyl-2-ethylbenzene	0.02
13	1,3-dimethyl-4-ethylbenzene	0.05
14	1,2-dimethyl-4-ethylbenzene	0.05
15	1,2-dimethyl-3-ethylbenzene	0.05
16	Hexachlorobutadiene	0.05

2.5.2. Linearity and working range

2.5.2.1. Preparation of primary (stock) solution from original standard solution

From original standard solutions, primary solutions (PS) were prepared, after suitable dilutions. PS were individually prepared and therefore there were 16 of them (Table 5). The dilution was done in HPLC grade methanol.

Table 5: Preparation of primary solutions

S.N.	Compounds	Density g/ml	Flask ml	Vol. Measured From original µl	Concentration mg/ml
1	MTBE	0.740	5	50	7.400
2	3-ethyltoluene	0.865	10	10	0.865
3	4-ethyltoluene	0.861	10	10	0.861
4	2-ethyltoluene	0.887	10	10	0.887
5	1,2,4-trimethylbenzene	0.867	10	10	0.867
6	4-isopropyltoluene	0.005	5	50	0.050
7	1,3-diethylbenzene	0.864	10	10	0.864
8	Indane	0.965	10	50	4.825
9	1,4-diethylbenzene	0.862	10	10	0.862
10	1,3-dimethyl-5-ethylbenzene	0.002	5	2,000	0.800
11	1,2-diethylbenzene	0.880	10	10	0.880
12	1,4-dimethyl-2-ethylbenzne	0.880	10	10	0.880
13	1,3-dimethyl-4-ethylbenzene	0.002	5	2,000	0.800
14	1,2-dimethyl-4-ethylbenzene	0.002	5	2,000	0.800
15	1,2-dimethyl-3-ethylbenzene	0.002	5	2,000	0.800
16	Hexaclorobutadiene	1.665	20	10	0.833

2.5.2.2. Preparation of Mix II from primary solutions

Primary solutions were diluted to prepare Mix II solutions. A specific volume of each primary solution was poured into a single flask of 50 ml to give a corresponding concentration (Table 6). The standard solution in this flask had 16 different compounds, each with its own concentration, and this solution was named Mix II. The dilution was carried out in HPLC grade methanol.

2.5.2.3. Preparation of Mix III from primary solution

Primary solutions were also diluted in HPLC grade methanol to prepare Mix III solution. As was done for Mix II, a certain volume of each primary solution was poured into a single flask of 10 ml (Table 7). Therefore, Mix III also contained 16 compounds whose concentrations were different from those in Mix II.

2.5.2.4. Preparation of Mix I from Mix II solution

Mix I solution was prepared by 10 folds dilution of Mix II in methanol. Therefore, this solution also contains all 16 compounds in different concentrations (Table 8).

Table 6: Preparation of Mix II solution

S.N.	Compounds	Vol. measured from PS µl	Volume of flask	Concentration mg/L
1	MTBE	50		7.4
2	3-ethyltoluene	5		0.087
3	4-ethyltoluene	5		0.086
4	2-ethyltoluene	5		0.089
5	1,2,4-trimethylbenene	5		0.088
6	4-isopropyltoluene	25		0.025
7	1,3-diethylbenzene	5	50 ml	0.086
8	Indane	5		0.483
9	1,4-diethylbenzene	5		0.086
10	1,3-dimethyl-5-ethylbenzene	5		0.080
11	1,2-diethylbenzene	5		0.088
12	1,4-dimethyl-2-ethylbenzene	5		0.088
13	1,3-dimethyl-4-ethylbenzene	5		0.080
14	1,2-dimethyl-4-ethylbenzene	5		0.080
15	1,2-dimethyl-3-ethylbenzene	5		0.080
16	Hexaclorobutadiene	5		0.083

Table 7: Preparation of Mix III solution

S.N.	Compounds	Vol. measured from PS µl	Volume of flask	Concentration mg/L
1	MTBE	100		74.00
2	3-ethyltoluene	20		1.73
3	4-ethyltoluene	20		1.72
4	2-ethyltoluene	20		1.77
5	1,2,4-trimethylbenene	20		1.75
6	4-isopropyltoluene	70		0.35
7	1,3-diethylbenzene	20	10 ml	1.73
8	Indane	20		9.65
9	1,4-diethylbenzene	20		1.72
10	1,3-dimethyl-5-ethylbenzene	20		1.60
11	1,2-diethylbenzene	20		1.76
12	1,4-dimethyl-2-ethylbenzene	20		1.76
13	1,3-dimethyl-4-ethylbenzene	20		1.60
14	1,2-dimethyl-4-ethylbenzene	20		1.60
15	1,2-dimethyl-3-ethylbenzene	20		1.60
16	Hexaclorobutadiene	20		1.67

Table 8: Preparation of Mix I solution

S.N.	Compounds	Vol. measured from Mix II	Volume	Concentration
		ml	of flask	mg/L
1	MTBE	5		0.74
2	3-ethyltoluene	5		0.01
3	4-ethyltoluene	5		0.01
4	2-ethyltoluene	5		0.01
5	1,2,4-trimethylbenene	5		0.01
6	4-isopropyltoluene	5		0.00
7	1,3-diethylbenzene	5	50 ml	0.01
8	indane	5		0.05
9	1,4-diethylbenzene	5		0.01
10	1,3-dimethyl-5-ethylbenzene	5		0.01
11	1,2-diethylbenzene	5		0.01
12	1,4-dimethyl-2-ethylbenzene	5		0.01
13	1,3-dimethyl-4-ethylbenzene	5		0.01
14	1,2-dimethyl-4-ethylbenzene	5		0.01
15	1,2-dimethyl-3-ethylbenzene	5		0.01
16	Hexaclorobutadiene	5		0.01

The three working solutions Mix I, Mix II and Mix III were prepared because initially the range of linearity intended for calibration was quite broad. Depending on the compound it ranged from 0.0016 to 0.148 µg/L in the lower end and 2.667 to 123.3 µg/L at the higher end. It was also assumed that drawing less than a volume of 3 µl of standard solution by the syringe could be too little a volume and may introduce volume error. Similarly, injecting into GC a sample with more than 25 µl of standard could make methanol having competitive advantage against target compounds in interaction with the stationary phase of the SPME fiber. The three types of working standards Mix I, Mix II and Mix III were prepared in order to match the three aspects of the analytical work just mentioned, wide range of initial calibration standards, volume of standards pipetted and volume of methanol injected.

Table 9: Calibration standards for the 16 VOCs

S.N.	Compound name	Calibration standards µg/L									
		P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
1	MTBE	4.93	5.92	7.40	8.39	9.87	10.85	12.33	13.32	8.88	11.84
2	3-ethyltoluene	0.06	0.07	0.09	0.10	0.12	0.13	0.15	0.16	0.21	0.28
3	4-ethyltoluene	0.06	0.07	0.09	0.10	0.11	0.13	0.14	0.15	0.21	0.28
4	2-ethyltoluene	0.06	0.07	0.09	0.10	0.12	0.13	0.15	0.16	0.21	0.28
5	1,2,4-trimethylbenzene	0.06	0.07	0.09	0.10	0.12	0.13	0.15	0.16	0.21	0.28
6	4-isopropyltoluene	0.02	0.02	0.03	0.03	0.03	0.04	0.04	0.05	0.04	0.06
7	1,3-diethylbenzene	0.06	0.07	0.09	0.10	0.12	0.13	0.15	0.16	0.21	0.28
8	Indan	0.32	0.39	0.48	0.55	0.64	0.71	0.81	0.87	1.16	1.54
9	1,4-diethylbenzene/ 1,3-dimethyl-5-ethylbenzene	0.06	0.07	0.09	0.10	0.11	0.13	0.14	0.15	0.21	0.28
10	1,2-diethylbenzene	0.06	0.07	0.09	0.10	0.12	0.13	0.15	0.16	0.21	0.28
11	1,4-dimethyl-2-ethylbenzene	0.06	0.07	0.09	0.10	0.12	0.13	0.15	0.16	0.21	0.28
12	1,3-dimethyl-4-ethylbenzene	0.05	0.06	0.08	0.09	0.11	0.12	0.13	0.14	0.19	0.26
13	1,2-dimethyl-4-ethylbenzene	0.05	0.06	0.08	0.09	0.11	0.12	0.13	0.14	0.19	0.26
14	1,2-dimethyl-3-ethylbenzene	0.05	0.06	0.08	0.09	0.11	0.12	0.13	0.14	0.19	0.26
15	Hexachlorobutadiene	0.06	0.07	0.08	0.10	0.11	0.12	0.14	0.15	0.20	0.26

For preparing calibration standards a calculated volume of either Mix I or Mix II or Mix III standard solutions were added into a vial containing 15 ml ultra pure water. The vial was then sealed with magnetic yellow crimp caps with natural rubber orange and vortexed for 10 seconds before placing it on the tray of the HS-SPME-GC-MS instrument for sample extraction followed by GC injection. In the chromatographic column of the GC instrument the compounds are separated and the eluted compounds are detected by the mass spectrometer.

First attempt of calibration was made with 18 calibration standards. However, after an initial run it was learnt that none of the compounds would produce linearity for such a big range and some calibration standards in both the ends were removed for each compound. Later, a narrow working range was determined for each compound which is shown in the table above (Table 9). The working range of concentration was divided into 10 calibration standards, named P9 to P18. In Table 9 the working range started with P9, the

lowest calibration concentration, because concentrations lower than P9 did not fall into the linear range of the calibration curve and were not used.

2.5.3. Standard preparation for repeatability, intermediate precision and recovery studies

Repeatability, intermediate precision, and recovery studies were carried out at two levels of concentrations; the lowest and highest calibration standards (Table 10). These standards were prepared the same way it was done for calibration standards. For repeatability and intermediate precision the standards were added into the vial containing ultrapure water. Recovery studies were carried out for tap water, river water and groundwater. For recovery studies 15 ml of the three types of matrix samples were poured into 20 ml vials, spiked with the standards and injected. A minimum of 7 and a maximum of 10 individual samples were used for each matrix.

The results of HS-SPME/GC-MS analysis of standards and samples and other information stored in the associated computer were retrieved on a hard copy. The data from the hard copy were typed in PC and processed. Information related to linearity, working range, LOD, LOQ, statistical tests and recovery was calculated by using appropriate software already available in the EPAL laboratory whereas precision data were manually processed by using excel spread sheet. Statistical tools like, Least square regression, Residual distribution, Mandel's test, Standardized area test, Grubb's outlier test, hypothesis testing, correlation coefficient, coefficient of determination and relative standard deviation were used to process and analyze the data and reach at a conclusion.

Table 10: Concentrations for repeatability, intermediate precision and recovery

S.N.	Compound name	Low Concentration	High Concentration
		µg/L	µg/L
1	MTBE	4.9	13.3
2	3-ethyltoluene	0.058	0.277
3	4-ethyltoluene	0.068	0.275
4	2-ethyltoluene	0.059	0.284
5	1,2,4-trimethylbenzene	0.058	0.28
6	4-isopropyltoluene	0.017	0.042
7	1,3-diethylbenzene	0.058	0.208
8	Indane	0.322	1.544
9	1,4-diethylbenzene/13DM5EBN	0.057	0.276
10	1,2-diethylbenzene	0.059	0.282
11	1,4-dimethyl-2-ethylbenzene	0.059	0.282
12	1,3-dimethyl-4-ethylbenzene	0.053	0.256
13	1,2-dimethyl-4-ethylbenzene	0.053	0.256
14	1,2-dimethyl-3-ethylbenzene	0.053	0.256
15	Hexachlorobutadiene	0.067	0.198

2.6. Solid-phase microextraction conditions

A robotic automatic head-space sampler was used to inject samples into injection port of gas chromatograph. Following SPME conditions were used for sample preparation. Gray fiber with DVB/CAR/PDMS (divinyl benzene, carboxen and polydimethylsiloxane) stationary phase was used for adsorption of the analytes.

Preincubation time	15 min
Incubation temperature	40^{o}C
Agitation speed	250 rpm
Agitation time	1 min
Extraction time	1 min
Desorption time	5 min

2.7. Gas chromatographic conditions

A splitless injector was used. Total run time was 20 min. Injector temperature was 200^{o}C.

Table 11: GC temperature profile

Oven ramp	Column temperature ^{o}C	Hold time (min)
^{o}C/min	35	0
15	160	0
20	250	7

2.8. Mass spectrometric condition

Detector type	MSD
Source temperature	230^{o}C
Quadrupole temperature	150^{o}C
Type of ionizaition	EI
Ionization energy	70ev

III. Results and Discussion

This study was conducted for validation of an assay method that is intended to be applied for identification and quantification of VOCs in different water matrices, namely, drinking water, surface water and groundwater using HS-SPME-GC/MS. This method has been used for the analysis of VOCs in numerous research studies for many years and has been the prescribed method of some official standard methods also. The VOCs included in this study can be used as an indicator of petroleum product contamination if detected in water samples and are potentially or possibly harmful to human health. Method validation included selectivity, linearity and working range, limit of detection, limit of quantification, precision and accuracy.

3.1. Selectivity

An in-house developed SPME, GC-MS conditions were applied in all of the experiments carried out in this study. The in-house established conditions have been already regarded as the most applicable one for the analysis of VOCs at the central laboratory of EPAL, Lisbon, where this research was conducted. The conditions used in this study have been given in 'Experimental Procedure' of this thesis. Interestingly, the conditions are even effective in separating isomers as many of the studied compounds are isomers. To begin with, arbitrarily, each of the original standards (procured from the vendor) were diluted to 20 μg/L and injected into GC one at a time in Full scan mode which scans all the compounds present in the sample.

In Full scan mode several peaks appeared in the chromatogram. The peaks were compared with NIST database. Except one, all the other peaks were regarded as impurity and they were either already present in ultra pure water or came from column bleeding. In most of the cases the tallest peak was the compound of interest which showed a specific retention time in the chromatogram. Normally, it is assumed that different compounds have different retention times during separation of the compound in gas chromatograph. Mass spectrometric detection also showed mass spectrum of them. Each

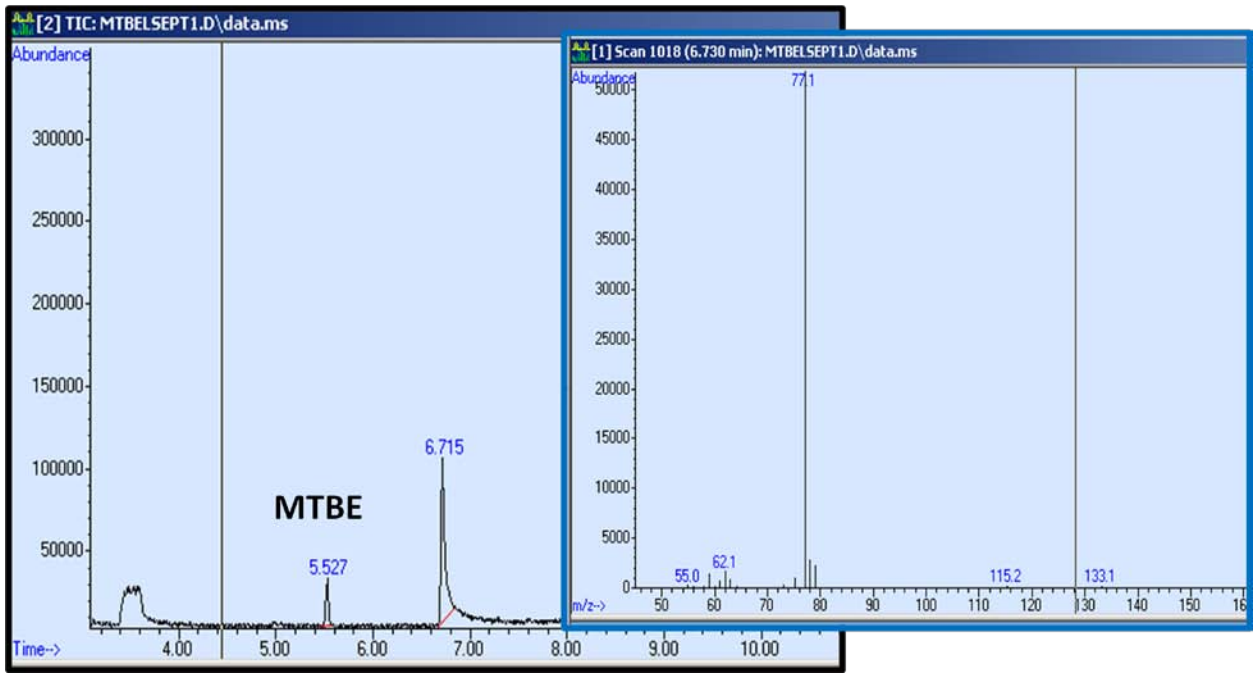
compound has its signature mass fragments (m/z) which is used to confirm its identification (Fig 7). Of all the fragments, 3 tallest or most abundant mass fragments were selected as the representative mass fragments for a compound. Matching retention time and mass fragments confirmed the compound of interest (Table 12).

SPME itself also participates in selectivity of a compound. Head-space SPME can adsorb only volatile compounds and thus separates them from non-volatile ones. Different stationary phase fibers used in SPME can select different compounds based on their polarity. For the selected 16 compounds DVB/CAR/PDMS stationary phase used in this study seems to be an appropriate one.

Table 12: Retention time and representative mass ions for each compound

S.N	Compound	Retention time tR	Representative ions		
1	MTBE	5.527	73.1	57.2	
2	3-ethyltoluene	11.091	105.1	120.1	91.1
3	4-ethyltoluene	11.133	105.1	120.1	91.1
4	2-ethyltoluene	11.353	105.1	120.1	91.1
5	1,2,4-trimethylbenzene	11.53	105.1	120.1	77.1
6	4-isopropyltoluene	11.767	119.1	134.2	91.1
7	1,3-diethylbenzene	11.994	119.1	105.1	134.2
8	Indane	12.051	117.1		91.1
9	1,4-diethylbenzene	12.088	119	105	134
10	1,3-dimethyl-5-ethylbenzene	12.107	119	134	91
11	1,2-diethylbenzene	12.195	105.1	119.1	134.2
12	1,4-dimethyl-2-ethylbenzene	12.357	119.1	134.2	91.1
13	1,3-dimethyl-4-ethylbenzene	12.398	119.1	134.2	91.1
14	1,2-dimethyl-4-ethylbenzene	12.45	119.1	134.2	91.1
15	1,2-dimethyl-3-ethylbenzene	12.719	119.1	134.2	91.1
16	Hexachlorobutadiene	13.802	225	190	260

Note: Retention time changed for the same compound in the different times of the study period because of the change brought by column repair or replacement.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MTBE
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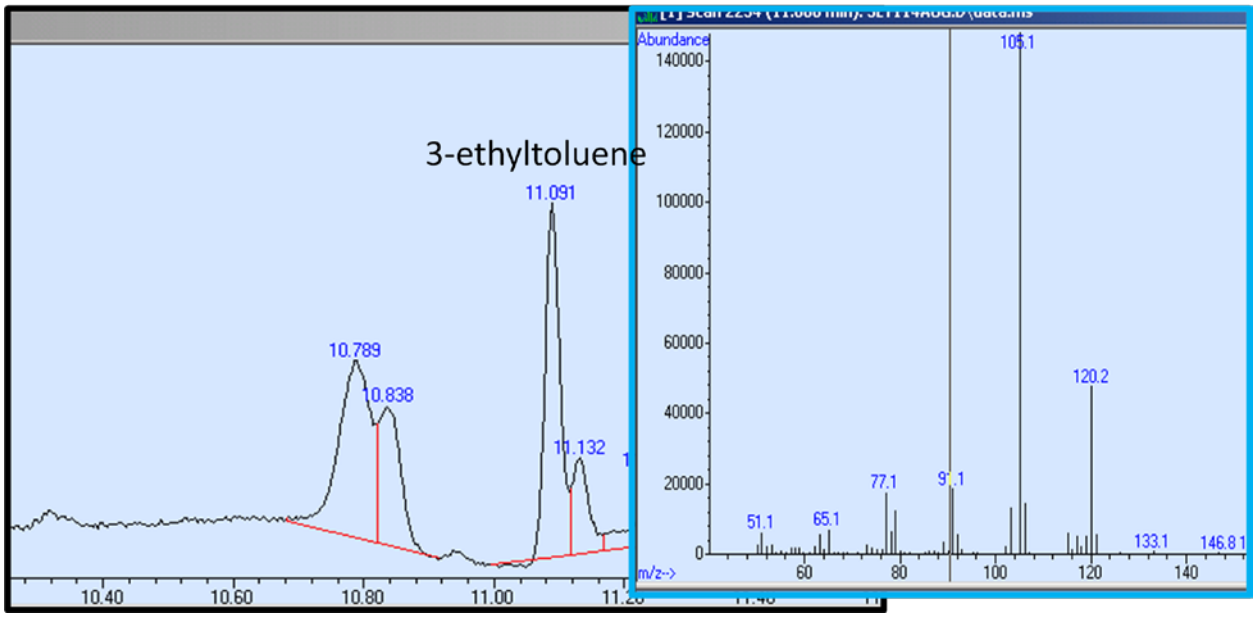
62.1
55.0
115.2
133.1
m/z-->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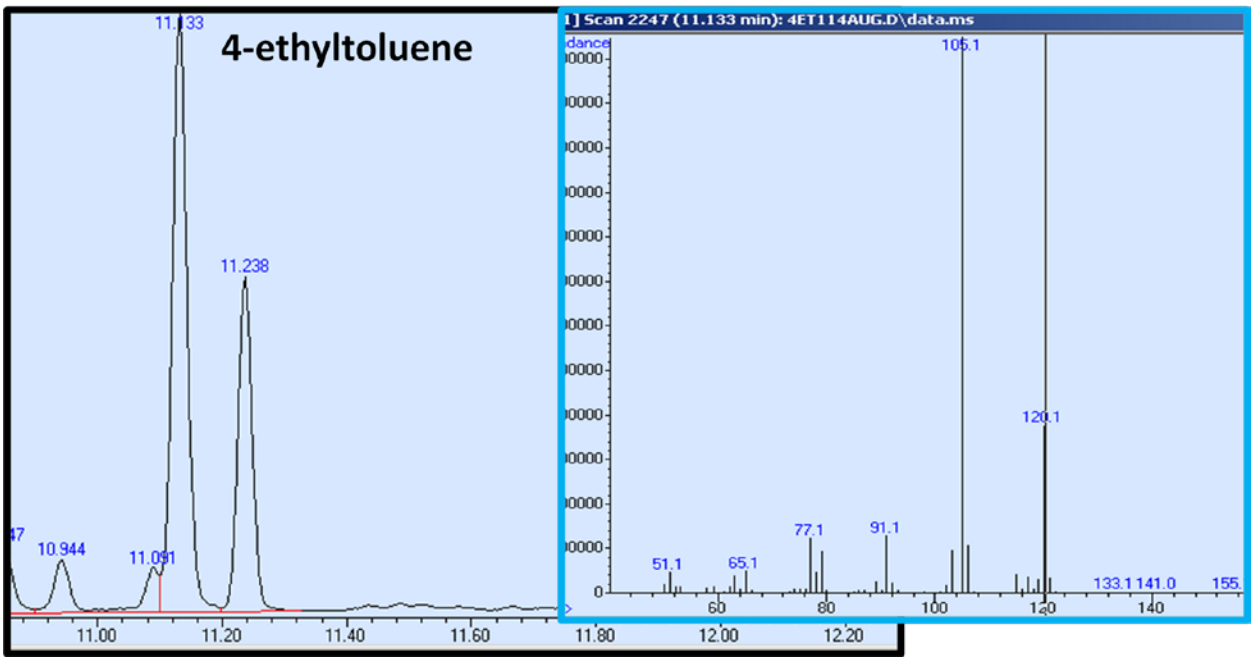
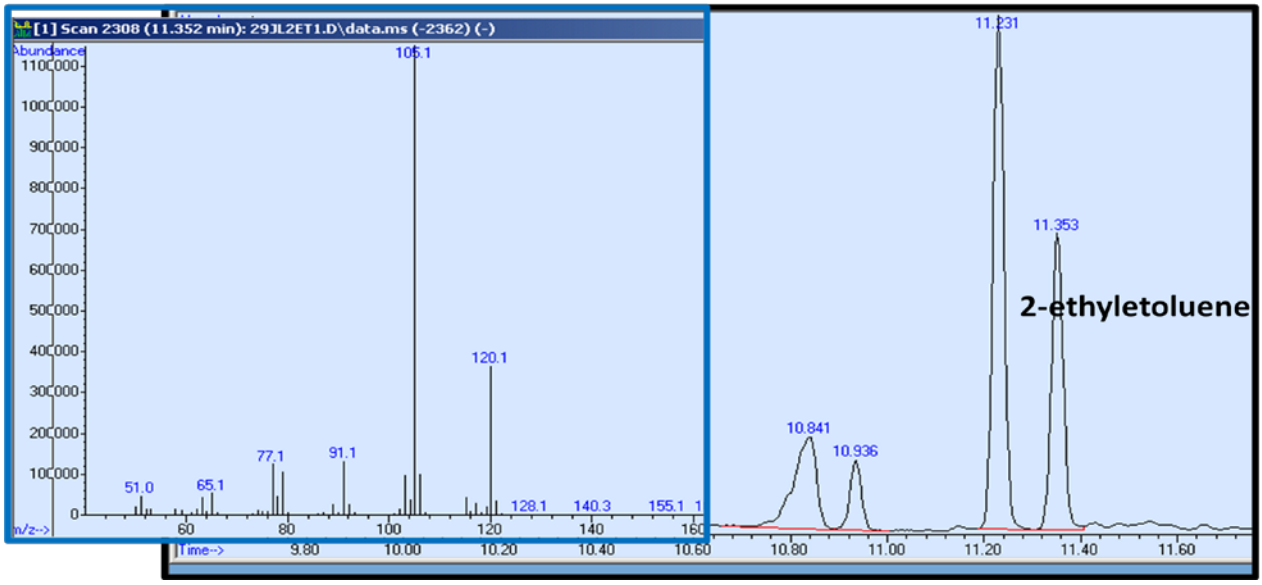
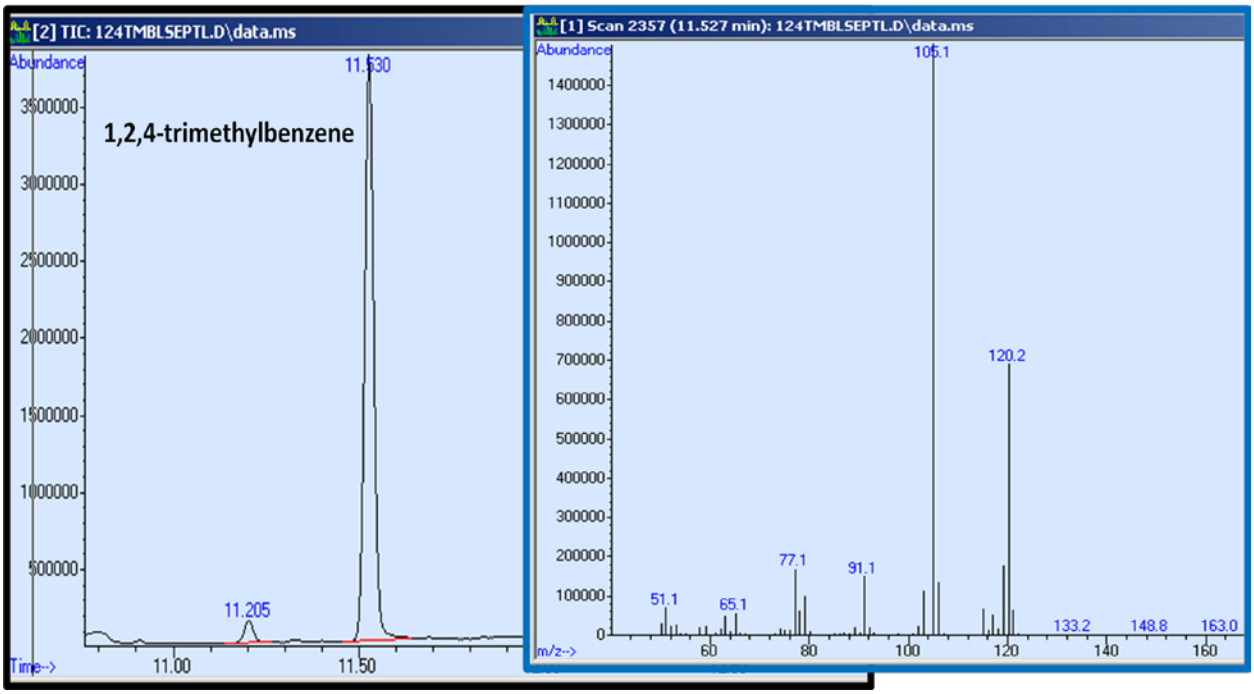
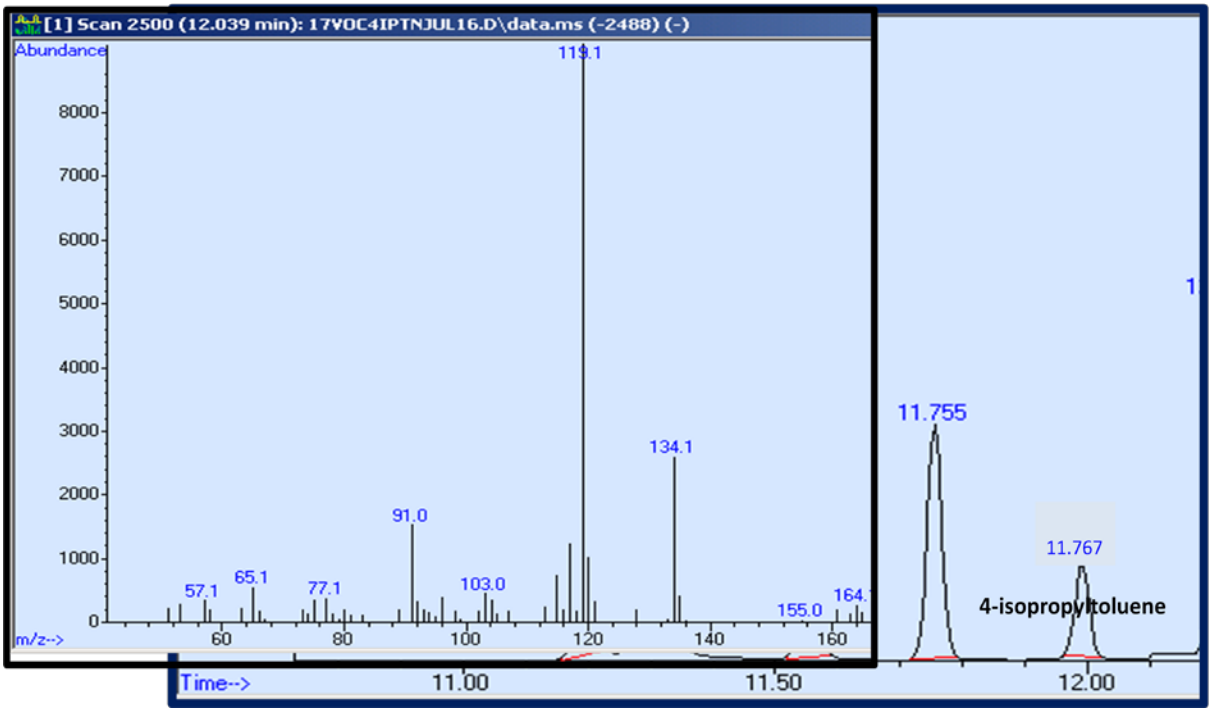
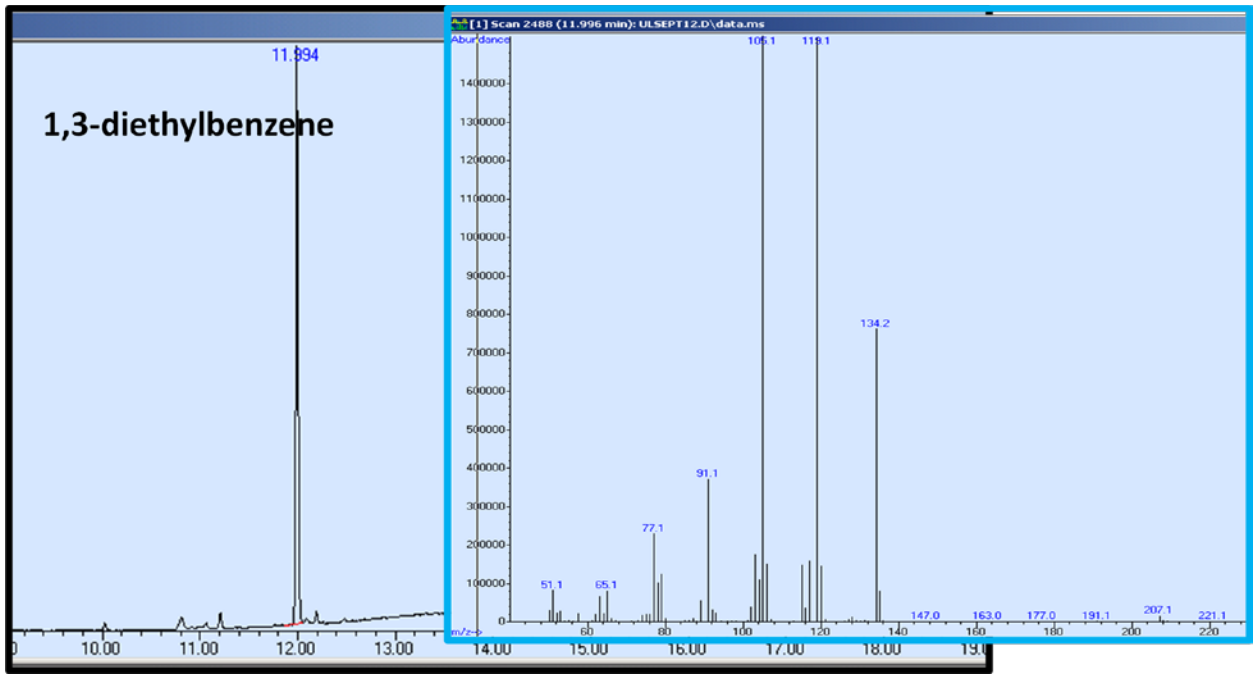
10.789
10.838
11.132
Abundance
105.1
120.2
77.1
51.1
65.1
133.1
146.81
m/z-->

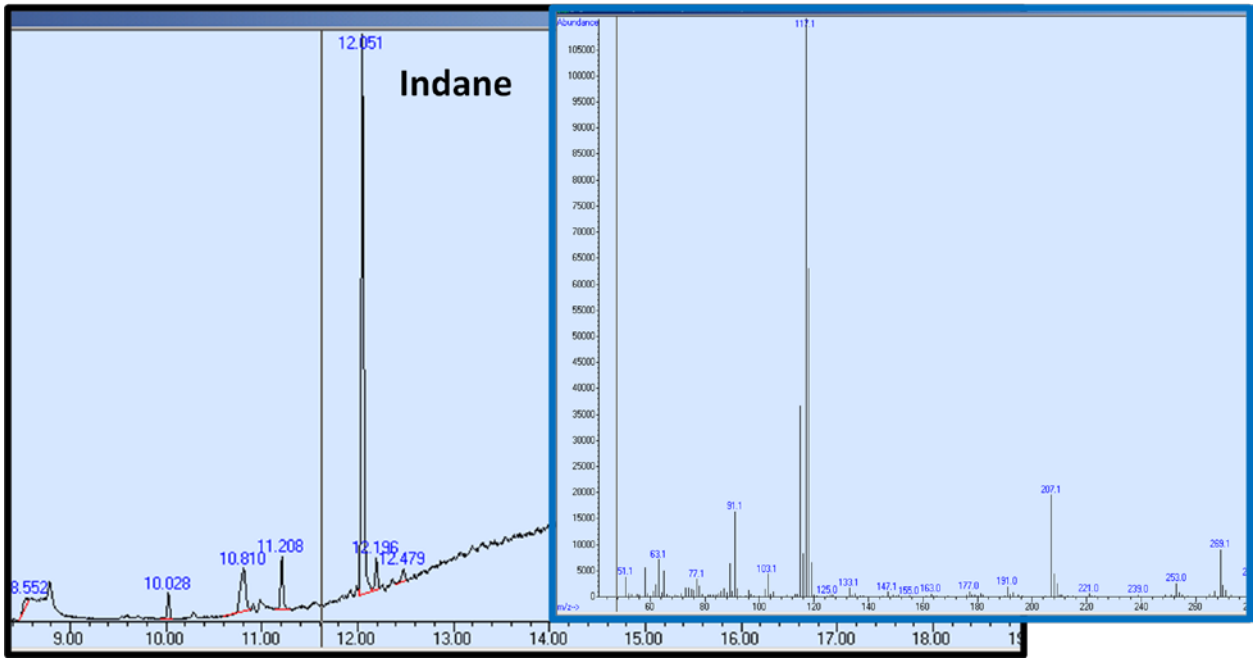

4-ethyltoluene
11.133
11.238
10.944
11.091
1] Scan 2247 (11.133 min): 4ET114AUG.D\data.ms
105.1
120.1
77.1
91.1
51.1
65.1
133.1
141.0
155.

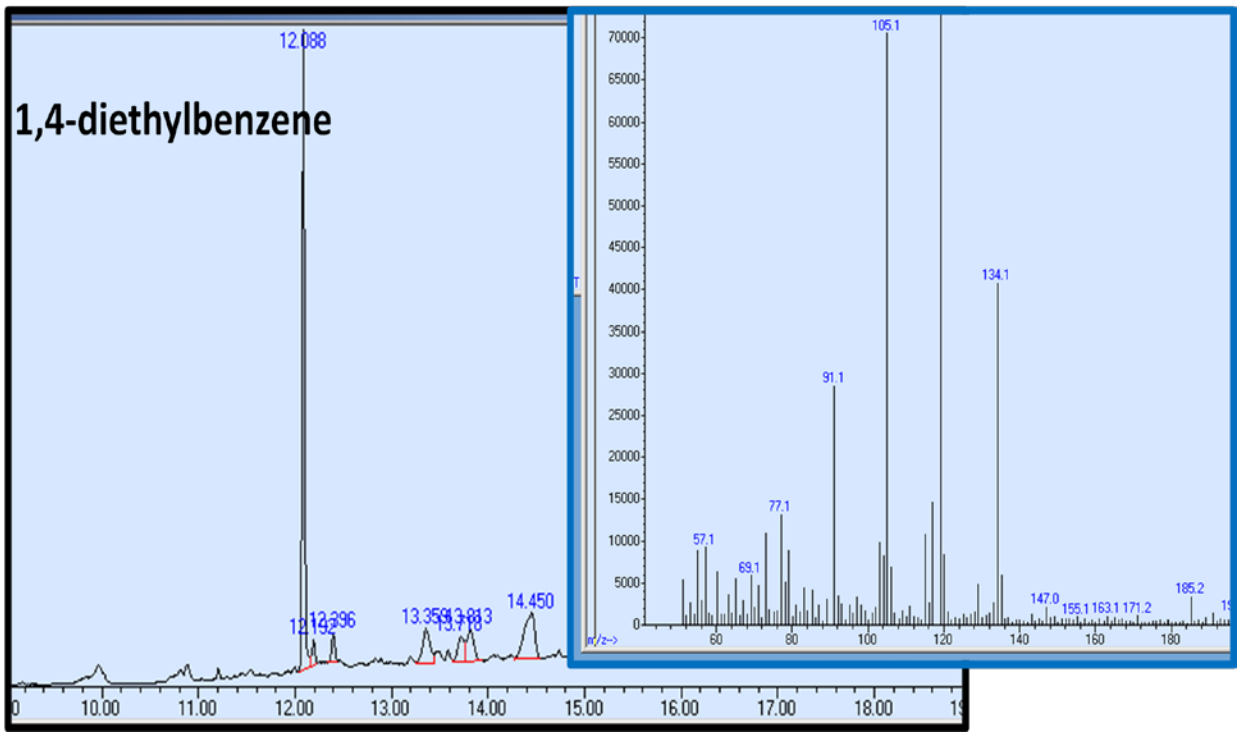

[1] Scan 2308 (11.352 min): 29JL2ET1.D\data.ms (-2362) (-)
105.1
120.1
77.1
91.1
51.0
65.1
128.1
140.3
155.1
11.231
11.353
2-ethyletoluene
10.841
10.936

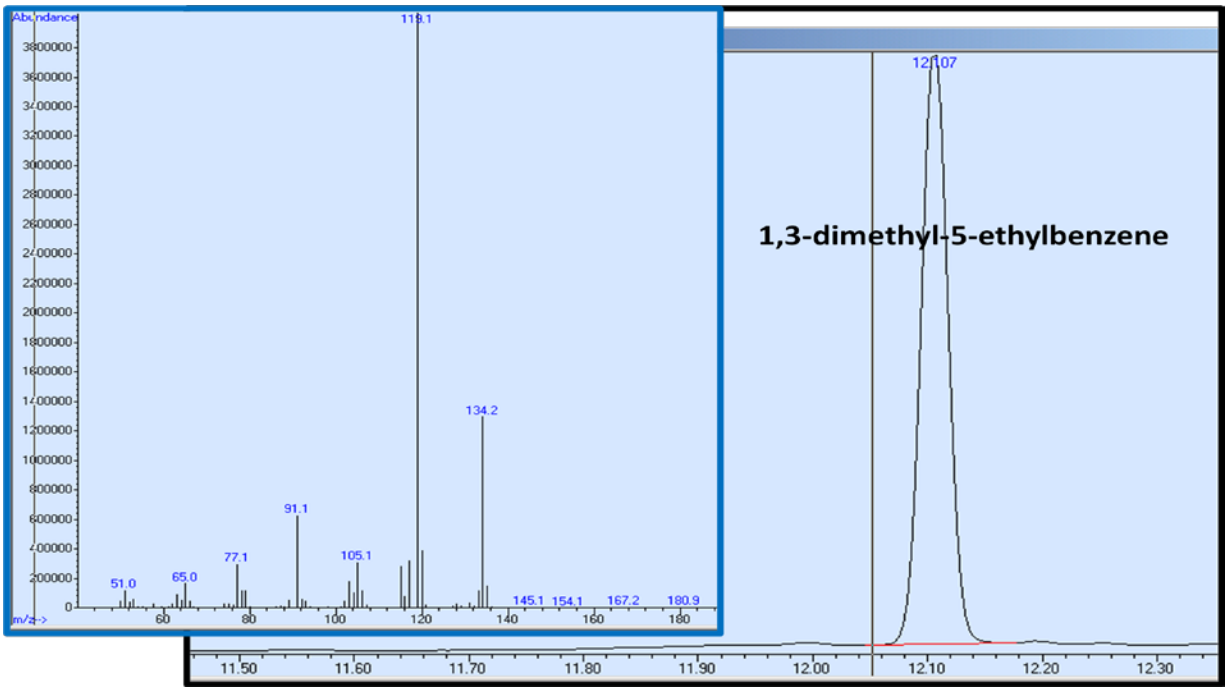

[2] TIC: 124TMBLSEPTL.D\data.ms
Abundance
11.530
1,2,4-trimethylbenzene
11.205
Time-->
11.00
11.50
[1] Scan 2357 (11.527 min): 124TMBLSEPTL.D\data.ms
Abundance
105.1
120.2
77.1
91.1
51.1
65.1
133.2
148.8
163.0
m/z-->

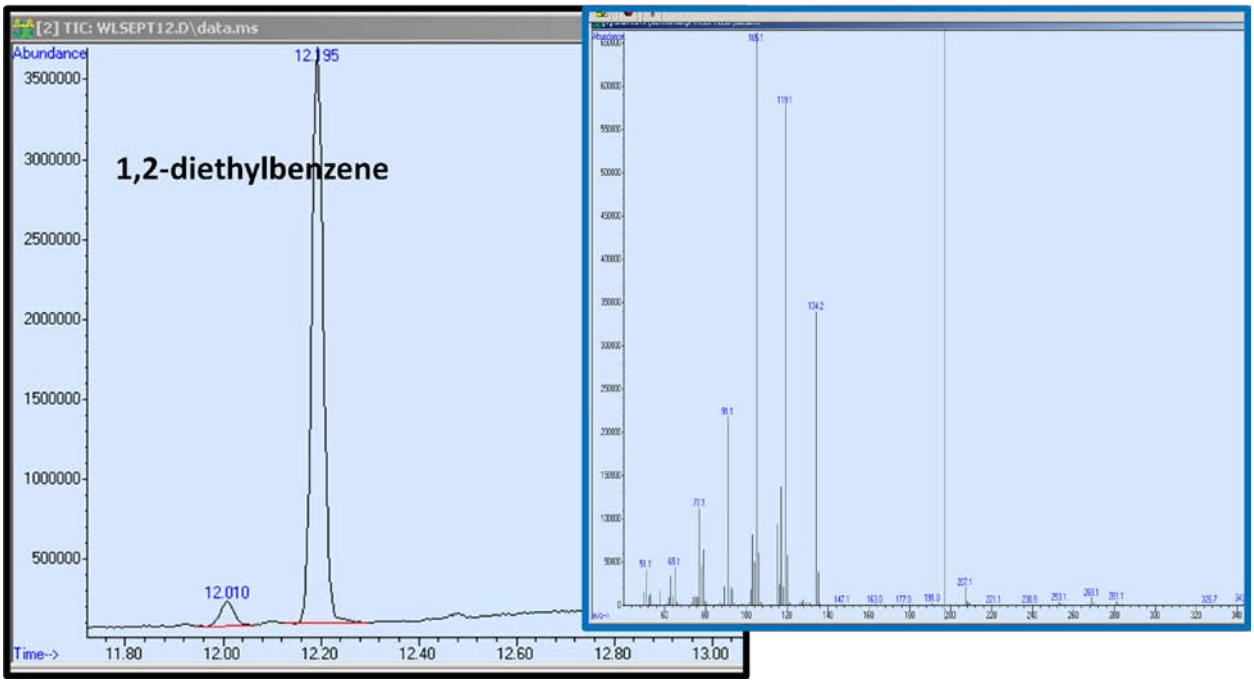

[1] Scan 2500 (12.039 min): 17VOC4IPTNJUL16.D\data.ms (-2488) (-)
Abundance
119.1
134.1
91.0
57.1
65.1
77.1
103.0
155.0
m/z-->
11.755
11.767
4-isopropyltoluene
Time-->
11.00
11.50
12.00

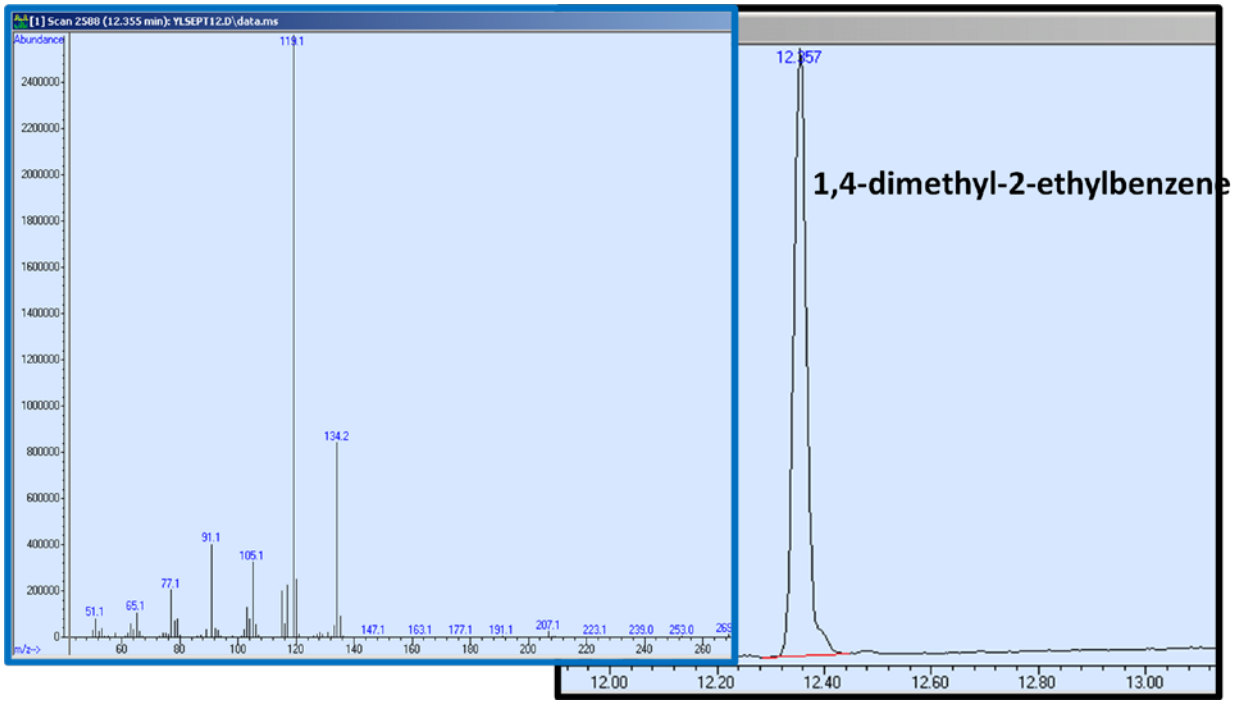

1,3-diethylbenzene
11.994
[1] Scan 2488 (11.996 min): ULSEPT12.D\data.ms
Abundance
105.1
119.1
134.2
91.1
77.1
51.1
65.1
147.0
163.0
177.0
191.1
207.1
221.1
m/z-->

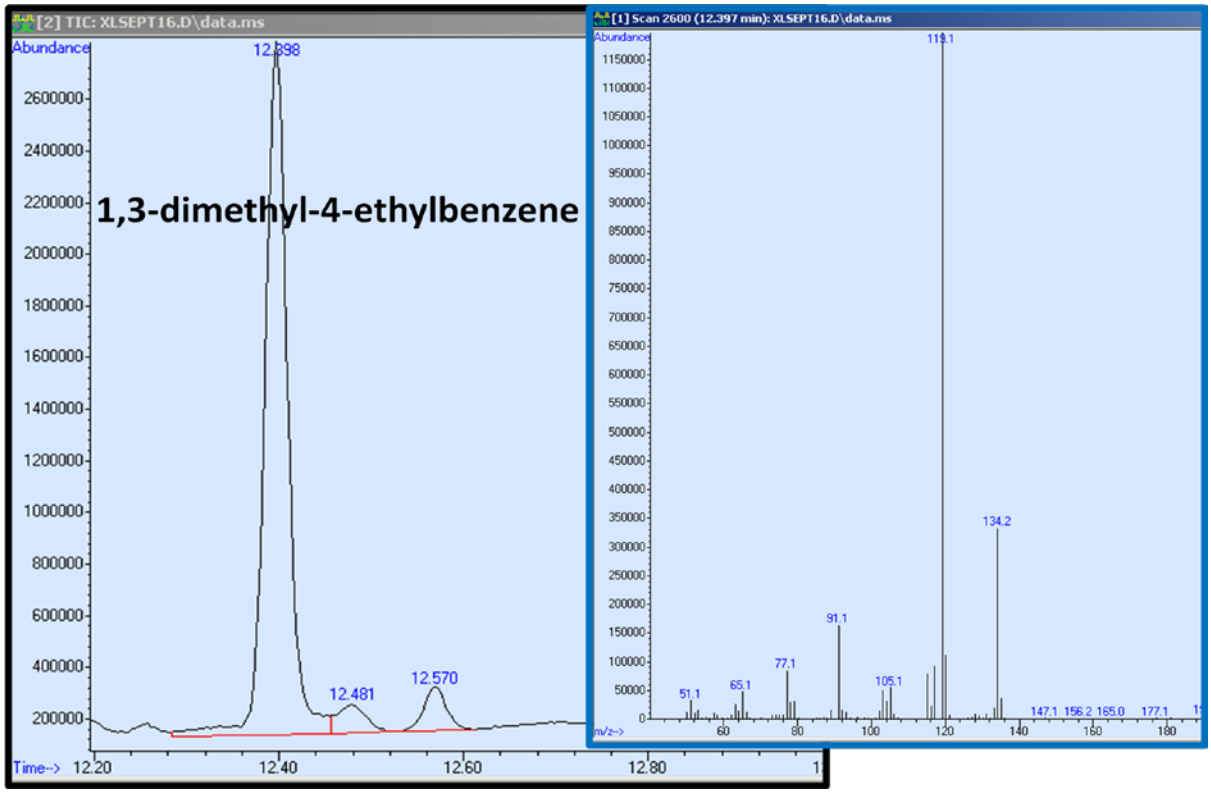

Indane
12.051
10.810
11.208
12.196
12.479
10.028
8.552
Abundance
117.1
91.1
207.1
269.1
63.1
51.1
77.1
103.1
m/z-->

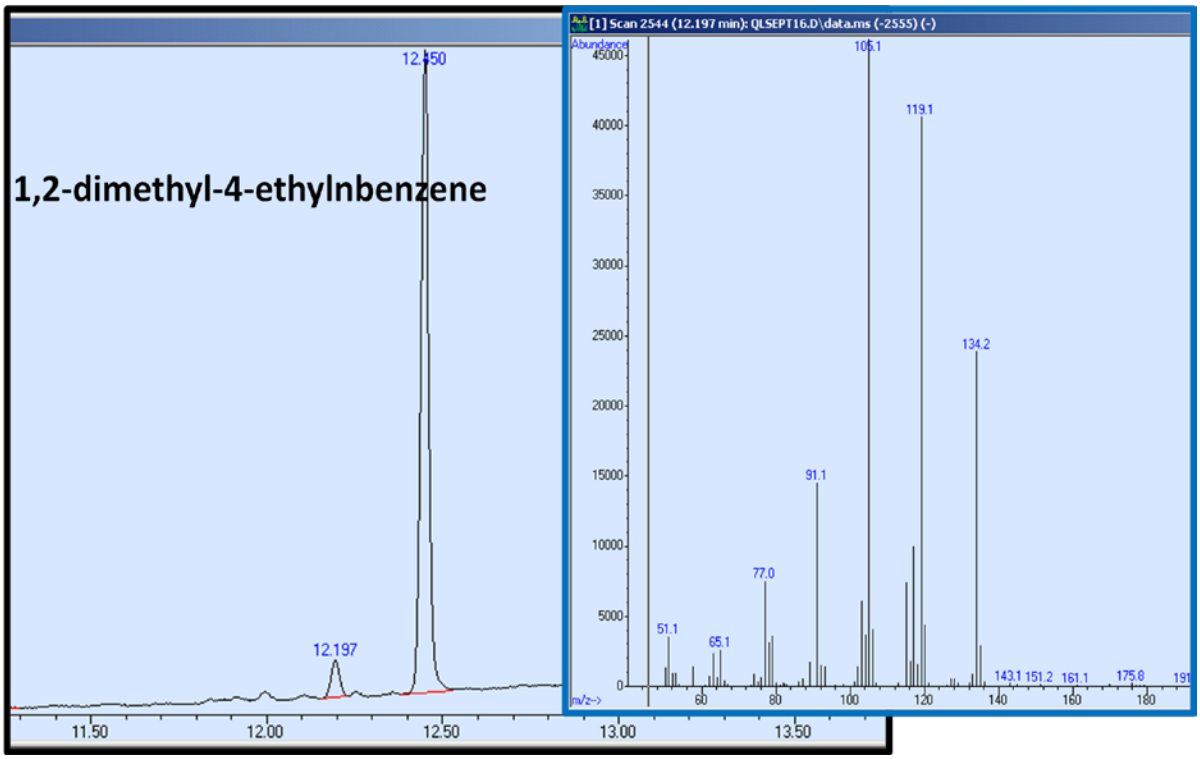

12.088
1,4-diethylbenzene
14.450
105.1
134.1
91.1
77.1
57.1
69.1
147.0
185.2

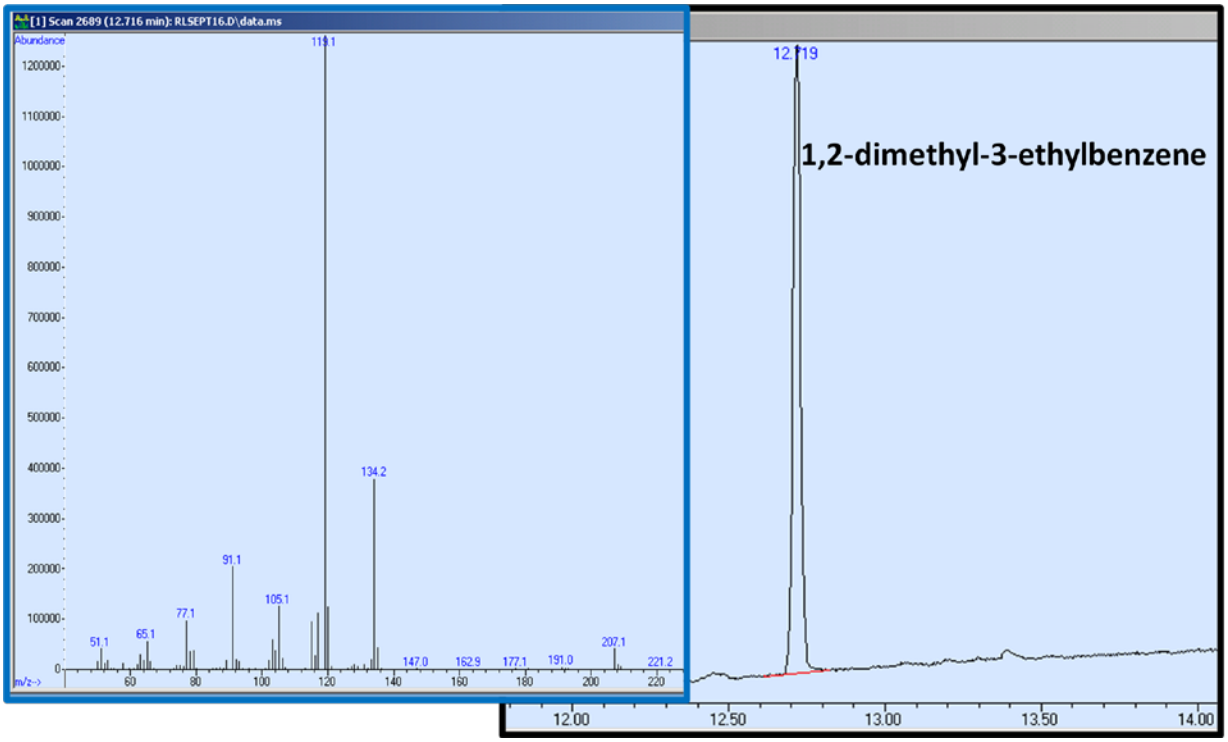

Abundance
119.1
134.2
91.1
77.1
105.1
51.0
65.0
12.107
1,3-dimethyl-5-ethylbenzene


[2] TIC: WLSEPT12.D\data.ms
Abundance
12.195
1,2-diethylbenzene
12.010
Time-->
11.80
12.00
12.20
12.40
12.60
12.80
13.00


[1] Scan 2588 (12.355 min): YLSEPT12.D\data.ms
Abundance
119.1
134.2
91.1
105.1
77.1
51.1
65.1
m/z-->
12.357
1,4-dimethyl-2-ethylbenzene
12.00
12.20
12.40
12.60
12.80
13.00


[2] TIC: XLSEPT16.D\data.ms
Abundance
12.398
1,3-dimethyl-4-ethylbenzene
12.481
12.570
Time-->
12.20
12.40
12.60
12.80
[1] Scan 2600 (12.397 min): XLSEPT16.D\data.ms
Abundance
119.1
134.2
91.1
77.1
105.1
51.1
65.1
m/z-->


12.450
1,2-dimethyl-4-ethylnbenzene
12.197
11.50
12.00
12.50
13.00
13.50
[1] Scan 2544 (12.197 min): QLSEPT16.D\data.ms (-2555) (-)
Abundance
105.1
119.1
134.2
91.1
77.0
51.1
65.1
m/z-->



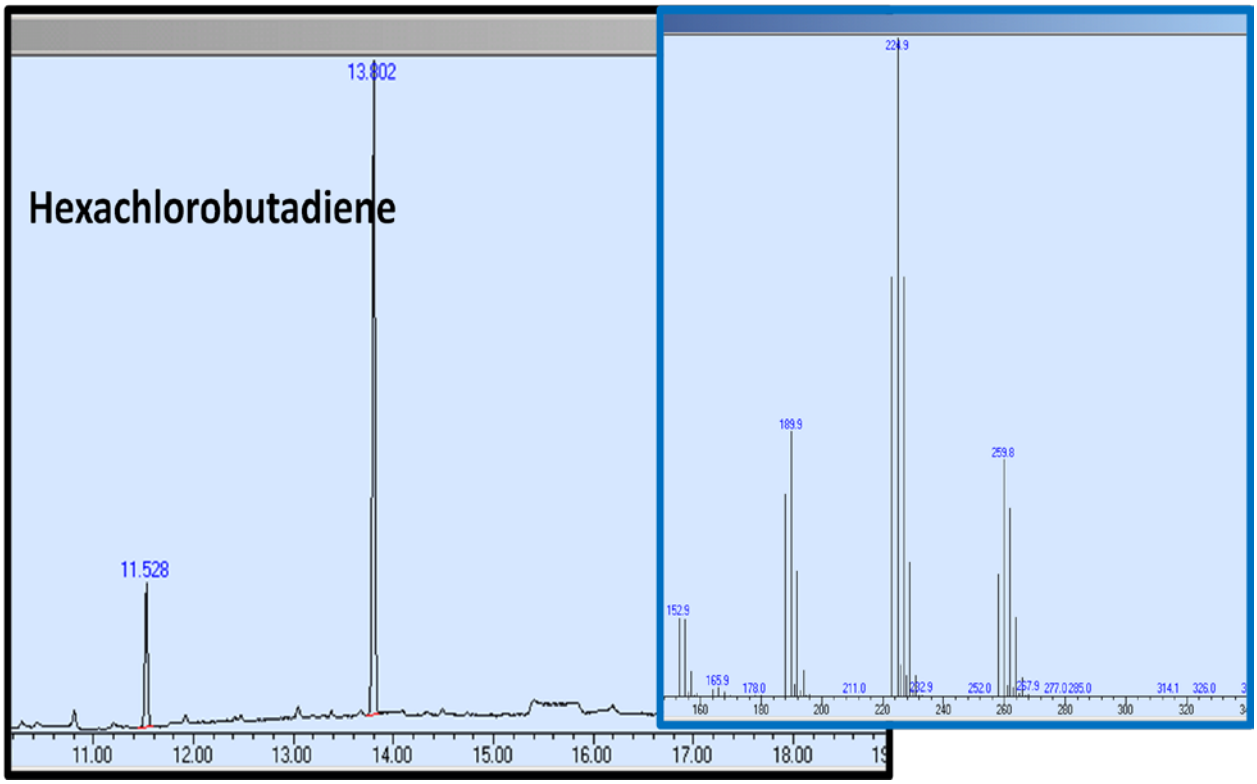


Figure 7: Individual chromatogram and mass spectrum of the VOCs

From here on, the MS was operated in SIM mode instead of FS mode. In SIM mode, in order to facilitate mass analyzer to select given masses only, a program consisting of separate window groups of the masses were designed. The program allows the MS to select the specified mass only which can pass through its mass analyzer and therefore detects only those ion masses (Table 13). In SIM mode the unwanted compounds were mostly undetected.

Table 13: Retention time window, the ion masses and the compounds

Group	Retention time window (min)	ions m/z	Compounds	Retention time (min)
1	3	57, 73	MTBE	5.7
2	8	65, 91, 105, 120	3-ethyltoluene	11.29
			4-ethyltoluene	11.33
			2-ethyltoluene	11.56
3	11.3	77, 105, 120	1,2,4-trimethylbenzene	11.76
4	11.5	91, 119, 134	4-isopropyltoluene	11.99
5	11.8	91, 105, 117, 118, 119, 134	1,3-diethylbenzene	12.216
			Indane	12.220
			1,4-diethylbenzene	12.32
			1,3-dimethyl-5-ethylbenzene	12.32
			1,2-diethylbenzene	12.42
6	12.25	91, 119, 134	1,4-dimethyl-2-ethylbenzene	12.58
			1,3-dimethyl-4-ethylbenzene	12.62
			1,2-dimethyl-4-ethylbenzene	12.67
			1,2-dimethyl-3-ethylbenzene	12.94
7	13.2	190, 225, 260	Hexachlorobutadiene	14.06

After confirmation of the compounds individually, based on retention time and mass spectrum, the compounds were investigated to find out how they would behave when injected together in a mixture. Separate mixtures of diethylbenzene, ethyltoluene and dimethylethylbenzene isomers and rest of the compounds (six) were injected in different groups in selected ion monitoring mode (SIM). Similarly, compounds were also injected in other mixture combination, for example, in a group of 12 compounds and in a group of isomers of dimethylethylbenzene only. The concentrations of compounds in the different mixtures ranged from 0.01 μg/L to 20 μg/L. In this trial and error attempt, the

concentration of compounds with larger peaks was reduced and those with smaller peaks were increased.

During all this trial and error exercise it was found that all the other compounds appear as separate peaks but indane, 1,4-diethylbenzene and 1,3-dimethyl-5-ethylbenzene masked each other in the chromatogram. Therefore, with an objective of separating these three compounds their concentrations were further manipulated and injected. It was finally determined that in the given conditions, indane can appear as a separate peak but 1,4-diethylbenzene and 1,3-dimethyl-5-ethylbenzene coelute and appear as a single peak in the chromatogram. It was then decided that one of the 15 peaks will represent both the compounds and their combined concentrations. Therefore, the complete chromatogram will show 15 peaks for 16 compounds (Fig 8a, 8b). The 'approximate' minimum concentration which was required for each compound to be detected and quantified by the chromatogram has been given in Table 4 above.

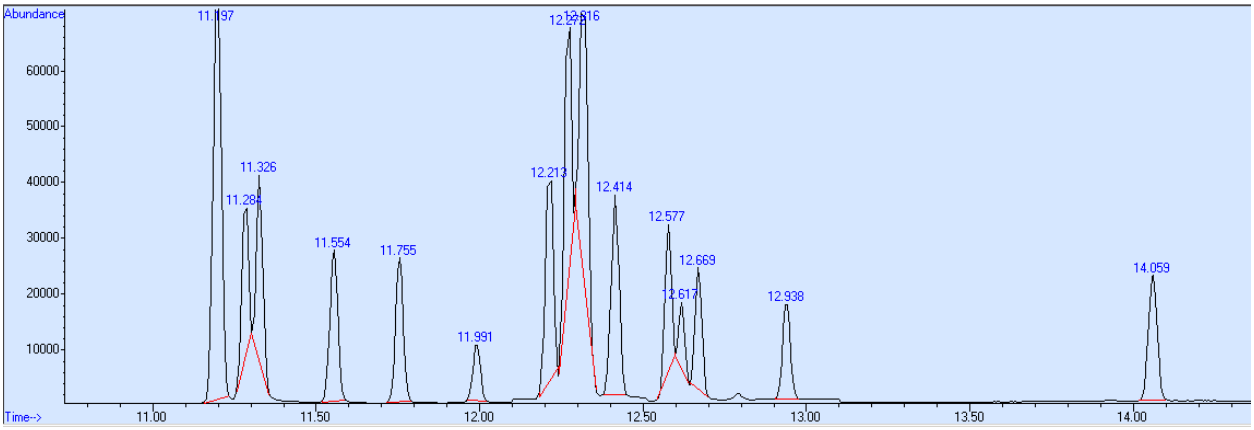


Figure 8a: Peaks of the 15 target compounds in SIM mode

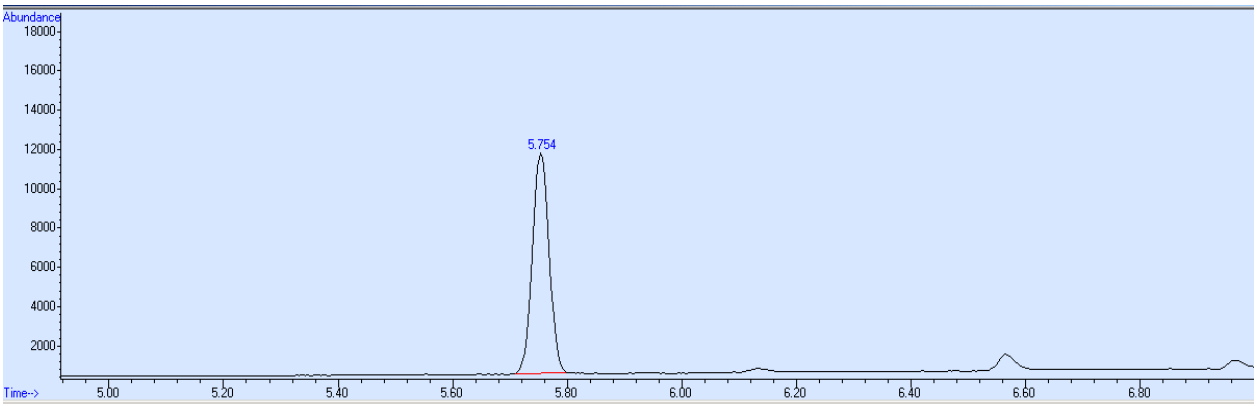


Fig 8b: Peak of MTBE

3.2. Linearity and working range

Calibration curve preparation is an indispensable function required for quantification of the VOCs using GC-MS. Linear working range was determined after trial and error from a relatively wider range of concentrations. For preparation of calibration curve 5 to 10 calibration standards were used. Measurement response given by the GC-MS was plotted against the calibration concentrations stipulated for each compound. Calibration curves for each compound have been shown in Figure 9. A summary of calibration curve information has been listed in the Table 14 given below.

Most of compounds have the lowest calibration concentration below 0.1 µg/L except for Indane which has 0.322 and MTBE which has 4.9 µg/L. Similarly, in the high end of the linearity range except for MTBE (13.3 µg/L) and Indane (1.5 µg/L) all the others have a concentration below 0.5 µg/L. It has been already mentioned earlier that 1,4-diethylbenzene and 1,2-dimethyl-5-ethylbenzene coelute. Therefore, these two compounds have been represented by one single peak and includes a sum of the concentration of the two compounds. Calibration curve was prepared using least square regression analysis. In order to provide further evidence for linearity of regression residual analysis, RIKILT test, Mandel's test, and Standardized area test were performed (Table 14).

Table 14: Calibration curve summary

S.N.	Compound name	No. of calibrations Standards	Linearity range	Coefficient of Determination	Residual analysis	RIKILT Test	Mandel's test		Standardized area test
		N	µg/L	r^2	%	%	VT	F	%
1	MTBE	6	4.9-13.319	0.993	-3.6:2.8	96-106	3.46	10.13	94-104
2	3-ethyltoluene	10	0.058-0.277	0.997	-6.0:6.0	93-108	3.76	5.59	96-109
3	4-ethyltoluene	5	0.057-0.143	0.978	-4.1:2.4	79-140	2.18	18.51	66-110
4	2-ethyltoluene	9	0.059-0.284	0.995	-6.2:7.8	91-110	1.83	5.99	90-109
5	1,2,4-trimethylbenzene	9	0.058-0.280	0.998	-3.4:4.8	91-107	1.44	5.99	89-105
6	4-isopropyltoluene	7	0.017-0.043	0.997	-3.4:4.6	88-107	-3.99	7.71	91-110
7	1,3-diethylbenzene	8	0.058-0.208	0.995	-4.3:6.4	84-109	1.22	6.61	84-109
8	Indane	8	0.322-1.544	0.998	-7.6:6.2	93-107	2.31	6.61	99-109
9	1,4-diethylbenzene/ 1,3-dimethyl-5-ethylbenzene	9	0.057-0.276	0.997	-6.7:5.6	89-108	1.42	5.99	92-112
10	1,2-diethylbenzene	9	0.059-0.282	0.997	-6.2:6.2	93-109	4.04	5.99	93-109
11	1,4-dimethyl-2-ethylbenzene	9	0.059-0.282	0.999	-8.4:4.3	91-104	1.95	5.99	96-110
12	1,3-dimethyl-4-ethylbenzene	6	0.053-0.144	0.996	-4.4:6.2	89-109	1.57	10.13	88-108
13	1,2-dimethyl-4-ethylbenzene	9	0.053-0.256	0.998	-4.4:4.6	90-109	1.31	5.99	94-113
14	1,2-dimethyl-3-ethylbenzene	7	0.053-0.320	0.999	-2.3:1.9	96-103	2.43	7.71	94-102
15	Hexachlorobutadiene	8	0.067-0.198	0.998	-4.6:2.2	94-106	5.1	6.61	98-111

3.2.1. Coefficient of determination r^2

In general, linearity is evaluated on the basis of coefficient of determination or r^2. An r^2 value of 0.990 or higher would indicate that the regression curve is linear. Of the 15 curves prepared in this study, all the compounds meet the criterion. A minimum of 5 calibration standards are needed to construct a calibration curve and all the calibration curves meet this criterion.

3.2.2. Residual analysis

Many believe that high coefficient of determination value and random residual distribution confirms the linearity of a curve. For all the compounds the residual distribution has been found random. Therefore, linearity of all the compounds meets this criterion. The plot of residual distribution has been given in annex (Annex I).

3.2.3. Mandel's test

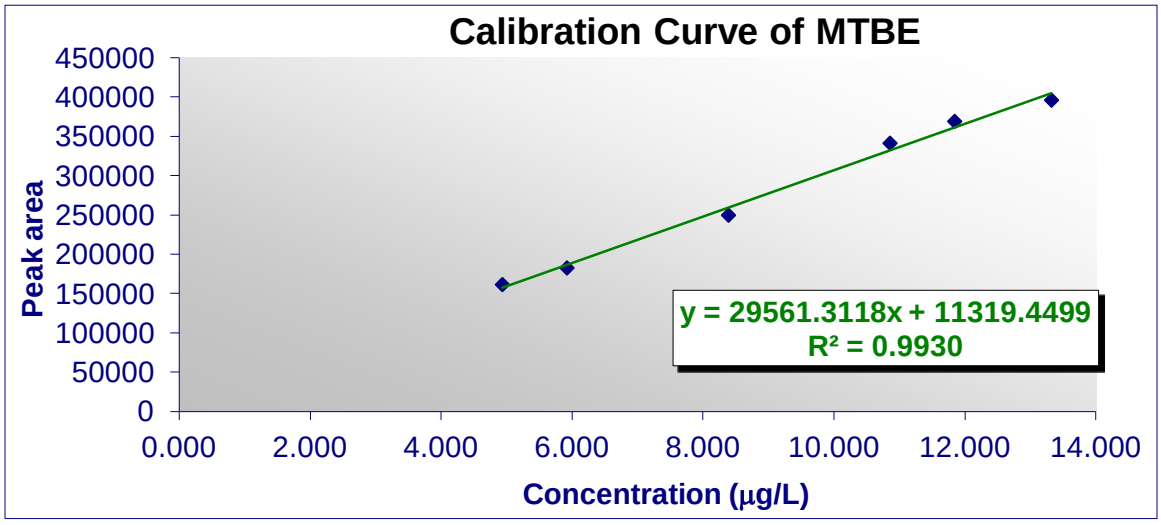
Many scholars are of the opinion that r^2 value alone may not be enough to ascertain linearity of a calibration curve. Mandel's test of residual variance can provide further evidence for or against linearity. An ISO reference for Mandel's test is ISO 8466-1:1990E. In Mandel's test residual variance of linear regression is compared with that of non-linear function through hypothesis testing (Annex II). All the calculated statistic values (VT) are smaller than F critical values and there is no significant difference between the residual variance of linear and non-linear curve. Therefore, according to Mandel's test all the 15 regression models fit linearity. For all the compounds, which have both r^2 value ≥ 0.990 and pass Mandel's test, the linearity is confirmed.

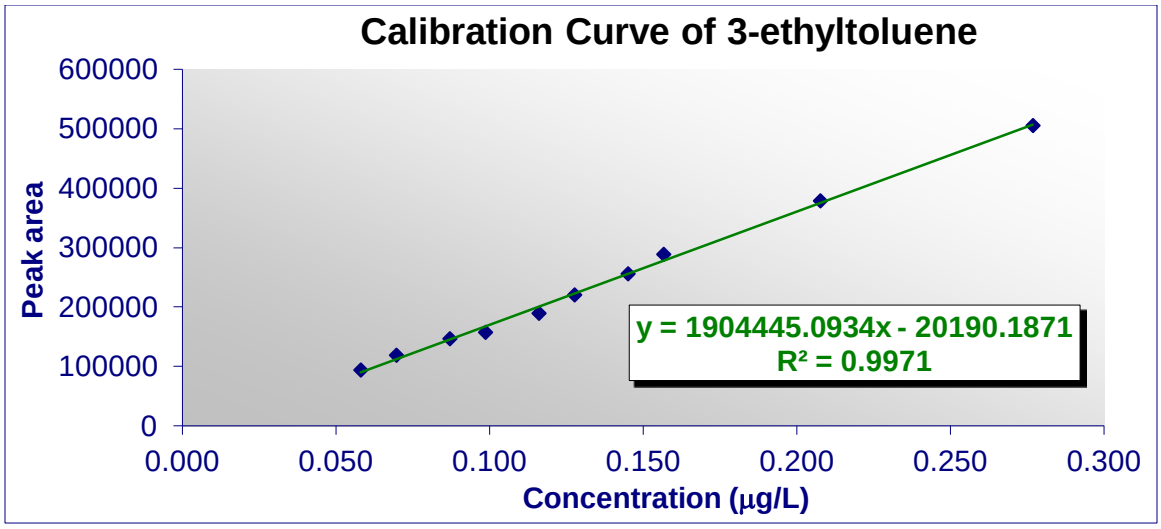
3.2.4. RIKILT test

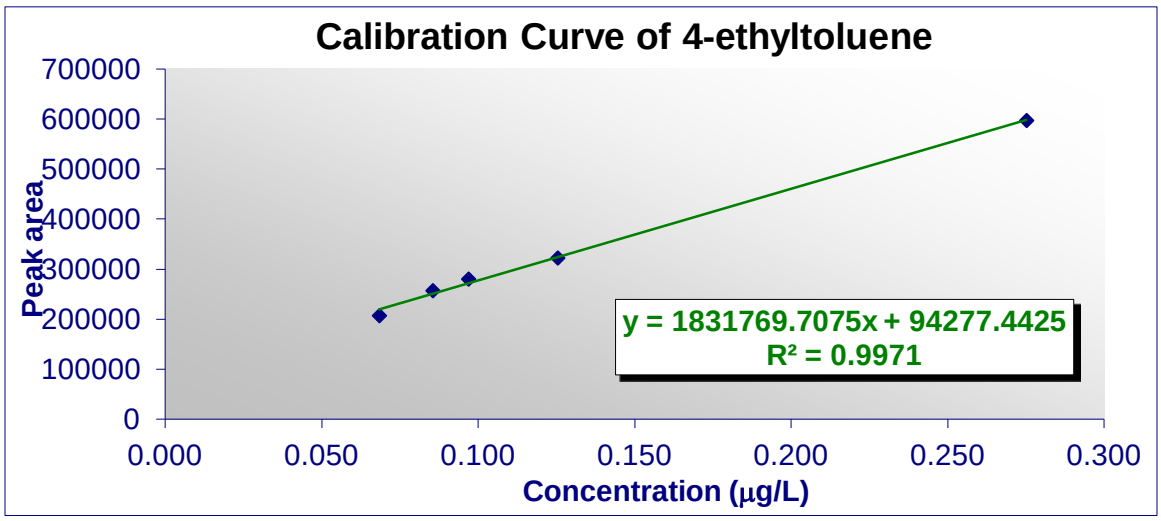
Chromatographic analysis of samples and standards can take significantly long time period. Therefore, it would be advisable to seek a valid single factor to determine sample concentration instead of using the calibration curve. RIKILT test tells us whether a single response factor, a ratio of concentration and response area (of the smallest calibration standard), can be used instead of the calibration curve for determination of the concentration of a sample analyte. The test has defined that the percentage of ratio of area and concentration for all the calibration points should fall between 90 and 110% for a response factor to be valid and applied (Annex III). For 4-ethyltoluene (79%) and 1,3-diethylbenzene (84%) the RIKILT percentage value violated the lower limit of 90% by a significant margin. Therefore, for these compounds calibration curve function may be used instead of response factor to determine unknown sample concentration or more investigation should be carried out on linearity.

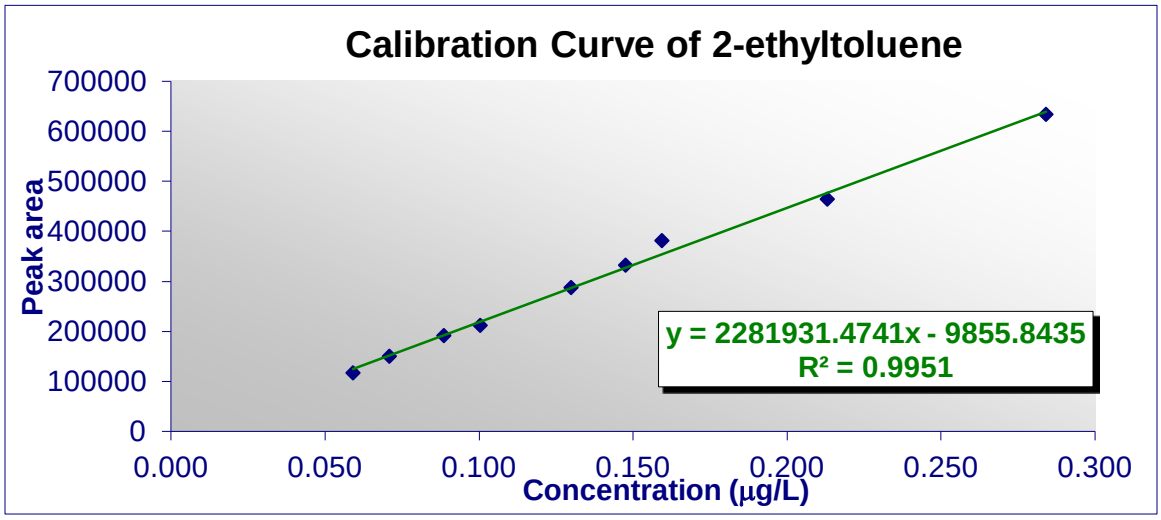
3.2.5. Standardized (Normalized) area test

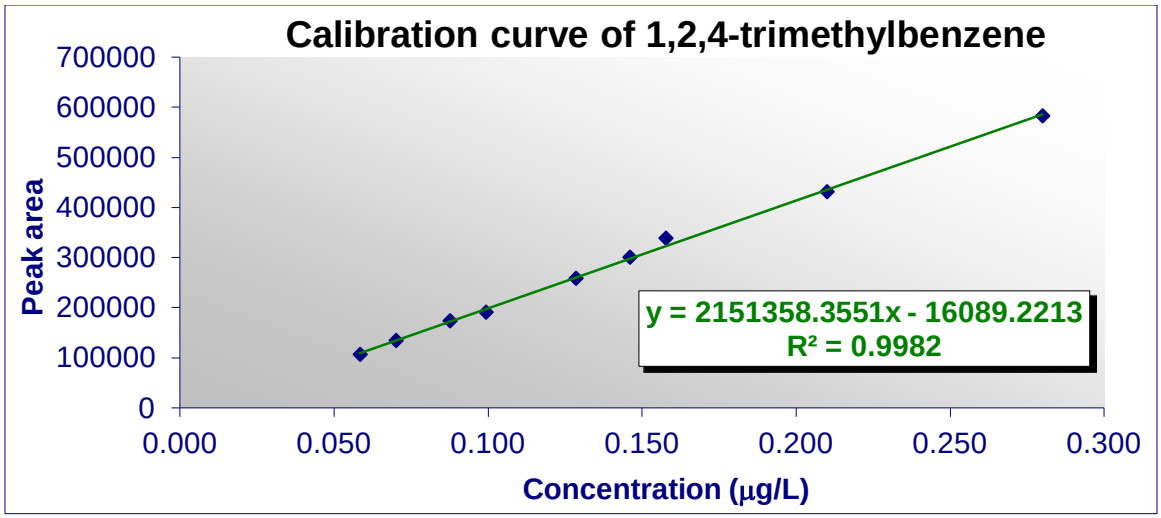
The regression equation tells us that what exact response areas should be for any concentration within the linear range. However, most of the times not all the experimental points fall exactly on the regression line. They spread around the line. The objective of the Standardized area test is to compare each of the experimental values with the best experimental (reference) value obtained among the calibration standards. The standardized values are plotted against the concentration of the standards (Annex IV). The values are then examined for their spread compared to the best experimental value. The test defines an acceptable range of 85% to 115% within which the standardized areas should fall. Standardized values for all the compounds except for 4-ethyltoluene and 1,3-diethylbenzene fall within 85 and 115%. For 1,3-diethylbenzene the lower limit value is 84% which is just below the threshold value of 85%. However, 4-ethyltoluene has exceeded the lower limit by a wider margin. Additional investigation may be recommended for 4-ethyltoluene on linearity according to the Standardized area test. In both the compounds the upper limit values are within the prescribed limit.

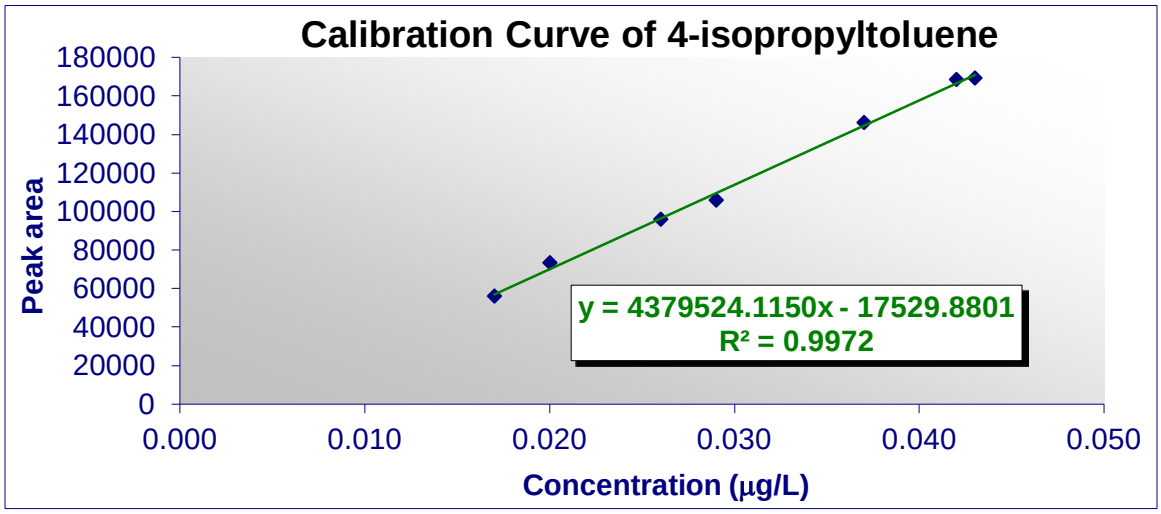


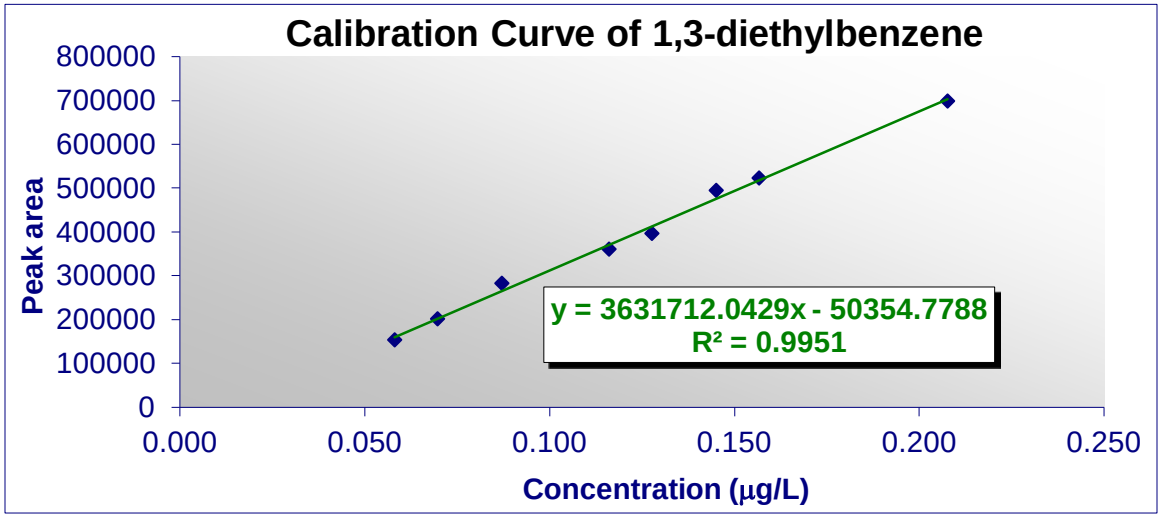

Calibration Curve of 3-ethyltoluene
Peak area
600000
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
Concentration (µg/L)
y = 1904445.0934x - 20190.1871
R² = 0.9971

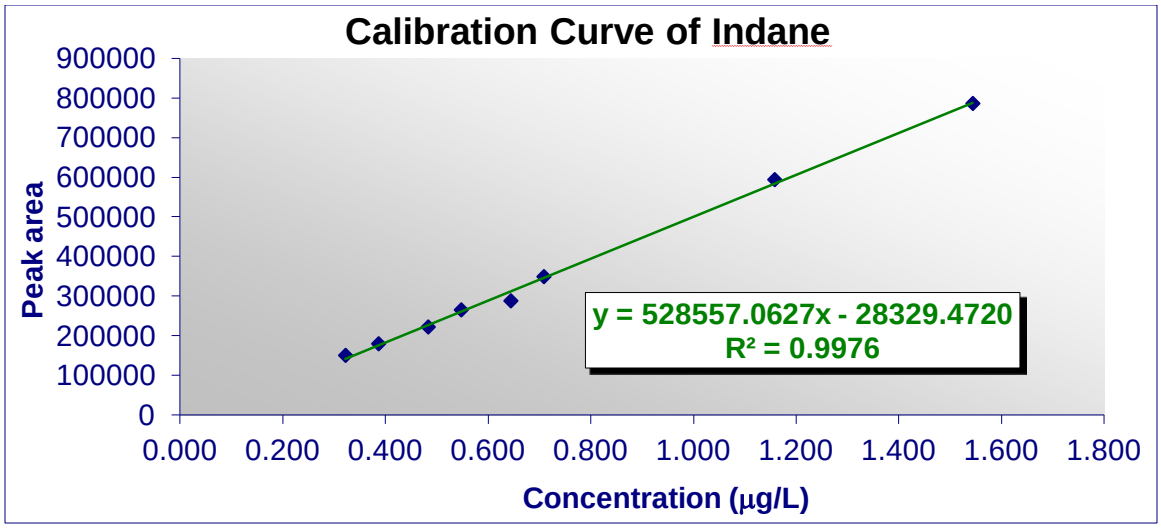

Calibration Curve of 4-ethyltoluene
Peak area
700000
600000
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
Concentration (µg/L)
y = 1831769.7075x + 94277.4425
R² = 0.9971

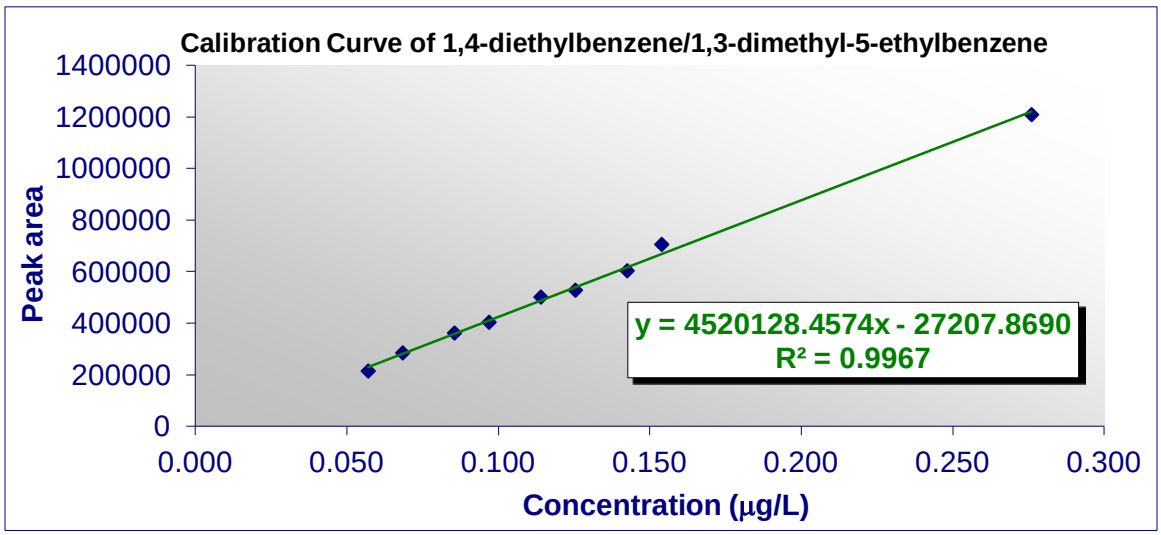

Calibration Curve of 2-ethyltoluene
Peak area
700000
600000
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
Concentration (µg/L)
y = 2281931.4741x - 9855.8435
R² = 0.9951

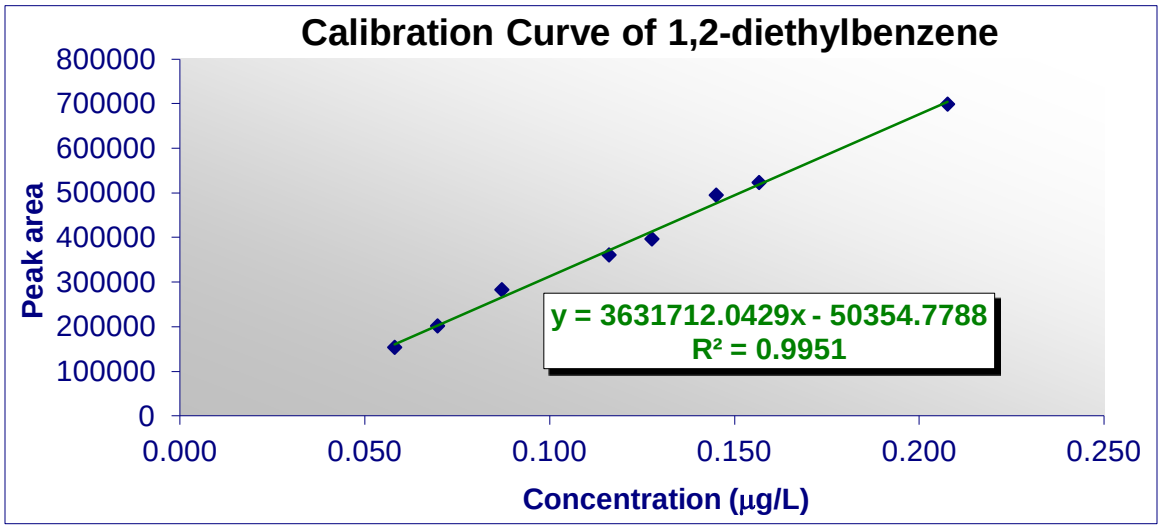

Calibration curve of 1,2,4-trimethylbenzene
Peak area
700000
600000
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
Concentration (µg/L)
y = 2151358.3551x - 16089.2213
R² = 0.9982

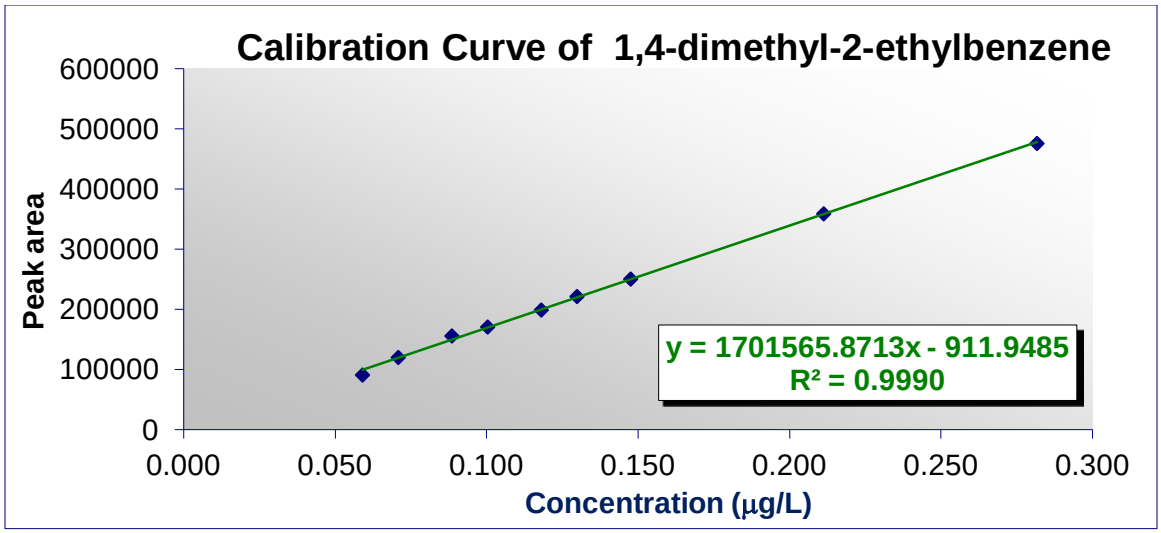

Calibration Curve of 4-isopropyltoluene
Peak area
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0.000
0.010
0.020
0.030
0.040
0.050
Concentration (µg/L)
y = 4379524.1150x - 17529.8801
R² = 0.9972

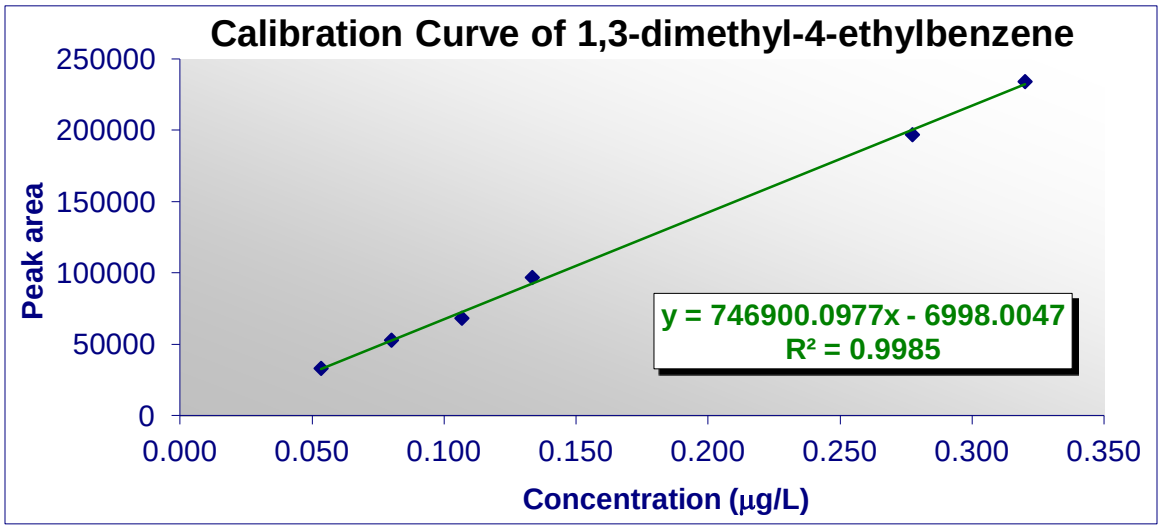

Calibration Curve of 1,3-diethylbenzene
Peak area
800000
700000
600000
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
Concentration (µg/L)
y = 3631712.0429x - 50354.7788
R² = 0.9951

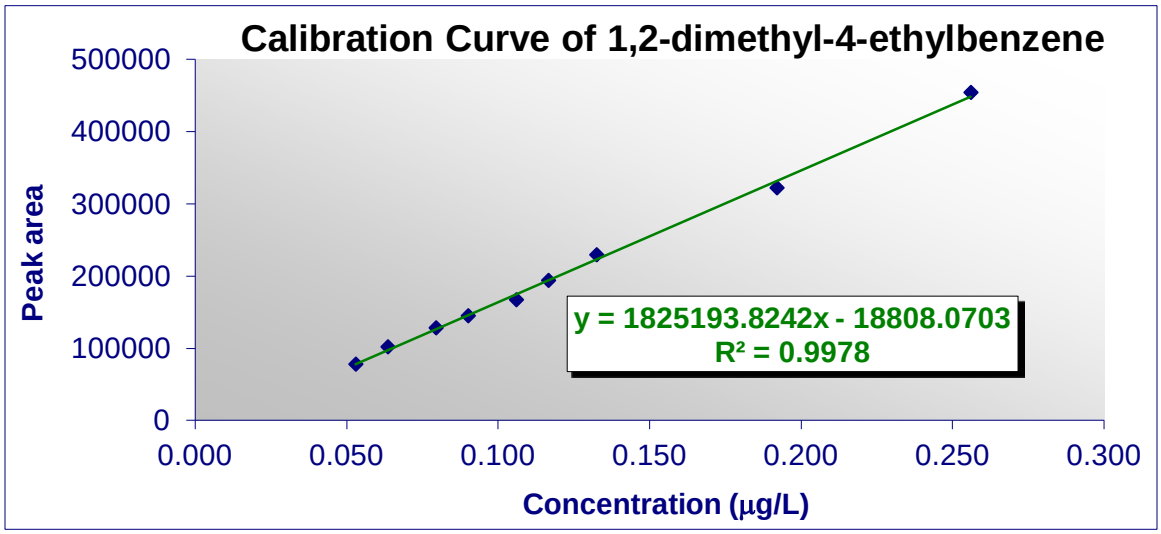

Calibration Curve of Indane
Peak area
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600
1.800
Concentration (µg/L)
y = 528557.0627x - 28329.4720
R² = 0.9976

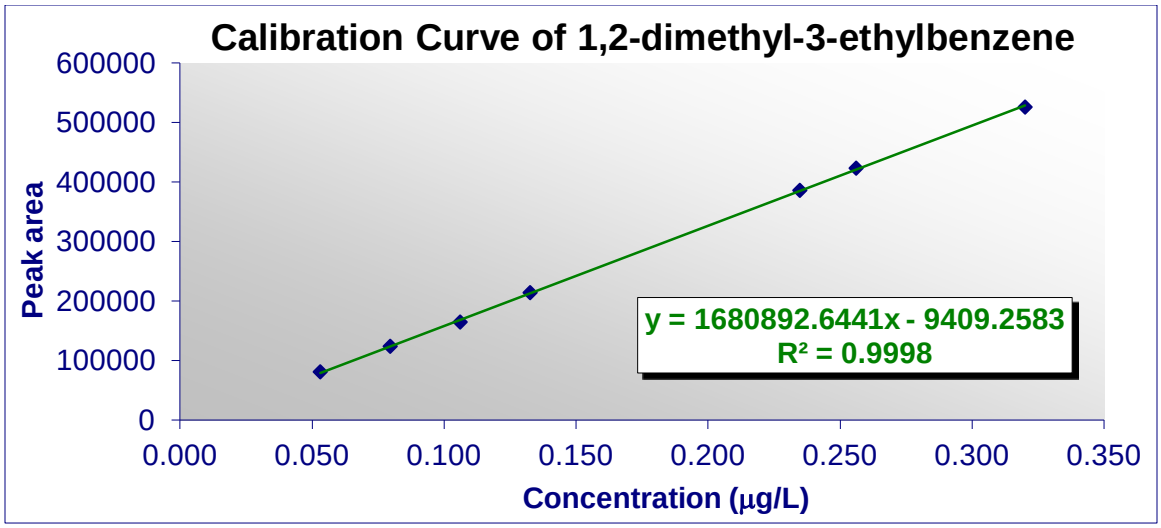

Calibration Curve of 1,4-diethylbenzene/1,3-dimethyl-5-ethylbenzene
Peak area
1400000
1200000
1000000
800000
600000
400000
200000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
Concentration (µg/L)
y = 4520128.4574x - 27207.8690
R² = 0.9967


Calibration Curve of 1,2-diethylbenzene
Peak area
800000
700000
600000
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
Concentration (µg/L)
y = 3631712.0429x - 50354.7788
R² = 0.9951


Calibration Curve of 1,4-dimethyl-2-ethylbenzene
Peak area
600000
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
Concentration (µg/L)
y = 1701565.8713x - 911.9485
R² = 0.9990


Calibration Curve of 1,3-dimethyl-4-ethylbenzene
Peak area
250000
200000
150000
100000
50000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
0.350
Concentration (µg/L)
y = 746900.0977x - 6998.0047
R² = 0.9985


Calibration Curve of 1,2-dimethyl-4-ethylbenzene
Peak area
500000
400000
300000
200000
100000
0
0.000
0.050
0.100
0.150
0.200
0.250
0.300
Concentration (µg/L)
y = 1825193.8242x - 18808.0703
R² = 0.9978



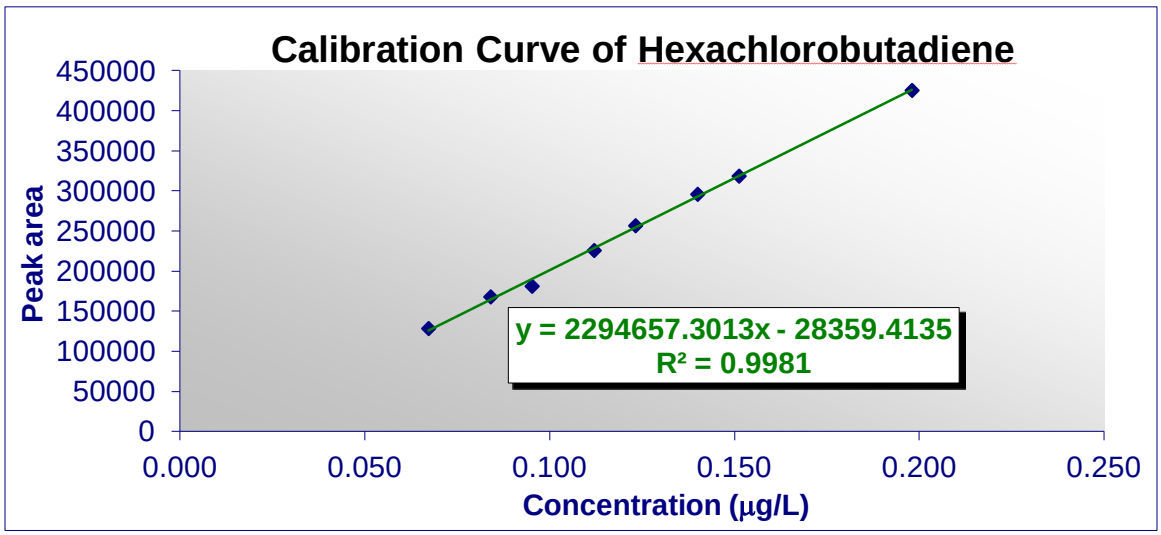


Figure 9: Calibration curve of the target compounds

3.3. Precision

3.3.1. Repeatability

Repeatability was obtained from 11 or 12 repeated measurements made for each compound. The measurements were made in one single batch on individual ultrapure water samples. Therefore, for repeatability observation same person, equipment, reagent, same condition and environment were used on the same day. Repeatability was obtained at the minimum and maximum concentration levels of the working range for each

compound. Repeatability is expressed as relative standard deviation % (coefficient of variation).

Before the calculation of repeatability the results were tested by Grubb's test for outliers. Any outlier detected is automatically discarded by the test. Repeatability in the low level of concentration ranged from 4.2 % for 1,4-dimethyl-2-ethylbenzene to 13.0% RSD for 4-ethyltoluene (Table 15). Similarly, in the high level of concentration, repeatability ranged from 1.6% for MTBE to 12.5% for 4-ethyltoluene. Repeatability values for all the measurements were below 15% and therefore are well within the limit of 25% prescribed by the Council Directive 98/83/EC for some of the organic compounds. Normally repeatability RSD would be higher at lower concentration than that at the higher concentration. However, this assumption is found true only for MTBE, 4-ethyltoluene, 4-isopropyltoluene, and 1,2-diethylbenzene.

Table 15: Repeatability at low and high concentrations

S.N.	Compound name	Repeatability			
		Low concentration		High concentration	
		Concentration µg/L	RSD (%) (n= 11)	Concentration µg/L	RSD (%) (n=12)
1	MTBE	4.9	7.5	13.3	1.6
2	3-ethyltoluene	0.058	6.9	0.277	7.5
3	4-ethyltoluene	0.068	13.0	0.275	12.5
4	2-ethyltoluene	0.059	6.9	0.284	9.2
5	1,2,4-trimethylbenzene	0.058	5.1	0.28	8.6
6	4-isopropyltoluene	0.017	6.6	0.042	2.9
7	1,3-diethylbenzene	0.058	4.8	0.208	6.6
8	Indane	0.322	11.2	1.544	12.1
9	1,4-diethylbenzene	0.057	5.5	0.276	10.9
	1,3-dimethyl-5-ethylbenzene				
10	1,2-diethylbenzene	0.059	6.0	0.282	5.7
11	1,4-dimethyl-2-ethylbenzene	0.059	4.2	0.282	9.2
12	1,3-dimethyl-4-ethylbenzene	0.053	9.2	0.256	11.5
13	1,2-dimethyl-4-ethylbenzene	0.053	5.2	0.256	9.9
14	1,2-dimethyl-3-ethylbenzene	0.053	6.2	0.256	6.9
15	Hexachlorobutadiene	0.067	7.1	0.198	9.0

3.3.2. Intermediate precision

For intermediate precision measurement, variation can be brought in any variable like day of analysis, person, instrument, reagent and environmental condition. In this study variation was brought for the day of analysis only. The repeated measurements were done in different days. Altogether 7 repeated measurements were made on 7 different days in both lowest concentration and highest concentration levels of working range. Intermediate precision is also expressed in percentage relative standard deviation (RSD). In the lowest concentration level RSD ranged from 3.5 % for MTBE to 19.1 % for hexachlorobutadiene. So at this level values for all 16 compounds were below 20 %. At the high level of concentration only 1,3-diethylbenzene has its RSD above 20 %. Intermediate precision RSD values also are within the EU limit of 25%. However, all the compounds have higher RSD values at high concentration level than at low concentration level except for indane and hexachlorobutadiene.

Table 16: Intermediate precision at low and high concentrations

S.N.	Compound name	Intermediate precision			
		Low concentration		High concentration	
		Concentration	RSD (%)	Concentration	RSD (%)
		µg/L	(n=7)	µg/L	(n= 7)
1	MTBE	4.9	3.5	13.3	9.3
2	3-ethyltoluene	0.058	8.8	0.277	12.2
3	4-ethyltoluene	0.068	14.9	0.275	16.2
4	2-ethyltoluene	0.059	5.8	0.284	11.6
5	1,2,4-trimethylbenzene	0.058	5.7	0.28	11.2
6	4-isopropyltoluene	0.017	8.1	0.042	8.8
7	1,3-diethylbenzene	0.058	15.8	0.208	22.0
8	Indane	0.322	11.8	1.544	9.3
9	1,4-diethylbenzene/ 1,3-dimethyl-5-ethylbenzene	0.057	13.6	0.276	18.4
10	1,2-diethylbenzene	0.059	7.7	0.282	12.0
11	1,4-dimethyl-2-ethylbenzene	0.059	6.4	0.282	11.5
12	1,3-dimethyl-4-ethylbenzene	0.053	5.6	0.256	12.2
13	1,2-dimethyl-4-ethylbenzene	0.053	7.4	0.256	12.6
14	1,2-dimethyl-3-ethylbenzene	0.053	5.0	0.256	10.1
15	Hexachlorobutadiene	0.067	19.1	0.198	11.0

3.4. Limit of detection (LOD)

Limit of detection can be determined by few methods. Objective of determining LOD is that it ensures that the minimum instrument response is clearly above chromatographic baseline and this implies that LOQ and reporting levels (minimum calibration concentration) are well above the baseline. LOD may be determined by 4 different methods: from baseline noise, from residual standard deviation and slope of calibration curve, repeatability standard deviation and intermediate precision standard deviation. LOD values have been given for three of the methods in the following table. Normally, standard deviation of intermediate precision data would be higher than that of repeatability. However, Table 17 shows that, for MTBE, 4-ethyltoluene, 2-ethyltoluene, indane and 1,3-dimethyl-4-ethylbenzne, 1,2-dimethyl-3-ethylbenzene and hexachlorobutadiene repeatability standard deviation is higher than intermediate precision standard deviation. It is advisable that more investigation be done on intermediate precision and repeatability.

Table 17: Comparison of LOD obtained from the three methods

		Limit of detection LOD (µg/L)		
S.N	Compound	Calibration	Repeatability	Intermediate precision
1	MTBE	0.944	1.292	0.516
2	3-ethyltoluene	0.011	0.014	0.015
3	4-ethyltoluene	0.017	0.033	0.015
4	2-ethyltoluene	0.016	0.020	0.009
5	1,2,4-trimethylbenzene	0.01	0.010	0.010
6	4-isopropyltoluene	0.002	0.004	0.004
7	1,3-diethylbenzene	0.011	0.010	0.027
8	Indane	0.066	0.128	0.111
9	1,4-diethylbenzene/13DM5EBN	0.012	0.011	0.024
	1,3-dimethyl-5-ethylbenzene			
10	1,2-diethylbenzene	0.012	0.013	0.015
11	1,4-dimethyl-2-ethylbenzene	0.007	0.008	0.012
12	1,3-dimethyl-4-ethylbenzene	0.007	0.018	0.009
13	1,2-dimethyl-4-ethylbenzene	0.012	0.010	0.011
14	1,2-dimethyl-3-ethylbenzene	0.005	0.011	0.008
15	Hexachlorobutadiene	0.006	0.018	0.015

3.5. Limit of quantification (LOQ)

Limit of quantification (LOQ) is an important parameter in method validation because the reporting level should be either equal to or greater than LOQ. LOQ is the minimum level at which the results can be quantified with acceptable precision and accuracy. LOQ can also be determined in same four different ways; from signal to noise ratio, from residual standard deviation and slope of calibration curve, from repeatability standard deviation and from intermediate precision standard deviation. For the comparison of LOQs refer to the Table 18. As it was for LOD, LOQs for intermediate precision are lower than that for repeatability for many compounds when normally intermediate precision readings would be more dispersed than repeatability readings (Table 18). Therefore, it is advisable to investigate further into this issue. For some compounds, that is for 4-ethyltoluene, 2-ethyltoluene, Indane, and 1,3-dimethyl-4-ethylbenzene, repeatability LOQ has been found higher than the lowest calibration standard. For intermediate precision LOQs for 1,3-diethylbenzene, Indane, and 1,4-diethylbenzene have been found higher than the minimum calibration standard of the working range. This is also a matter to look into.

Table 18: Comparison of LOQ obtained from the three methods

S.N	Compound	Working range µg/L	Limit of quantification (µg/L)		
			Calibration	Repeatability	Intermediate precision
1	MTBE	4.9-13.319	3.147	4.306	1.720
2	3-ethyltoluene	0.058-0.277	0.038	0.048	0.050
3	4-ethyltoluene	0.057-0.143	0.056	0.110	0.050
4	2-ethyltoluene	0.059-0.284	0.054	0.066	0.030
5	1,2,4-trimethylbenzene	0.058-0.280	0.032	0.034	0.032
6	4-isopropyltoluene	0.017-0.043	0.006	0.014	0.014
7	1,3-diethylbenzene	0.058-0.208	0.037	0.032	0.090
8	Indane	0.322-1.544	0.221	0.426	0.370
9	1,4-diethylbenzene/ 1,3-dimethyl-5-ethylbenzene	0.057-0.276	0.04	0.037	0.080
10	1,2-diethylbenzene	0.059-0.282	0.041	0.042	0.050
11	1,4-dimethyl-2-ethylbenzene	0.059-0.282	0.024	0.027	0.039
12	1,3-dimethyl-4-ethylbenzene	0.053-0.144	0.023	0.061	0.030
13	1,2-dimethyl-4-ethylbenzene	0.053-0.256	0.033	0.032	0.035
14	1,2-dimethyl-3-ethylbenzene	0.053-0.320	0.015	0.038	0.028
15	Hexachlorobutadiene	0.067-0.198	0.02	0.060	0.050

3.6. Accuracy

Accuracy expresses how close an experimentally measured value is with a true value. Accuracy has two components to it, systematic error is shown by the relative error to 100% and random error is shown by the relative standard deviation. Recovery is the ratio of the experimental value and a true value (spiking concentration). In order to determine the accuracy of the method, measurements were made in three different matrices: tap water, surface water (Tagus river) and groundwater. The matrix samples were spiked with the standards for highest and lowest levels of the working range for each compound. Concentrations for the spiked samples were obtained by multiplying the spiked sample area by the response factor obtained in the same batch with calibration standards. Ratio of spiked concentration and true concentration of a compound gave the recovery %. An RSD of recovery was obtained by making 10 repeated measurements of the spiked samples in the same batch.

3.6.1. Recovery in tap water

Recovery for tap water ranged from 100% for 1,4-diethylbenzene to 118% for 1,2,4-trimethylbenzene in the lowest concentration level and 79 % for 1,2-dimethyl-4-ethylbenzene to 108 % for 1,3-dimethyl-4-ethylbenzene in the highest concentration level. Recoveries of 1,2-dimethyl-4-ethylbenzene and 4-ethyltoluene were found relatively low at the highest concentration level (Table 19).

Table 19: Recovery of the compounds from the tap water

S.N.	Compound name	Tap water recovery					
		Low concentration			High concentration		
		Concentration µg/L	Recovery %	RSD (%) (n= 10)	Concentration µg/L	Recovery %	RSD (%) (n=10)
1	MTBE	4.9	102.6	7.1	13.3	97.2	3.6
2	3-ethyltoluene	0.058	108.2	11.41	0.277	104.9	10
3	4-ethyltoluene	0.068	104.4	7	0.275	82.4	8.3
4	2-ethyltoluene	0.059	104.2	9.2	0.284	100	8.3
5	1,2,4-trimethylbenzene	0.058	118.1	8.55	0.28	99.6	7.8
6	4-isopropyltoluene	0.017	109.6	14.6	0.042	96.2	16.4
7	1,3-diethylbenzene	0.058	101.5	13.7	0.208	105.5	7.7
8	Indane	0.322	113.2	9.76	1.544	101.6	4.4
9	1,4-diethylbenzene/ 1,3-dimethyl-5-ethylbenzene	0.057	100.1	15.5	0.276	101.3	14.8
10	1,2-diethylbenzene	0.059	103.7	11.4	0.282	102.1	10.6
11	1,4-dimethyl-2-ethylbenzene	0.059	103	9.4	0.282	97.7	9.1
12	1,3-dimethyl-4-ethylbenzene	0.053	112.3	9.5	0.256	108.2	8.9
13	1,2-dimethyl-4-ethylbenzene	0.053	106	9.2	0.256	79.2	8.9
14	1,2-dimethyl-3-ethylbenzene	0.053	108.1	7	0.256	98.6	2.6
15	Hexachlorobutadiene	0.067	101.8	7.9	0.198	101.5	17

3.6.2. Recovery in river water

Recovery of river water ranged from 84.5% for Indane to 102 % for 1,2,4-trimethylbenzene and 4-ethyltoluene at the lowest concentration level. Actually, barring indane recovery (84.5 %), recovery for other compounds is fairly good. The other recoveries are between 90 and 102 %. RSD for river water was all below 20% except for 1,4-diethylbenzene. At highest concentration level the recovery was between 92.3% for 1,2-dimethyl-4-ethylbenzne and 122% for 1,4-diethylbenzene and 3-ethyltoluene. Indane had 120%. Comparatively, according to the data, more compounds in low concentration level have under recovered (just above 90%) and more compounds in high concentration level have over recovery above 110 %. RSD in high concentration is low except for hexachlorobutadiene.

Table 20: Recovery of the compounds from river water

S.N.	Compound name	River water recovery					
		Low concentration			High concentration		
		Concentration	Recovery	RSD (%)	Concentration	Recovery	RSD (%)
		µg/L	%	(n=10)	µg/L	%	(n=10)
1	MTBE	4.9	95.5	5.2	13.3	100.8	2.8
2	3-ethyltoluene	0.058	101.1	12.95	0.277	122.1	3.4
3	4-ethyltoluene	0.068	102.4	17.5	0.275	108	4.1
4	2-ethyltoluene	0.059	95.4	9.35	0.284	110.7	3.3
5	1,2,4-trimethylbenzene	0.058	102.6	7.9	0.28	113.1	3.4
6	4-isopropyltoluene	0.017	98.3	10.9	0.042	106	5.1
7	1,3-diethylbenzene	0.058	92.3	10.6	0.208	102.6	11
8	Indane	0.322	84.5	17.5	1.544	120.1	12.2
9	1,4-diethylbenzene/13DM5EBN	0.057	95	20.14	0.276	122.4	7.3
	1,3-dimethyl-5-ethylbenzene						
10	1,2-diethylbenzene	0.059	93.6	9.9	0.282	114.9	3.7
11	1,4-dimethyl-2-ethylbenzene	0.059	91.5	9	0.282	109	2.7
12	1,3-dimethyl-4-ethylbenzene	0.053	91.7	7.5	0.256	115.2	5.3
13	1,2-dimethyl-4-ethylbenzene	0.053	95.6	9.7	0.256	92.3	3.5
14	1,2-dimethyl-3-ethylbenzene	0.053	94	8.14	0.256	108.1	3
15	Hexachlorobutadiene	0.067	99.2	18.5	0.198	99.4	20.6

3.6.3. Recovery in groundwater

Recovery of the compounds in groundwater at low concentration level can be considered efficient as shown by the range of recovery between 94.3 for 1,4-diethylbenzene and 104.2 % for hexachlorobutadiene and RSD is also below 10% except that in 3-ethyltoluene and Indane. At high concentration more compounds have under recovery than over recovery. The compound 1,2-dimethyl-4-ethylbenzene has about 80% recovery and Indane has 112%. Recoveries for rest of the compounds are between 90 and 105%.

Table 21: Groundwater recovery at low and high concentrations

S.N.	Compound name	Groundwater recovery							
		Low concentration				High concentration			
		Conc	Recovery	RSD		Conc	Recovery	RSD	
		µg/L	%	%	n	µg/L	%	%	n
1	MTBE	4.9	96.0	4.7	9	13.3	97	1.9	10
2	3-ethyltoluene	0.058	95.3	11.3	8	0.277	103.5	7.8	10
3	4-ethyltoluene	0.068	101.7	8.8	10	0.275	99.5	10	9
4	2-ethyltoluene	0.059	98.2	7.1	8	0.284	97.3	5.5	10
5	1,2,4-trimethylbenzene	0.058	98.6	6.4	8	0.28	98.5	5	10
6	4-isopropyltoluene	0.017	102.4	8.0	7	0.042	105.4	4.8	9
7	1,3-diethylbenzene	0.058	96.4	8.0	7	0.208	95.9	8.2	10
8	Indane	0.322	97.5	12.5	8	1.544	112.1	13.6	10
9	1,4-diethylbenzene/ 1,3-dimethyl-5-ethylbenzene	0.057	94.3	9.4	7	0.276	92.1	12.5	10
10	1,2-diethylbenzene	0.059	101.7	6.6	7	0.282	98.5	6.8	10
11	1,4-dimethyl-2-ethylbenzene	0.059	101.8	7.1	7	0.282	97.9	6.4	10
12	1,3-dimethyl-4-ethylbenzene	0.053	103.0	9.8	7	0.256	105.4	7.3	10
13	1,2-dimethyl-4-ethylbenzene	0.053	99.6	5.3	7	0.256	79.9	5.6	10
14	1,2-dimethyl-3-ethylbenzene	0.053	98.2	6.6	9	0.256	96.9	4.1	10
15	Hexachlorobutadiene	0.067	104.2	9.1	10	0.198	99.7	10.1	10

3.6.4. Comparison of recovery in the three types of water

Indane has not so good recovery in river water both at low and high concentration levels. Both in tap water and groundwater 1,2-dimethyl-4-ethylbenzene has recoveries below 80% at high concentration level. Some of the low and high recoveries have already been mentioned. Other high and low recoveries are fairly sporadic. Not any compound has particularly shown less than satisfactory recovery. At the highest concentration level, comparatively river water shows higher recovery than tap water or groundwater do. A total of only four recovery results are close to 80 % and four are close to 120%. Others are between 90% and 115%. Therefore, on the whole, the recovery study results have been fairly good. The target compounds have been recovered well in the three different water matrices and the recovery value meets the European Union limit value of 25% trueness.

Table 22: Comparison of recovery in the three types of water matrices

S.N.	Compound name	Recovery					
		Low concentration			High concentration		
		%			%		
		Tap water	Groundwater	River water	Tap water	Groundwater	River water
1	MTBE	102.6	96	95.5	97.2	97	100.8
2	3-ethyltoluene	108.2	95.3	101.1	104.9	103.5	122.1
3	4-ethyltoluene	104.4	101.7	102.4	82.4	99.5	108
4	2-ethyltoluene	104.2	98.2	95.4	100	97.3	110.7
5	1,2,4-trimethylbenzene	118.1	98.6	102.6	99.6	98.5	113.1
6	4-isopropyltoluene	109.6	102.4	98.3	96.2	105.4	106
7	1,3-diethylbenzene	101.5	96.4	92.3	105.5	95.9	102.6
8	Indane	113.2	97.5	84.5	101.6	112.1	120.1
9	1,4-diethylbenzene/13DM5EBN	100.1	94.3	95	101.3	92.1	122.4
10	1,2-diethylbenzene	103.7	101.7	93.6	102.1	98.5	114.9
11	1,4-dimethyl-2-ethylbenzene	103	101.8	91.5	97.7	97.9	109
12	1,3-dimethyl-4-ethylbenzene	112.3	103	91.7	108.2	105.4	115.2
13	1,2-dimethyl-4-ethylbenzene	106	99.6	95.6	79.2	79.9	92.3
14	1,2-dimethyl-3-ethylbenzene	108.1	98.2	94	98.6	96.9	108.1
15	Hexachlorobutadiene	101.8	104.2	99.2	101.5	99.7	99.4

3.7. Blank studies

It was mentioned in selectivity that it is very important that the peaks observed for the target compounds in the chromatogram are indeed from the target compounds themselves and not from a foreign compound or an interference. So, the study also included ultrapure water blanks which was were analyzed in every batch. Similarly matrix blank for tap water, river water and groundwater and an atmosphere blank (empty vial) were also used during recovery study. At times chromatogram of these blanks consisted of a few measurable peaks. Appearance of these peaks were mostly random and their retention times were different from the retention times of compounds of interest. One peak with retention time very close to 11.751 min (t_R of 1,2,4-trimethylbenzene)appeared for many times during the recovery study. However, for most of the times size of its area was significantly small although for a couple of times it was observed that the area was less

than 10% of the area observed in the standard injected. Therefore, possible effects of 1,2,4-trimethylbenzene contamination may be investigated further.

3.8. Measurement uncertainty

Reporting laboratory generated results with measurement uncertainty is one of the technical requirement of ISO 17025.[94] Any measurement has some level of inherent uncertainty and implies the imperfection involved in analysis. Therefore, the measurement result of a laboratory is an estimate of the true value of the measurand (concentration in this study). The quality of this estimate is given by the uncertainty range.[100] Uncertainty is a width within which a true value lies with a certain level of confidence.

Uncertainty may arise from a variety of possible sources, for example, from sample collection and processing, matrix effects and interference, environmental conditions, instruments, masses, volumetric apparatuses, and reference values. Possible sources of uncertainty should be taken into account when measurement uncertainty is determined. Each source of uncertainty is known as a component uncertainty and is expressed in terms of standard deviation called standard uncertainty. Determining measurement uncertainty involves identifying source of uncertainty (component uncertainty), quantifying component uncertainty, calculating combined uncertainty and final step is the calculation of the expanded uncertainty. Some component is taken from the standard deviation of precision studies and calibration uncertainty of the apparatus used while others derived from assumed probability distributions based on experience or other information. It is understood that the result is the best estimate of a true value and all the component uncertainty, including those arising from systematic errors such as associated with reference standards contribute to the dispersion of the results.[101]

In the present study, no mass was used. So this excludes uncertainty associated with weighing operation. Three sources of uncertainty have been accounted in this study, they are volumetric operation, instrument quantification and precision of the analytical method

used. These component uncertainties are added to give combined uncertainty which in turn is multiplied by a coverage factor to give the expanded uncertainty.[102]

$u_c = \sqrt{u^2_{pre} + u^2_{std} + u^2_{inter}}$

u_c = combined uncertainty

u^2_{pre} = uncertainty due to precision studies (from recovery data in this study)

u^2_{std} = uncertainty due to preparation of calibration standards

u^2_{inter} = uncertainty due to interpolation of calibration curve

u_c * 2 or 3 = U

U= expanded uncertainty

K = 2 or 3 (coverage factors)

For a normally distributed data a coverage factor of 2 is used for 95% confidence level and 3 is used for 99% confidence level. Uncertainty of the method accompanies the test result in the form of "± range". Uncertainty should be expressed in absolute value, i.e., in the unit in which the test results are reported.

Table 23: Uncertainty in the three water matrices (95%, k=2)

		Uncertainty (%)		
S.N	Compound name	Tap water	River water	Groundwater
1	MTBE	15.9	15.6	15.5
2	3-ethyltoluene	16.2	16.6	16.6
3	4-ethyltoluene	20.1	22.9	20.4
4	2-ethyltoluene	23	23.0	22.8
5	1,2,4-trimethylbenzene	14.2	14.0	13.9
6	4-isopropyltoluene	12.7	11.1	10.6
7	1,3-diethylbenzene	17.8	16.9	16.7
8	Indane	16.1	18.6	17.2
9	1,4-diethylbenzene	19.8	21.4	18.6
	1,3-dimethyl-5-ethylbenzene			
10	1,2-diethylbenzene	17.2	17.1	17.2
11	1,4-dimethyl-2-ethylbenzene	12.2	12.1	11.9
12	1,3-dimethyl-4-ethylbenzene	22.3	22.0	22.7
13	1,2-dimethyl-4-ethylbenzene	14.9	15.5	14.3
14	1,2-dimethyl-3-ethylbenzene	8.2	8.7	8.2
15	Hexachlorobutadiene	9	13.9	9.4

Uncertainty was calculated for all three matrices and the table above shows the expanded uncertainty calculated (percentage) for the water matrices. The lowest uncertainty observed was 8.2% in groundwater and tap water for 1,2-dimethyl-3-ethylbenzene and the highest was 23% for ethyl toluene. The table clearly shows that for the same compound, uncertainty values for the different matrices are similar except for hexachlorobutadiene.

IV. CONCLUSION AND FUTURE PERSPECTIVE

HS-SPME/GC-MS method is a well established technique for VOC analysis. In this study the method was successfully validated and used to detect and quantify 16 VOCs that are mainly found in petroleum products. Most of these compounds have not been regulated for human consumption but the method can be used to detect the VOCs which can be used as indicators of petroleum contamination in drinking and natural waters. Each compound has its own retention time and mass spectrum which can be used to identify the compounds. Even isomers of ethyltoluene, diethylbenzene and dimethylethylbenzne could be separated by the method. Because of overlapping of retention times, 1,4-diethylbenzene and 1,3-dimethyl-5-ethylbenzene coelute and their concentration is the sum of the individual concentrations.

Each method has its own working range. Most of the compounds can be detected at ppt level also. A linear working range could be ascertained for each target compound. Linearity of the calibration curves were supported by high r^2 values, random residual distribution, Mandel's test, RIKILT test and Standardized area test. The three different methods of determination have given different values of LOD and LOQ. Among the three methods calibration method gives the lowest LOD and LOQ. Repeatability of all the compounds is acceptable because RSD of the compounds are below the prescribed value of 25%. Except for hexachlorobutadiene and 1,3-diethylbenzene, intermediate precision of all the other compounds is fairly low. For the three different matrices; tap water, river water and groundwater, the method is well suited because most of the recovery values are between 90 % and 115 %.

Since optimization of HS-SPME and temperature profile of gas chromatograph was not carried out they should be done in fut3ure. Attempts should be made to separate coelution of 1,4-diethylbenzene and 1,3-dimethyl-5-ethylbenzene. More investigation should be done to find out the effect and source of possible contamination due to 1,2,4-

trimethylbenzene. In some cases LOD and LOQ of intermediate precision have been found lower than that of repeatability and this should be further investigated.
HS-SPME sampling is solvent free and less time consuming and therefore is preferable. There have been only limited research in method validation of many target VOCs. So this study contributes to methods of analysis used to detect VOCs in different water matrices.

Reference

1. Pollution Issues. www.pollutionissues.com/Na-Ph/Petroleum.html (accessed 6/20/2015), Petroleum.
2. Petroleum.co.uk. www.petroleum.co.uk (accessed 6/20/2015), An introduction to petroleum.
3. USEPA, Petroleum hydrocarbons and chlorinated solvents differ in their potential for vapor intrusion. 2012, Office of Underground Storage Tanks, Washington D.C., www.epa.gov/oust/cat/pvi/pvicvi.pdf (accessed 20/6/2015).
4. Cwiertny, D.M.; Scherer, M.M. Chlorinated solvent chemistry: structures, nomenclatures and properties, In In situ remediation of chlorinated solvent plumes, Stroo, H.F.,Ward, C.H. Eds, Springer, 2010,Chapter 2, pp 29-37.
5. ALS Environmental. www.caslab.com/Petroleum-Hydrocarbon-Ranges, (20/6/2015), Petroleum hydrocarbon ranges.
6. Moran, M.J.; Zogorski, J.S.; Rowe, B.L. Approach to an assessment of volatile organic compounds in the Nations groundwater and drinking water supplies, 2006, USGS. www.pubs.usgs.gov (accessed 20/6/2015).
7. Crude oil contamination in the shallow subsurface: Bemidji, Minnesota, Enviornmental Health – Toxic Substances, USGS, http://toxics.usgs.gov/sites/bemidji_page.html, (accessed 7/16/2015).
8. Nadim, F.; Hoag, G.E.; Liu, S.; Carley, R.J.; Zack, P. Detection and remediation of soil and aquifer systems contaminated with petroleum products: an overview. Journal of Petroleum and Science and Engineering, 2000, 26, 169-178.
9. Pollution Issues. Underground storage tank. http://www.pollutionissues.com/Te-Un/Underground-Storage-Tank.html, (6/20/2015).
10. MDH Minnesota Department of Health. VOCs: volatile organic chemicals in private drinking water wells. www.health.state.mn.us/divs/eh/haxardous/topics/vocs.html (accessed 20/6/2015).
11. Masters, G.M. *Introduction to environmental science and engineering*. 1991. Prentice Hall.

12. Drinking water standards and health advisories, USEPA, 2004. Washington DC, Office of Water, EPA 822-R-04-005, http://water.epa.gov/action/advisories/drinking/upload/2009_04_27_criteria_drinking_dwstandards2004.pdf, (accessed 16/7/2015).
13. Gorlach, B.; Interwies, E. Economic assessment groundwater protection: a survey of the literature. 2003. Berlin. Ecologic.
14. Volatile organic compounds (VOCs). NIH Tox Town, US National Library of Medicine, http://toxtown.nlm.nih.gov/text_version/chemicals.php?id=31 (accessed 20/6/2015).
15. New Jersey Department of Health. Volatile organic compounds in drinking water. http://www.state.nj.us/health/eoh/hhazweb/vocs.pdf, (accessed 7/16/2015).
16. IARC, Some halogenated hydrocarbons. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. 1979,Vol 20.
17. WHO. Guidelines for drinking-water quality. 2011, World Health Organization. Geneva.
18. European Union, DIRECTIVE 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
19. USEPA. Drinking water advisory: consumer acceptability advice and health effects analysis on methyl-tert-butyl-ether (MTBE). 1997, EPA-822-F-97-009.
20. P-Cymene; Material safety data sheet. ScienceLab.com. http://www.sciencelab.com/msds.php?msdsId=9923637 (accessed 7/22/ 2015)
21. ChemicalBook; CAS database list; 4-isopropyltoluene; 2010; http://www.chemicalbook.com/ChemicalProductProperty_EN_CB9262508.htm (accessed 7/22/2015).
22. Source Watch; Isopropyltoluene; swww.sourcewatch.org/index.php/Isopropyltoluene (accessed 22/7/ 2015)
23. New York State; Human health fact sheet; Ambient water quality value for protection of sources of potable water; http://www.epa.gov/gliclear/pdfs/ny_hh_637_w_03121998.pdf (accessed on 7/16/ 2015)

24. AME 436; http://ronney.usc.edu/AME436-PS1.pdf (accessed 7/22/ 2015)
25. Craig, Mathew; Classification of hazardous and non-hazardous substances in groundwater. Environment Protection Agency, 2010, Ireland. https://www.epa.ie/pubs/reports/water/ground/Classification%20of%20Hazardous%20and%20Non-Hazardous%20Substances%20in%20Groundwater.pdf (accessed 7/16/2015)
26. Center for Disease Control and Prevention, NIOSH Pocket Guide to Chemical Hazards. 1,2,4-trimethylbenzene; http://www.cdc.gov/niosh/npg/npgd0638.html (accessed 7/22/2015).
27. Commonwealth of Pennsylvania, Department of Environmental Protection, Bureau of Point and Non-point Source, Rationale for the development of ambient water quality criteria for the protection of human health use. http://www.portal.state.pa.us/portal/server.pt/community/BPNPSM/10535
28. Philip Howard;Handbook of environmental fate and exposure for organic chemicals. 1997; Volume 5. CRC Press. Florida.
29. IPCS; International Program on Chemical Safety. INCHEM, European Commission. 2004-2012; http://www.inchem.org/documents/icsc/icsc/eics0445.htm (accessed 7/16/2015).
30. Occupational health database, National Library of Medicine, National Institute of Health, US Department of Health and Human Services. http://hazmap.nlm.nih.gov/category-details?id=12170&table=copytblagents (accessed 7/16/2015)
31. Scottish pollutant release inventory, Scottish Environment Protection Agency, http://apps.sepa.org.uk/spripa/Pages/SubstanceInformation.aspx?pid=53 (accessed 7/16/2015).
32. Parameters of water quality, interpretation and standards, 2001. Environment Protection Agency, Ireland. https://www.epa.ie/pubs/advice/water/quality/Water_Quality.pdf (accessed 7/16/2015).
33. Surface Water Directive 75/440/EEC. Council Directive of 16 June 1975 concerning the quality required of surface water intended for the abstraction of

drinking water in member states. Official Journal of the European Communities. 25.7.75.

34. Bathing Water Directive 76/160/EEC. Council Directive of 8 December 1975 concerning the quality of bathing water. Official Journal of the European Communities. 5.2.76.
35. Groundwater Directive 2006/118/EC. Directive of the European Parliament and the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. Official Journal of the European Union. 27.12.2006.
36. Drinking Water Directive 80/778/EEC. Council Directive of 15 July 1980 relating to the quality of water intended for human consumption. Official European Communities. 1980L0078-EN-01.011995-001.001-1.
37. Council Directive 98/83/EC. Council Directive of 3 November 1998 on the quality of water intended for human consumption. Official Journal of European Communities. 5.12.98.
38. Commission Decision 2002/657/EC. Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities. 17.8.2002.
39. Directive 2008/105/EC. Directive of European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy. Official Journal of the European Union. 24.12.2008.
40. Standard guidelines for the environmental monitoring of chemicals. Chpater III Analytical methods. Government of Japan, Ministry of Environment. https://www.env.go.jp/en/chemi/pops/Appendix/04-GuideLine/guidelines.pdf (accessed date 16/7/2015.)

41. Brito, N.M.; Navickiene, S.; Polese' L.; Jardim,E.F.G.; Abakerli, R.B.; Ribeiro, M.L. Determination of pesticide residues in coconut water by liquid–liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high-performance liquid chromatography with ultraviolet detection. J. of Chromatogr. A. 2002, 957, 201-9.

42. Usher, K.M.; Simmons, C.R.; Keating, D.W.; Rossi, H.F. Determination of niacinamide in lotions and creams using liquid-liquid extraction and high performance liquid chromatography, J. of Chem. Educ. 2015, 92, 907-910.
43. Robles-Molina, J.; Gilbert-Lemopez, B.; Garcia-Reyes, J.F.; Molina-Diaz, A. Comparative evaluation of liquid-liquid extraction, solid-phase extraction and solid-phase microextraction for the gas chromatography-mass spectrometry determination of multiclass priority organic contaminants in wastewater. Talanta, 2013, 11,382-391.
44. Mottaleb, M.A.; Abedin, M.Z.; Islam, M.S. Determination of volatile organic compounds in river water by solid phase extraction and gas chromatography, J. of Environmental Science, 2004, 16, 497-501.
45. Balakrishnan, V.K.; Terry, K.A. ; Toito, J. Determination of sulfonamide antibiotics in wastewater: A comparison of solid phase microextraction and solid phase extraction methods. J. of Chromatogr. A, 2006, 1131,1-10.
46. Moret, S. and Conte, L.S. A rapid method for polycyclic aromatic hydrocarbon determination in vegetable oils. J. Sep. Sci. 2002, 25, 96-100.
47. Ghaffar, S.; Hashmi, I.; Awan, M.A.; Nasir, H.; Amjad, H. Determination of Volatile Organic Compounds (VOCs) in Potable Water Using Solid Phase Micro Extraction-Gas Chromatography (SPME-GC). Arab J of Science and Engineering, 2012, 37, 1255-62.
48. Menéndeza, J.C.F.; Sanchez, M.L.F.; Sánchez, Ur´ıa, J.E.; Martinez, E.F.; Sanz-Medel, A. Static headspace, solid-phase microextraction and headspace solid-phase microextraction for BTEX determination in aqueous samples by gas chromatography. Anal. Chim. Acta. 2000, 415, 9-20.
49. Lambropoulou, D.A.; Albanis, T.A. Headspace Solid Phase Microextraction Applied to the Analysisof Organophosphorus Insecticides in Strawberry and Cherry Juices. J. of Agricultural and Food Chemistry, 2002, 50, 3359-3365.
50. Zhang, Z.; Pawliszyn, J. Analysis of organic compounds in environmental samples by headspace solid phase microextraction. J. of High Resolution Chromatography, 1993, 16:689-692.

51. Martinez, C.; Ramirez, N.; Gomez, V.; Pocurull, E.; Borrull, F. Simultaneous determination of 76 micropollutants in water samples by headspace solid phase microextraction and gas chromatography-mass spectrometry. Talanta, 2013, 116, 937-945.
52. Zhang, Z.; Pawliszyn, J. Headspace solid-phase microextraction. Anal. Chem., 1993, 65, 1843-52.
53. Arthur, C.L.; Killam, L.M.; Buchholz, K.D.; Pawliszyn, J. Automation and optimization of solid-phase microextraction. Anal. Chem., 1992, 64,1960-1966.
54. Vas, G.; Vekey, K. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. J. Mass. Spectrom. 2004, 39, 233-254.
55. Lord, H.; Pawliszyn, J. Evolution of solid-phase microextraction technology. J. Chromatogr A. 2000, 885, 153-193.
56. Manura, J.J. and Overton, S. SIS application Note # 39. Scientific Instrument Services.
www.sisweb.com/reference/applnote/app-39.html (accessed 20/6/2015).
57. Stringfellow, W.T. and Oh, K.C. Comparison of SPME Headspace Analysis to U.S. EPA Method 5030/8260B for MTBE Monitoring. Groundwater Monitoring and Remediation, 2005, 25, 52-58.
58. Rosell, M.; Lacorte, I.; Ginebreda, A.; Barcelo, D. Simultaneous determination of MTBE and its degradation products, other gasoline oxygenates and benzene, toluene, ethylbenzene and xylenes in Catalonian groundwater by purge-and-trap-gas chromatography-mass spectrometry. Journal of Chromatogr. A. 2003, 995, 171-184.
59. Leo, K.J.; Pyo, H.; Park, S.J.; Yoo, E.A.; Lee, D.W. A study on purge efficiency in purge and trap analysis of VOCs in water. Bull. Korean Chem. Soc. 2001, 22, 171-178.
60. Luo, T.; Zhou, Z.R.; Lin, S.B. Determination of volatile organic compounds in drinking water by purge and trap-gas chromatography mass spectrometry. Wei Sheng Yan Jiu, 2006, 35, 504-507.
61. Kusch, P.; Knupp, G. Analysis of residual styrene monomer and other volatile organic compounds in expanded polystyrene by headspace solid-phase

microextraction followed by gas chromatography and gas chromatography/mass spectrometry. J. of Sep. Sci. 2003, 25, 539-542.

62. Ceballos, L.; Pino, G.A.; Quijano-Celis, E.C.; Dago, A. Optimization of a HS-SPME/GC-MS method for determination of volatile compounds in some Cuban unifloral honeys. Journal of Food Quality, 2010, 33, 507-528.
63. Kotowska, U.; Zalikowski, M.J.; Isidorov, V.A. HS-SPME/GC–MS analysis of volatile and semi-volatile organic compounds emitted from municipal sewage sludge. Environmental Monitoring and Assessment, 2012, 184, 2893-2907.
64. Michell, F.; Werres, F.; Chen, Y.; Shirey, B. Determination of volatile organic compounds in water by SPME and GC/MS: validation of new ISO Standard 17943. Sigma Aldrich.
65. ASTM D6520. Standard practice for the solid phase microextraction (SPME) of water and its headspace for the analysis of volatile and semivolatile organic compounds.
66. ASTM D6889. Standard practice for fast screening for volatile organic compounds in water using solid phase microextraction.
67. EPA Method 8272. Parent and alkyl polycyclic aromatics in sediment pore water by solid phase microextraction and gas chromatography/mass spectrometry in selected ion monitoring mode.
68. ISO 27108:2010. Water Quality – Determination of selected plant treatment agents and biocide products – Method using solid phase microextraction (SPME) followed by gas chromatography – mass spectrometry (GC-MS).
69. Bessonneau, V; Bojko, B; Pawliszyn J. Analysis of human saliva metabolome by direct immersion solie-phase microextraction LC and benchtop orbitrap MS. Bioanalysis, 2013, 5, 783-792.
70. Moder, M.; Popp, P; Pawliszyn, J. Characterization of water-soluble components of slurries using solid-phase microextraction coupled to liquid chromatography-mass spectrometry. J of Microcolumn Separations, 1998, 10, 225-234.
71. Bocato, M.Z; Simoes, R.A.; Calixto, L.A.; Gaitani, M.C.; Pupo, M.T.; Oliveira, A.R.M. Solid phase microextraction and LC-MS/MS for the determination of

paliperidone after stereoselective fungal biotransformation of resperidone. Anal. Chim. Acta. 2012, 742, 80-89.

72. Gomez-belinchon, J.I.; Grimalt, J.O.; Albaiges, J. Volatile organic compounds in two polluted rivers in Barcelona (Catalonia, Spain). Water. Res. 1991, 25, 577-589.
73. Bianchi, A.P.; and Varney, M.S. Volatile organic compounds in the surface waters of a British Estuary Part 1. Occurrence, distribution and variation. Water. Res. 1998, 32, 352-370.
74. Nikolaou, A.D.; Golfinopoulos, S.K.; Kostopoulou, M.N.; Koloythas, G.A.; Lekkas, T.D. Determination of volatile organic compounds in surface waters and treated wastewater in Greece. Water Res., 2002, 36, 2883-2890.
75. Martinez, C.; Ramirez, N.; Gomez, V.; Pocurull, E.; Borrull, F. Simultaneous determination of 76 micropollutants in water samples by headspace solid phase microextraction and gas chromatography-mass spectrometry. Talanta, 2013, 116, 937-945.
76. Robles-Molina, J.; Gilbert-Lopez, B.; Garcia-Reyes, J.F.; Molina-Diaz, A. Comparative evaluationof liquid-liquid extraction, solid-phase extraction and solid-phase microextraction for the gas chromatography-mass spectrometry determination of multiclass priority organic contaminants in wastewater. Talanta, 2013, 11, 382-391.
77. Samuesson, R.; Nilsson, C.; Burvall, J. Sampling and GC-MS as a method for analysis of volatile organic compounds (VOC) emitted during oven drying of biomass materials. Biomass and Bioenergy, 2006, 30, 923-928.
78. Siddique, S.; Azad, S.A.; Bakar, F.A.; Nahel, L.; Kumar, S.V. Separation and identification of hydrocarbons and other volatile compounds from cultures of Aspergillus niger by GC-MS using two different capillary columns and solvents. Journal of Saudi Chemical Society, 2015, 19, 243-256.
79. Pellati, F.; Prencipe, F.P.; Benvenuti, S. Headspace solid-phase microextraction-gas chromatography-mass spectrometry characterization of propolis volatile compounds. J. Pharm. Biomed. Anal. 2013, 84, 103-111.

80. Ciganek, M.; Pisarikova, B.; Zraly, Z. Determination of volatile organic compounds in the curde and heat treated amaranth samples. Veterinari Medicina, 2007, 52, 111-120.
81. Rood, D., A practical guide to the care, maintenance and trouble shooting of capillary gas chromatographic systems, Wiley: Weinheim, 1999.

82. Mani, V. Properties of commercial SPME coatings; In Applications solid phase microextraction; Pawliszyn, J. Ed.; The Royal Society of Chemistry: Cambridge, 1999.
83. Mann, P.S. *Introductory statistics*; John Wiley; New Delhi, 2010.
84. ISO 8466-1:1990. Water quality – calibration of analytical methods and estimation of performance characteristics. Part 1: Statistical evaluation of the linear calibration function
85. Caroli, S. and Loco, J.V. Method validation for food analysis, concepts and use of statistical techniques, In The determination of chemical elements in food: applications for atomic and mass spectrometry; Caroli, S.; John Wiley, 2007.
86. Loco, J.V.; Elskens, M;, Croux, C.; Beernaert, H. Linearity of calibration curves: use and misuse of the correlation coefficient. Accred. Qual. Assur. 2002, 7, 281-285.
87. Andrade, J. M.; Gomez-Carracedo, M. P. Notes on the use of Mandel's test to check for non-linearity in laboratory calibrations. 2013, Anal. Methods, 5, 1145-1149.
88. Trijp, J.M.P. V.; Roos, A. H. "RIKILT-DLO, Model for the calculation of calibration curves," RIKILT report 91.02, Wageningen, Netherlands, 1991.
89. Grubb, F.E. Sample criteria testing for outlying observations. The Annals of Mathematical Statistics, 1950, 21, 27-58.
90. Eurachem Guide, The fitness for purpose analytical methods, a laboratory guide to method validation and related topics; LGC; 1998.
91. Bouque, R.; Morato, A.; Riu, J.; Rius, F.X. Validation of analytical methods. 2002, Grasas y Aceites, 53, 128-143.

92. Thompson, M.; Ellison, S.L.R. ; Wood, R. Harmonized guidelines for single laboratory validation of methods of analysis. Pure Appl. Chem., 2002, 74, 835–855.
93. Huber, L. Validation of analytical methods. Agilent Technologies, 2010 https://www.chem.agilent.com/Library/primers/Public/5990-5140EN.pdf (accessed date 16/7/2015).
94. ISO/IEC 17025:2005. General requirement for the competence of testing and calibration laboratories. International Standard; ISO, 2005.
95. Lab Network. Method Validation. http://www.labnetwork.org/en/chemical-lab-recent/111-method-validation.
96. Reewijk, L.P.V. and Houba V.J.G. Quality of analytical procedures. Guidelines for quality management in soil and plat laboratories. FAO Soils Bulletin 74. 1998, FAO and ISRIC. http://www.fao.org/docrep/w7295e/w7295e09.htm#7 quality of analytical procedures
97. Particle Sciences. Analytical method development and validation. Technical Brief, Volume 5. 2009. http://www.particlesciences.com/news/technical-briefs/2009/analytic-method-development-and-validation.html. (accessed 16/7/2015).
98. Valid Analytical Method (VAM). Introduction to method validation. LGC, 2006. http://www.lgcgroup.com/LGCGroup/media/PDFs/Our%20science/posters/Introduction-to-method-validation.pdf (accessed 16/7/2015).
99. Karla, K. Method development and validation of analytical procedures. Quality control of herbal medicines and related areas, Shoyam, Y. Ed. In Tech., 2011. http://cdn.intechopen.com/pdfs-wm/23463.pdf 6/25/2015
100. ISO 11352. Water quality – Estimation of measurement uncertainty based on validation and quality control data. 2012. ISO.
101. EURAHEM. URACHEM/CITAC Guide, Quantifying uncertainty in analytical measurement. Editors: S.L.R. Ellison and A. Williams. 2011.
102. Henriques, M., Cardoso, V.V., Rodrigues, A.M., Ferreira, E., Benoliel, M.J., and Almeida, C.M.M. Simultaneous determination of ten endocrine

hormone disrupters in water using SPE/LC-(ESI)Ms-MS. Journal of Water Resource and Protection, 2010, 2:818-829.

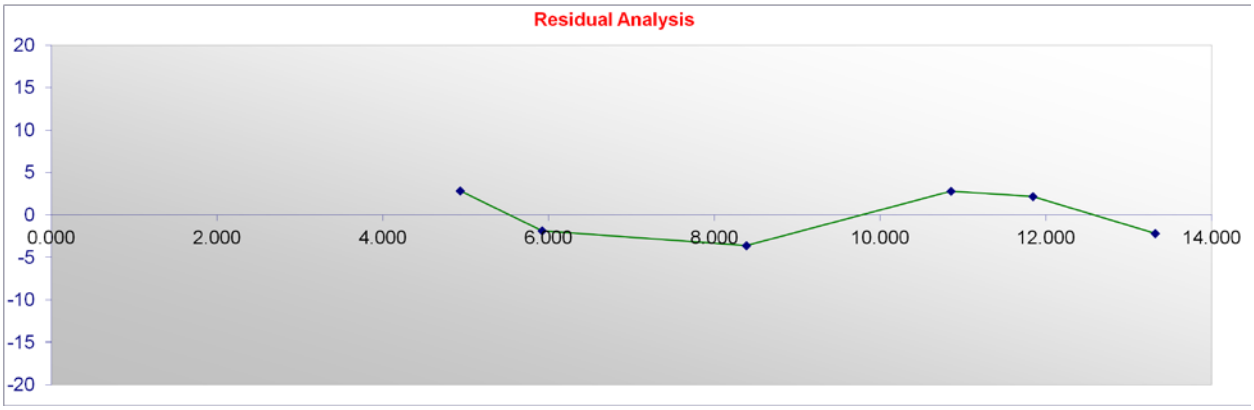
ANNEX I RESIDUAL ANALYSIS

MTBE

Concentration (μg/L)	Peak area
4.933	161614
5.920	182793
8.386	249883
10.853	341446
11.840	369215
13.319	396240

Slope =	29561.3			
Intercept =	11319.4			
R =	0.9965			
X mean =	9.208			
Sy/x =	9302.0	LOD =	0.944μg/L	CA<1.644
S_{xo} =	0.3147	LOQ =	3.147μg/L	CA<4.933
V_{xo} (%) =	3.42			

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
157145.4	1.03	4.933	2.8
186310.6	0.98	5.920	-1.9
259223.6	0.96	8.386	-3.6
332136.5	1.03	10.853	2.8
361325.4	1.02	11.840	2.2
405049.5	0.98	13.319	-2.2



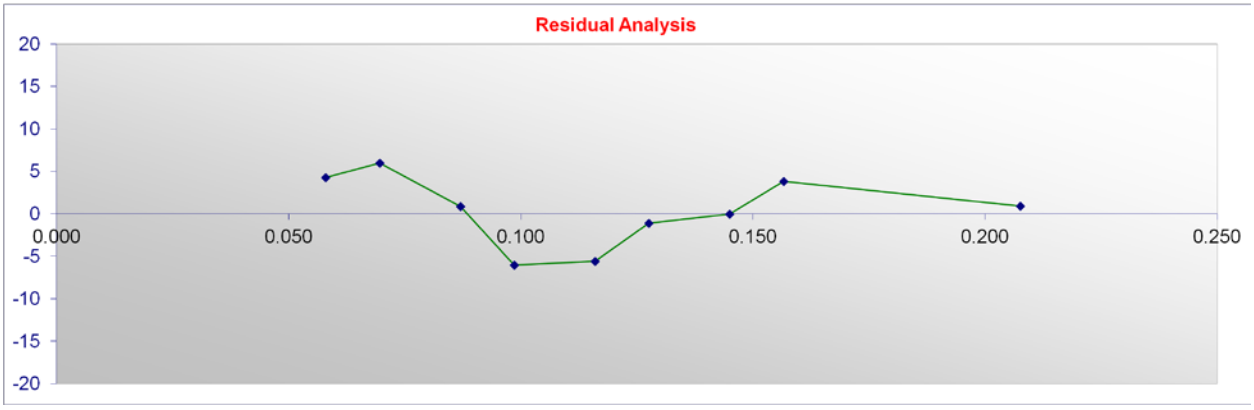
3-ethyltoluene

Concentration (µg/L)	Peak area
0.058	94139
0.070	119049
0.087	146757
0.099	157504
0.116	189488
0.128	220397
0.145	255875
0.157	288627
0.208	378514
0.277	505037

Slope = 1904445.1
Intercept = -20190.2
R = 0.9986
X mean = 0.134
Sy/x = 7262.8
S_{xo} = 0.0038
V_{xo} (%) = **2.84**

LOD = **0.011µg/L** **CA<0.019**
LOQ = **0.038µg/L** **CA<0.058**

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
90267.6	1.04	0.058	4.3
112359.2	1.06	0.070	6.0
145496.5	1.01	0.087	0.9
167588.1	0.94	0.099	-6.0
200725.4	0.94	0.116	-5.6
222817.0	0.99	0.128	-1.1
255954.4	1.00	0.145	0.0
278045.9	1.04	0.157	3.8
375172.6	1.01	0.208	0.9



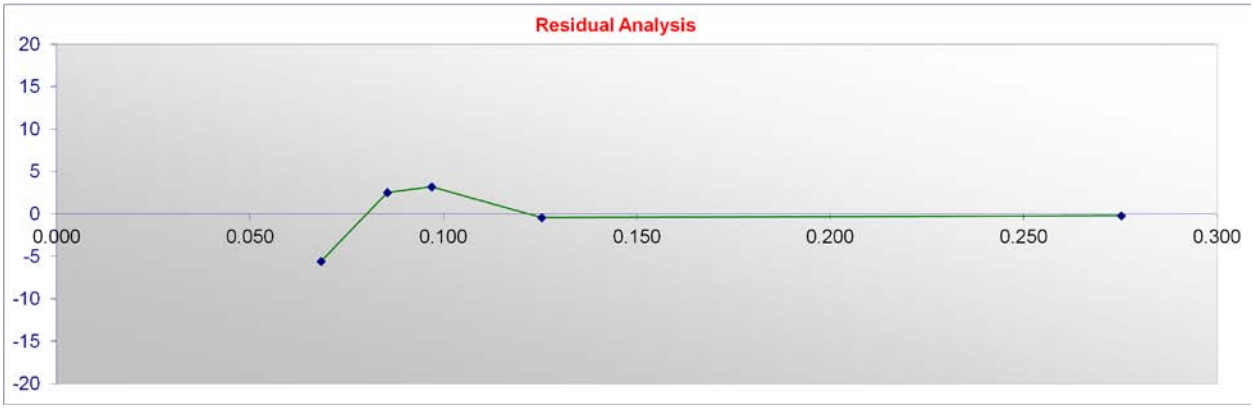
4-ethyltoluene

Concentration (μg/L)	Peak Area
0.068	207342
0.086	257246
0.097	280436
0.125	322511
0.275	597067

Slope = 1831769.7
Intercept = 94277.4
R = 0.9986
X mean = 0.130
Sy/x = 9465.2
S_{xo} = 0.0052
V_{xo} (%) = 3.97

LOD = 0.016μg/L CA<0.023
LOQ = 0.052μg/L CA<0.068

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
219570.5	0.94	0.068	-5.6
250893.8	1.03	0.086	2.5
271775.9	1.03	0.097	3.2
323981.4	1.00	0.125	-0.5
598380.5	1.00	0.275	-0.2



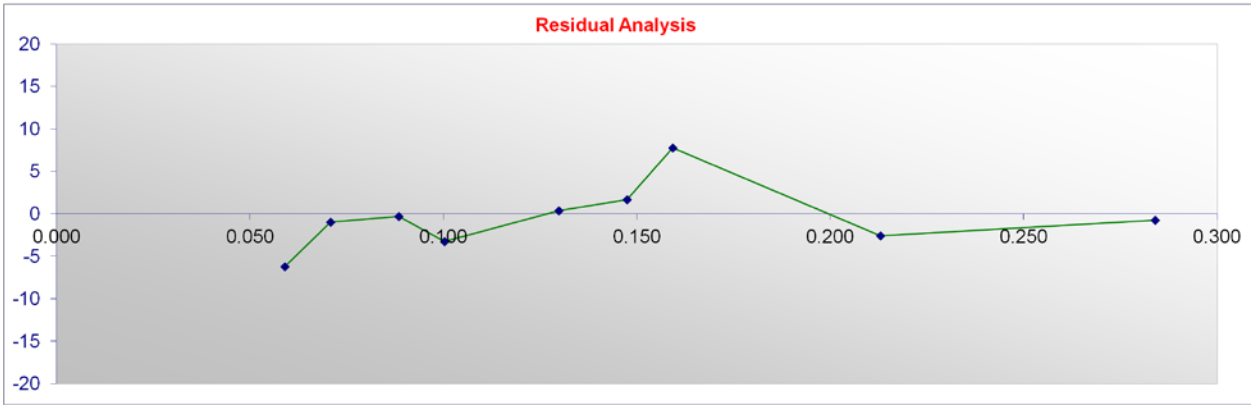
2-ethyltoluene

Concentration (μg/L)	Peak area
0.059	117046
0.071	150234
0.089	191517
0.100	211924
0.130	287436
0.148	332128
0.159	381173
0.213	463980
0.284	633294

Slope = 2281931.5
Intercept = -9855.8
R = 0.9976
X mean = 0.139
Sy/x = 12384.5
S_{xo} = 0.0054
V_{xo} (%) = 3.90

LOD = 0.016μg/L CA<0.020
LOQ = 0.054μg/L CA<0.059

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
124778.1	0.94	0.059	-6.2
151704.9	0.99	0.071	-1.0
192095.1	1.00	0.089	-0.3
219021.9	0.97	0.100	-3.2
286338.9	1.00	0.130	0.4
326729.0	1.02	0.148	1.7
353655.8	1.08	0.159	7.8
476195.6	0.97	0.213	-2.6
638212.7	0.99	0.284	-0.8



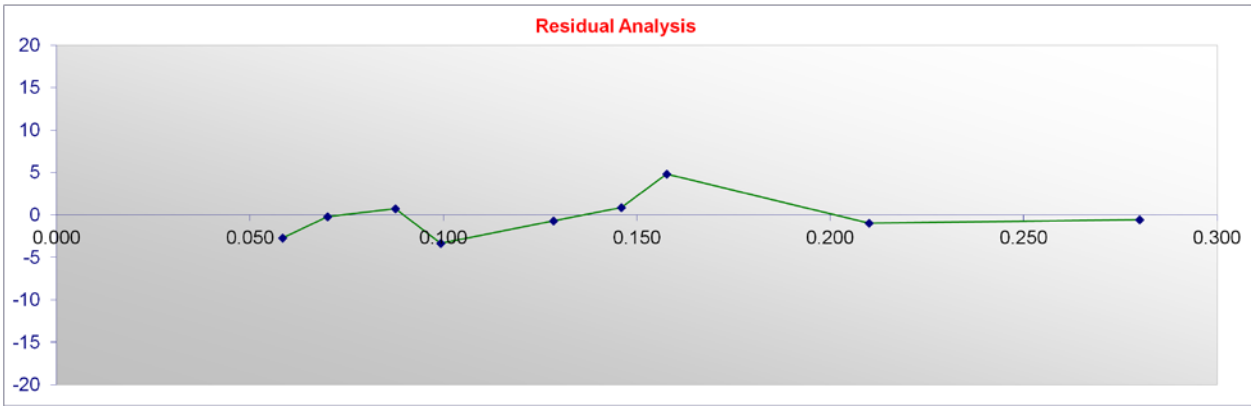
1,2,4-trimethylbenzene

Concentration (μg/L)	Peak Area
0.058	106586
0.070	134369
0.088	173641
0.099	190850
0.128	258488
0.146	300636
0.158	338676
0.210	431452
0.280	582848

Slope = 2151358.4
Intercept = -16089.2
R = 0.9991
X mean = 0.138
Sy/x = 6930.9
S_{xo} = 0.0032
V_{xo} (%) = 2.34

LOD = 0.010μg/L CA<0.019
LOQ = 0.032μg/L CA<0.058

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
109550.1	0.97	0.058	-2.7
134678.0	1.00	0.070	-0.2
172369.8	1.01	0.088	0.7
197497.6	0.97	0.099	-3.4
260317.3	0.99	0.128	-0.7
298009.1	1.01	0.146	0.9
323137.0	1.05	0.158	4.8
435696.0	0.99	0.210	-1.0
586291.1	0.99	0.280	-0.6



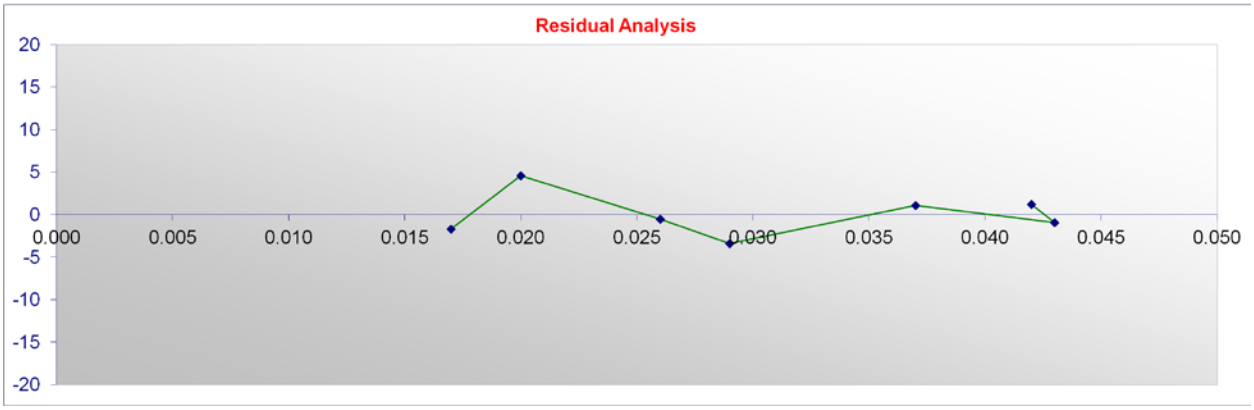
4-isopropyltoluene

Concentration (μg/L)	Peak area
0.017	55964
0.020	73270
0.026	95852
0.029	105766
0.037	146061
0.043	169182
0.042	168414

Slope = 4379524.1
Intercept = -17529.9
R = 0.9986
X mean = 0.031
Sy/x = 2616.0
S_{xo} = 0.0006
V_{xo} (%) = 1.95

LOD = 0.002μg/L CA<0.006
LOQ = 0.006μg/L CA<0.017

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
56922.0	0.98	0.017	-1.7
70060.6	1.05	0.020	4.6
96337.7	0.99	0.026	-0.5
109476.3	0.97	0.029	-3.4
144512.5	1.01	0.037	1.1
170789.7	0.99	0.043	-0.9
166410.1	1.01	0.042	1.2



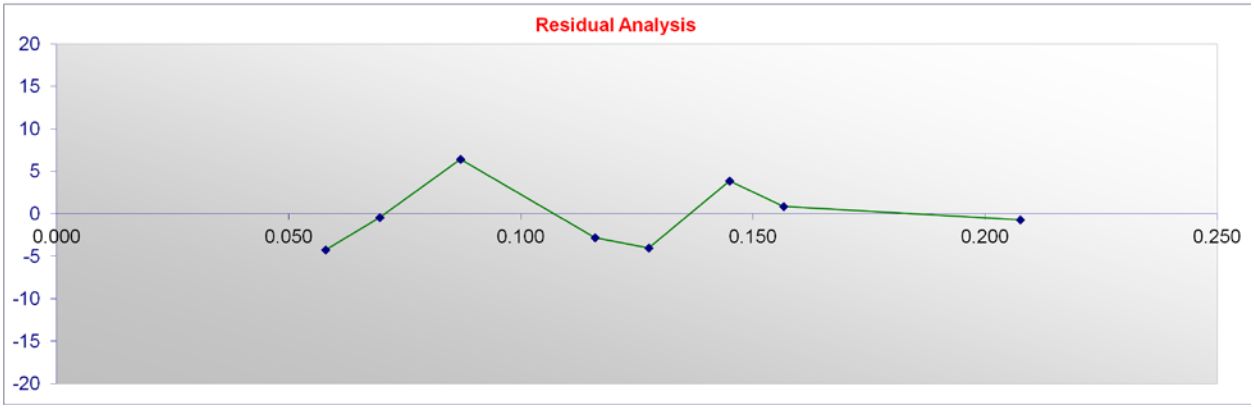
1,3-diethylbenzene

Concentration (μg/L)	Peak area
0.058	153472
0.070	201486
0.087	282603
0.116	360539
0.128	396392
0.145	494636
0.157	522873
0.208	698479

Slope = 3631712.0
Intercept = -50354.8
R = 0.9976
X mean = 0.121
Sy/x = 13578.6
S_{xo} = 0.0037
V_{xo} (%) = 3.09

LOD = 0.011μg/L CA<0.019
LOQ = 0.037μg/L CA<0.058

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
160284.5	0.96	0.058	-4.3
202412.4	1.00	0.070	-0.5
265604.2	1.06	0.087	6.4
370923.8	0.97	0.116	-2.8
413051.7	0.96	0.128	-4.0
476243.5	1.04	0.145	3.9
518371.3	1.01	0.157	0.9
703588.6	0.99	0.208	-0.7



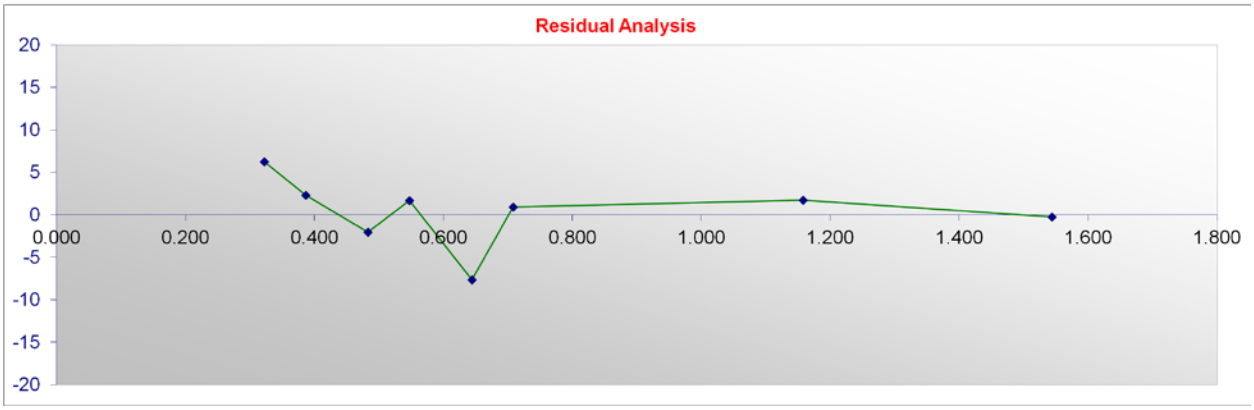
Indane

Concentration (μg/L)	Peak area
0.322	150724
0.386	179980
0.483	222316
0.547	265415
0.644	288193
0.708	349218
1.158	593761
1.544	785794

Slope = 528557.1
Intercept = -28329.5
R = 0.9988
X mean = 0.724
Sy/x = 11689.4
S_{xo} = 0.0221

LOD = 0.066μg/L CA<0.107
LOQ = 0.221μg/L CA<0.322

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
141865.9	1.06	0.322	6.2
175905.0	1.02	0.386	2.3
226963.6	0.98	0.483	-2.0
261002.7	1.02	0.547	1.7
312061.3	0.92	0.644	-7.6
346100.4	1.01	0.708	0.9
583739.6	1.02	1.158	1.7
787762.6	1.00	1.544	-0.2



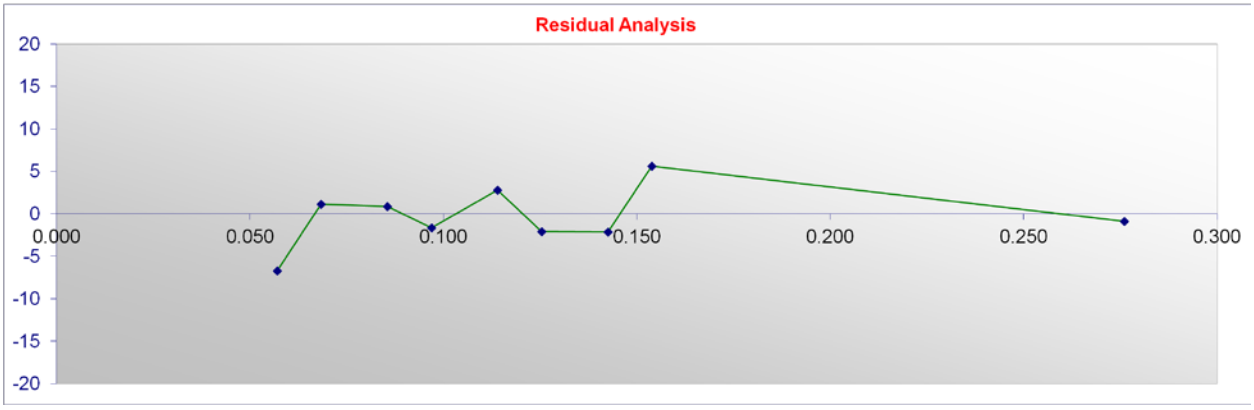
1,4-diethylbenzene/1,3-dimethyl-5-ethylbenzene

Concentration (μg/L)	Peak area
0.057	214972
0.068	285176
0.086	362353
0.097	404024
0.114	501707
0.125	528329
0.143	603784
0.154	706040
0.276	1209480

Slope = 4520128.5
Intercept = -27207.9
R = 0.9983
X mean = 0.124
Sy/x = 18212.9
S_{xo} = 0.0040
V_{xo} (%) = 3.24

LOD = 0.012μg/L CA<0.019
LOQ = 0.040μg/L CA<0.057

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
230439.5	0.93	0.057	-6.7
281968.9	1.01	0.068	1.1
359263.1	1.01	0.086	0.9
410792.6	0.98	0.097	-1.6
488086.8	1.03	0.114	2.8
539616.2	0.98	0.125	-2.1
616910.4	0.98	0.143	-2.1
668439.9	1.06	0.154	5.6
1220347.6	0.99	0.276	-0.9



x

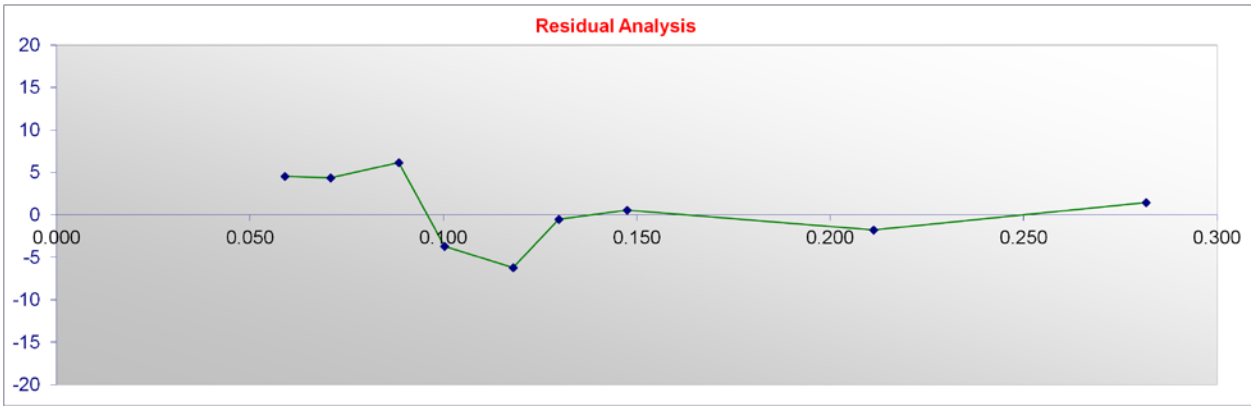
1,2-diethylbenzene

Concentration (μg/L)	Peak area
0.059	148437
0.071	186020
0.089	246986
0.100	258927
0.118	303120
0.130	357716
0.148	416227
0.211	598780
0.282	838055

Slope = 3073020.8
Intercept = -39327.4
R = 0.9986
X mean = 0.134
Sy/x = 12458.5
S_{xo} = 0.0041
V_{xo} (%) = 3.02

LOD = 0.012μg/L CA<0.020
LOQ = 0.041μg/L CA<0.059

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
141980.9	1.05	0.059	4.5
178242.5	1.04	0.071	4.4
232635.0	1.06	0.089	6.2
268896.6	0.96	0.100	-3.7
323289.1	0.94	0.118	-6.2
359550.7	0.99	0.130	-0.5
413943.2	1.01	0.148	0.6
609694.6	0.98	0.211	-1.8
826035.3	1.01	0.282	1.5



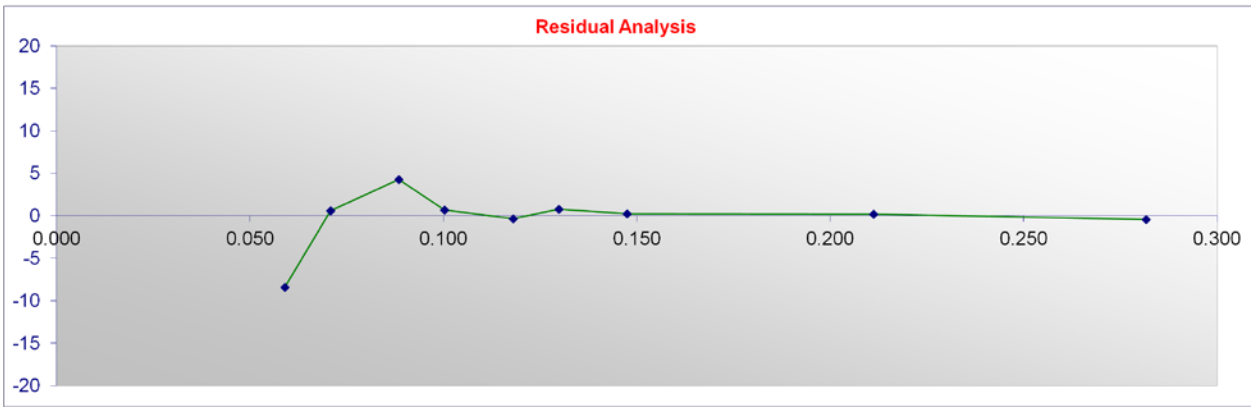
1,4-dimethyl-2-ethylbenzene

Concentration (µg/L)	Peak Area
0.059	91131
0.071	120275
0.089	156052
0.100	170908
0.118	199170
0.130	221631
0.148	250664
0.211	359137
0.282	476104

Slope = 1701565.9
Intercept = -911.9
R = 0.9995
X mean = 0.134
Sy/x = 4156.4
S_{xo} = 0.0024
V_{xo} (%) = 1.82

LOD = 0.007µg/L CA<0.020
LOQ = 0.024µg/L CA<0.059

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
99480.4	0.92	0.059	-8.4
119558.9	1.01	0.071	0.6
149676.6	1.04	0.089	4.3
169755.1	1.01	0.100	0.7
199872.8	1.00	0.118	-0.4
219951.3	1.01	0.130	0.8
250069.0	1.00	0.148	0.2
358458.8	1.00	0.211	0.2
478249.0	1.00	0.282	-0.4



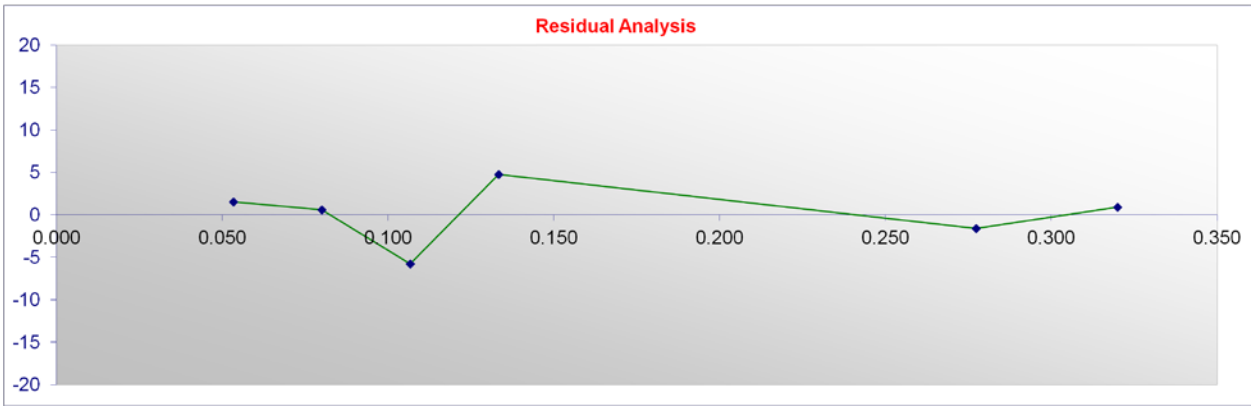
1,3-dimethyl-4-ethylbenzene

Concentration (μg/L)	Peak area
0.053	33339
0.080	53077
0.107	68467
0.133	96984
0.277	196978
0.320	234158

Slope = 746900.1
Intercept = -6998.0
R = 0.9992
X mean = 0.162
Sy/x = 3604.8
S_{xo} = 0.0048
V_{xo} (%) = 2.98

LOD = 0.014μg/L CA<0.018
LOQ = 0.048μg/L CA<0.053

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
32836.7	1.02	0.053	1.5
52754.0	1.01	0.080	0.6
72671.3	0.94	0.107	-5.8
92588.7	1.05	0.133	4.7
200142.3	0.98	0.277	-1.6
232010.0	1.01	0.320	0.9



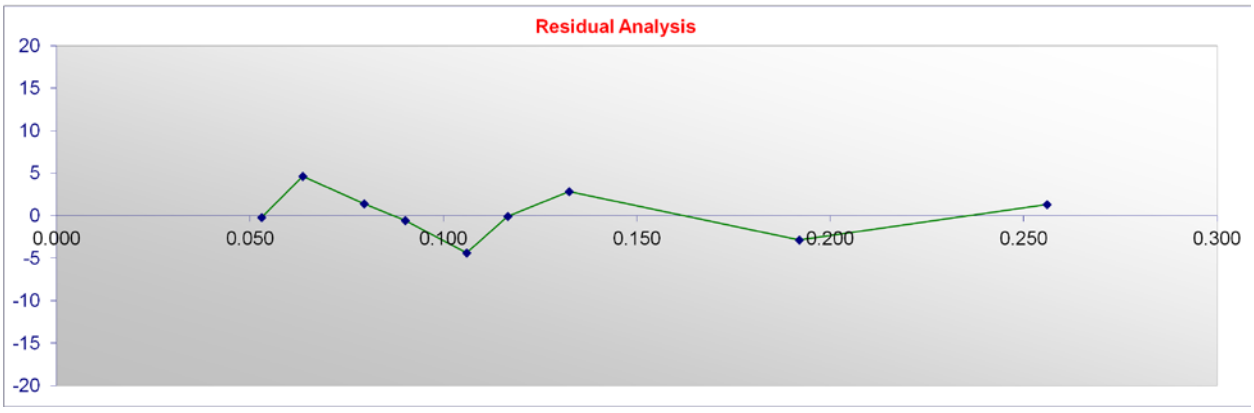
1,2-dimethyl-4-ethylbenzene

Concentration (µg/L)	Peak area
0.053	77746
0.064	101772
0.080	128089
0.090	144810
0.106	167002
0.117	193819
0.133	229338
0.192	322088
0.256	454247

Slope = 1825193.8
Intercept = -18808.1
R = 0.9989
X mean = 0.121
Sy/x = 5945.2
S_{xo} = 0.0033
V_{xo} (%) = 2.69

LOD = 0.010µg/L CA<0.018
LOQ = 0.033µg/L CA<0.053

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
77927.2	1.00	0.053	-0.2
97274.3	1.05	0.064	4.6
126294.8	1.01	0.080	1.4
145641.9	0.99	0.090	-0.6
174662.5	0.96	0.106	-4.4
194009.5	1.00	0.117	-0.1
223030.1	1.03	0.133	2.8
331629.1	0.97	0.192	-2.9
448441.5	1.01	0.256	1.3



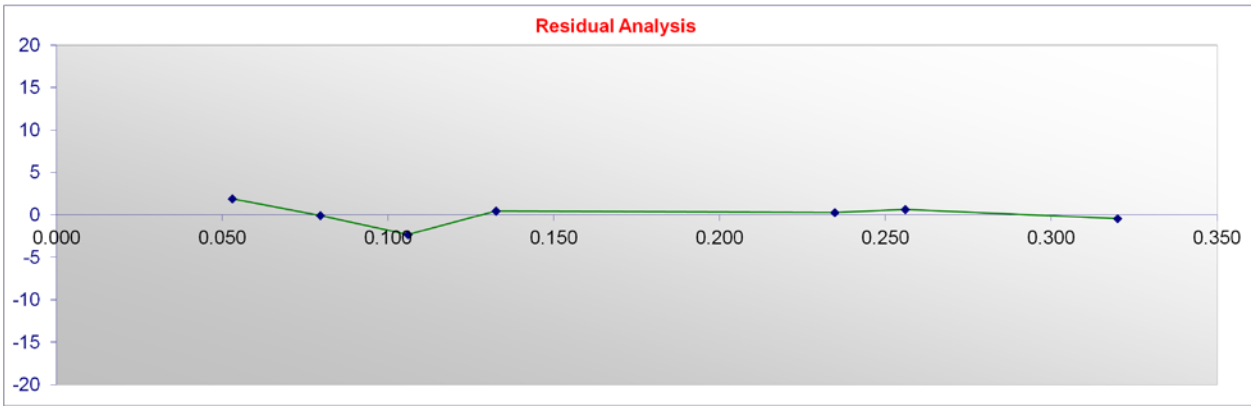
1,2-dimethyl-3-ethylbenzene

Concentration (µg/L)	Peak area
0.053	81195
0.080	124118
0.106	164884
0.133	214289
0.235	386201
0.256	423520
0.320	526183

Slope = 1680892.6
Intercept = -9409.3
R = 0.9999
X mean = 0.169
Sy/x = 2522.4
S_{xo} = 0.0015
V_{xo} (%) = 0.89

LOD = 0.005µg/L CA<0.018
LOQ = 0.015µg/L CA<0.053

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Error (%)
79678.1	1.02	0.053	1.9
124221.7	1.00	0.080	-0.1
168765.4	0.98	0.106	-2.3
213309.0	1.00	0.133	0.5
385040.2	1.00	0.235	0.3
420899.3	1.01	0.256	0.6
528476.4	1.00	0.320	-0.4



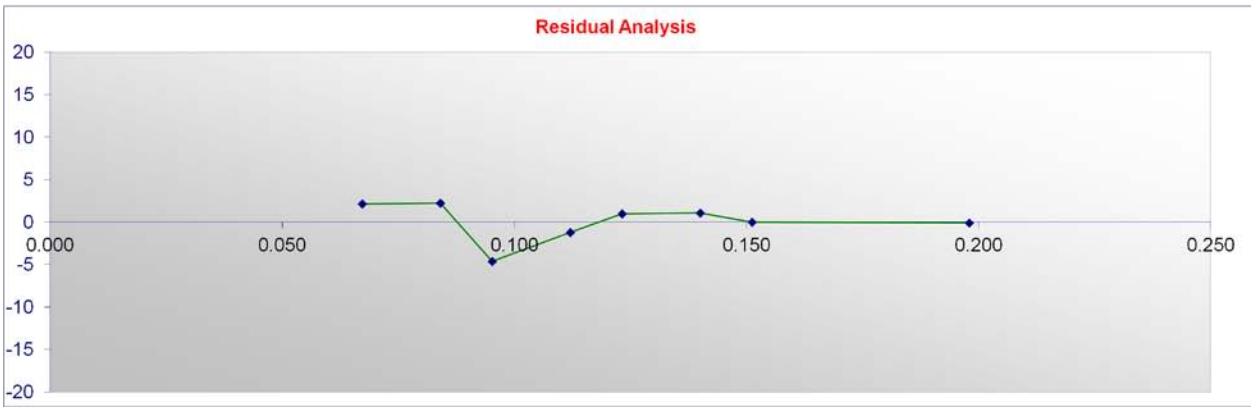
Hexachlorobutadiene

Concentration (μg/L)	Peak area
0.067	128571
0.084	168042
0.095	181293
0.112	225898
0.123	256828
0.140	295985
0.151	318638
0.198	425523

Slope = 2294657.3
Intercept = -28359.4
R = 0.9991
X mean = 0.121
Sy/x = 4503.3
S_{xo} = 0.0020
V_{xo} (%) = 1.62

LOD = 0.006μg/L CA<0.022
LOQ = 0.020μg/L CA<0.067

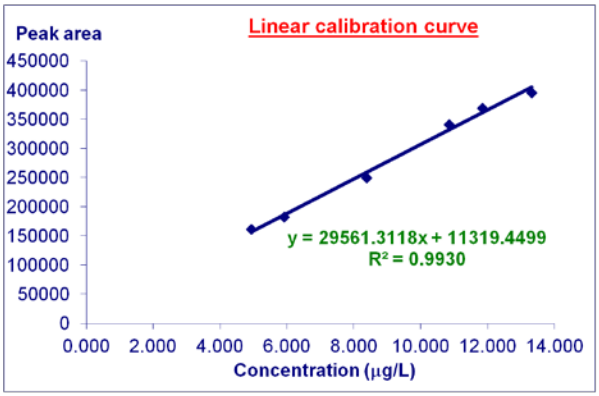
Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Error (%)
125841.6	1.02	0.067	2.2
164391.8	1.02	0.084	2.2
190092.0	0.95	0.095	-4.6
228642.2	0.99	0.112	-1.2
254342.4	1.01	0.123	1.0
292892.6	1.01	0.140	1.1
318592.8	1.00	0.151	0.0
425982.7	1.00	0.198	-0.1

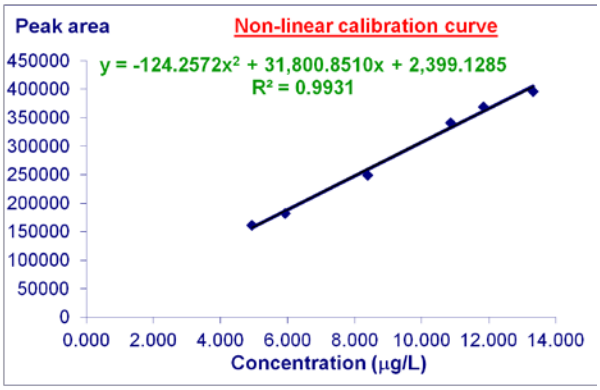


ANNEX II MANDEL'S FITTING TEST

MTBE

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
4.933	161614	157145	156249
5.920	182793	186311	186293
8.386	249883	259224	260346
10.853	341446	332137	332886
11.840	369215	361325	361502
13.319	396240	405050	403915





Linear calibration function

$(y-y_i)^2$
1.997E+07
1.237E+07
8.725E+07
8.667E+07
6.225E+07
7.761E+07

Sum =	3.461E+08
N-2 =	4
$S_{y/x}$ =	9.302E+03

Non-linear calibration function

$(y-y_i)^2$
2.878E+07
1.225E+07
1.095E+08
7.327E+07
5.949E+07
5.890E+07

Sum =	3.422E+08
N-3 =	3
$S_{y/x(2^a)}$ =	1.068E+04

$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^a)}$$

DS^2= 3.944E+06

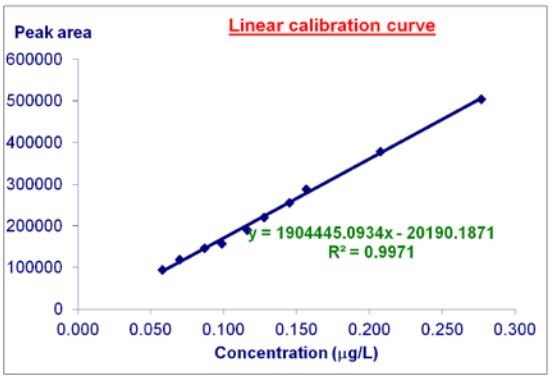
$$VT = DS^2 / S^2_{y/x(2^a)}$$

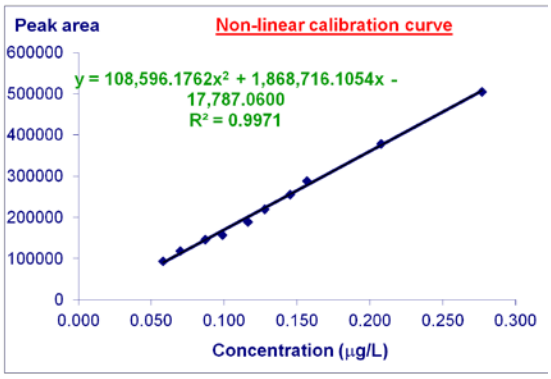
VT= 3.458E-02

$F_{(1,3)95\%}$ = 10.13

3-ethyltoluene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.058	94139	90268	90964
0.070	119049	112359	112802
0.087	146757	145497	145613
0.099	157504	167588	167524
0.116	189488	200725	200445
0.128	220397	222817	222429
0.145	255875	255954	255460
0.157	288627	278046	277517
0.208	378514	375173	374839
0.277	505037	506960	507794





Linear calibration function

	$(y-y_i)^2$
	1.499E+07
	4.475E+07
	1.589E+06
	1.017E+08
	1.263E+08
	5.856E+06
	6.297E+03
	1.120E+08
	1.116E+07
	3.699E+06
Sum =	4.220E+08
N-2 =	8
$S_{y/x}$ =	7.263E+03

Non-linear calibration function

	$(y-y_i)^2$
	1.008E+07
	3.903E+07
	1.308E+06
	1.004E+08
	1.201E+08
	4.130E+06
	1.723E+05
	1.234E+08
	1.351E+07
	7.601E+06
Sum =	4.197E+08
N-3 =	7
$S_{y/x(2^a)}$ =	7.743E+03

$DS^2 = (N - 2)\ S^2_{y/x} - (N - 3)\ S^2_{y/x(2^o)}$

$DS^2 =$ 2.258E+06

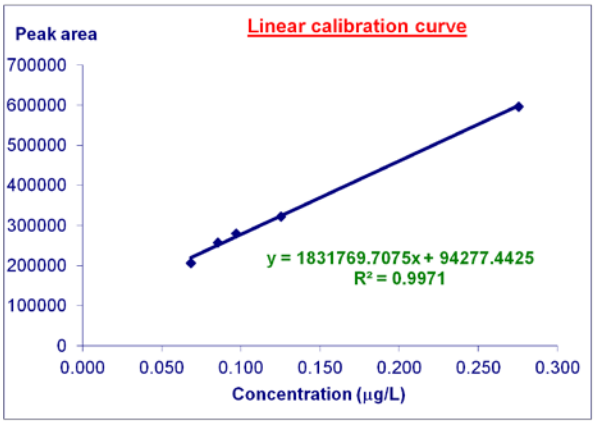
$VT = DS^2 / S^2_{y/x(2^o)}$

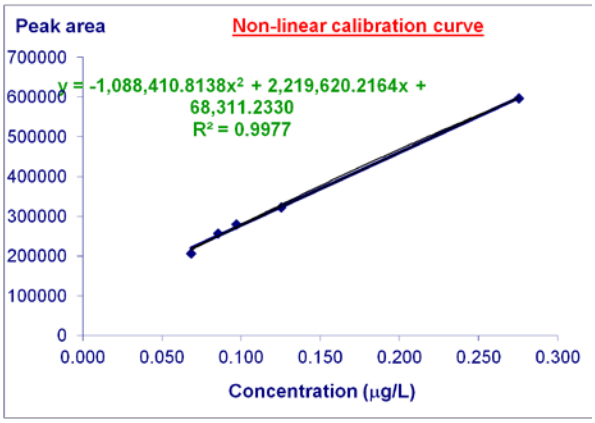
VT= 3.765E-02

$F_{(1,7)95\%}$ = 5.59

4-ethyltoluene

Concentration (µg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.068	207342	219570	215041
0.086	257246	250894	250132
0.097	280436	271776	273173
0.125	322511	323981	329536
0.275	597067	598380	596720





Linear calibration function

$(y-y_i)^2$
1.495E+08
4.035E+07
7.500E+07
2.162E+06
1.725E+06

Sum =	2.688E+08
N-2 =	3
$S_{y/x}$ =	9.465E+03

Non-linear calibration function

$(y-y_i)^2$
5.928E+07
5.061E+07
5.276E+07
4.935E+07
1.205E+05

Sum =	2.121E+08
N-3 =	2
$S_{y/x(2^a)}$ =	1.030E+04

$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^a)}$$

DS^2= 5.666E+07

$$VT = DS^2 / S^2_{y/x(2^a)}$$

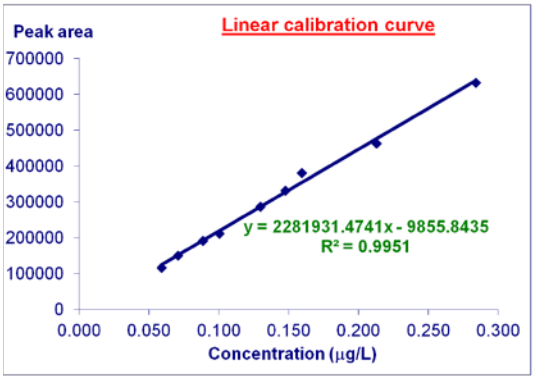
VT= 5.342E-01

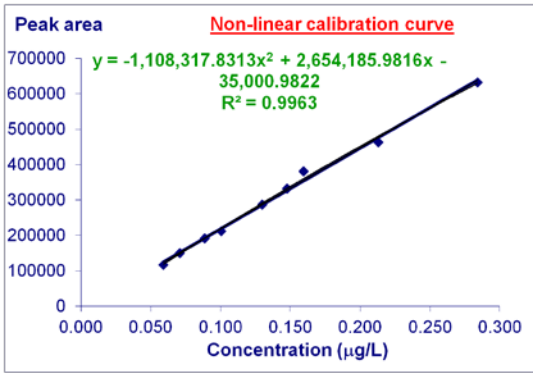
$F_{(1,2)95\%}$= 18.51

xx

2-ethyltoluene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.059	117046	124778	117738
0.071	150234	151705	147360
0.089	191517	192095	191214
0.100	211924	219022	220064
0.130	287436	286339	290839
0.148	332128	326729	332379
0.159	381173	353656	359686
0.213	463980	476196	480057
0.284	633294	638213	629395





Linear calibration function

$(y-y_i)^2$
5.979E+07
2.164E+06
3.342E+05
5.038E+07
1.204E+06
2.915E+07
7.572E+08
1.492E+08
2.419E+07

Sum =	1.074E+09
N-2 =	7
$S_{y/x}$ =	1.238E+04

Non-linear calibration function

$(y-y_i)^2$
4.789E+05
8.261E+06
9.185E+04
6.626E+07
1.158E+07
6.285E+04
4.617E+08
2.585E+08
1.520E+07

Sum =	8.221E+08
N-3 =	6
$S_{y/x(2^a)}$ =	1.171E+04

$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^a)}$$

DS^2= 2.515E+08

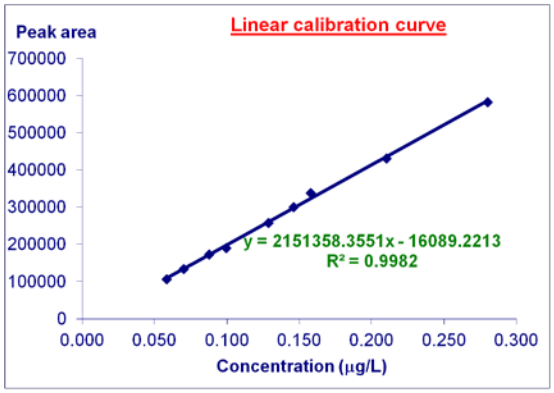
$$VT = DS^2 / S^2_{y/x(2^a)}$$

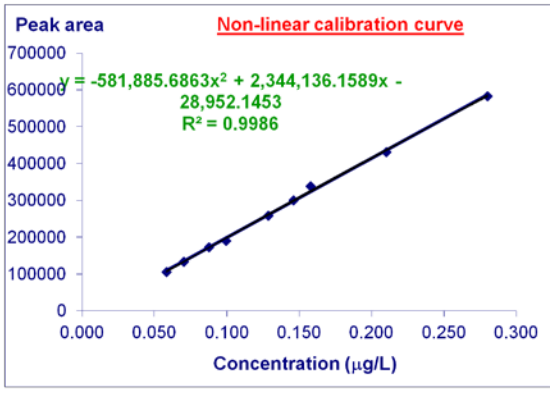
VT= 1.835E+00

$F_{(1,6)95\%}$= 5.99

1,2,4-trimethylbenzene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.058	106586	109550	105961
0.070	134369	134678	132467
0.088	173641	172370	171929
0.099	190850	197498	198038
0.128	258488	260317	262617
0.146	300636	298009	300888
0.158	338676	323137	326204
0.210	431452	435696	437655
0.280	582848	586291	581786





Linear calibration function

$(y-y_i)^2$
8.786E+06
9.546E+04
1.616E+06
4.419E+07
3.346E+06
6.901E+06
2.415E+08
1.801E+07
1.186E+07

Sum =	3.363E+08
N-2 =	7
$S_{y/x}$ =	6.931E+03

Non-linear calibration function

$(y-y_i)^2$
3.908E+05
3.617E+06
2.931E+06
5.167E+07
1.705E+07
6.366E+04
1.556E+08
3.848E+07
1.127E+06

Sum =	2.709E+08
N-3 =	6
$S_{y/x(2^a)}$ =	6.719E+03

$DS^2 = (N - 2)\ S^2_{y/x} - (N - 3)\ S^2_{y/x(2^o)}$

DS^2= 6.538E+07

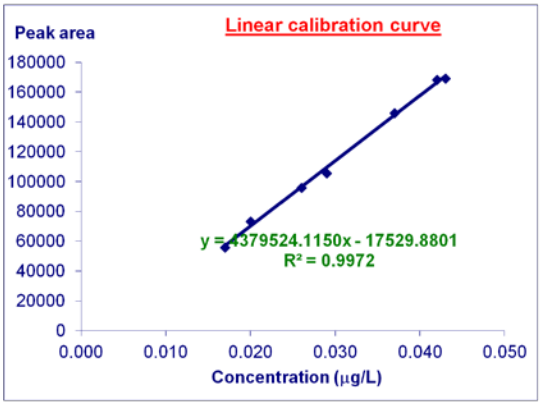
$VT = DS^2 / S^2_{y/x(2^o)}$

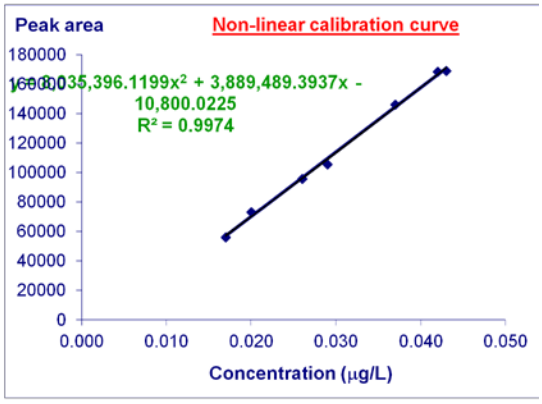
VT= 1.448E+00

$F_{(1,6)95\%}$= 5.99

4-isopropyltoluene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.017	55964	56922	-4614
0.020	73270	70061	-4546
0.026	95852	96338	-4408
0.029	105766	109476	-4339
0.037	146061	144513	-4156
0.043	169182	170790	-4018
0.042	168414	166410	-4041





Linear calibration function

$(y-y_i)^2$
9.178E+05
1.030E+07
2.360E+05
1.377E+07
2.398E+06
2.585E+06
4.015E+06

Sum =	3.422E+07
N-2 =	5
$S_{y/x}$ =	2.616E+03

Non-linear calibration function

$(y-y_i)^2$
3.670E+09
6.055E+09
1.005E+10
1.212E+10
2.257E+10
3.000E+10
2.974E+10

Sum =	1.142E+11
N-3 =	4
$S_{y/x(2^a)}$ =	1.690E+05

$DS^2 = (N - 2)\ S^2_{y/x} - (N - 3)\ S^2_{y/x(2^o)}$

DS^2= -1.142E+11

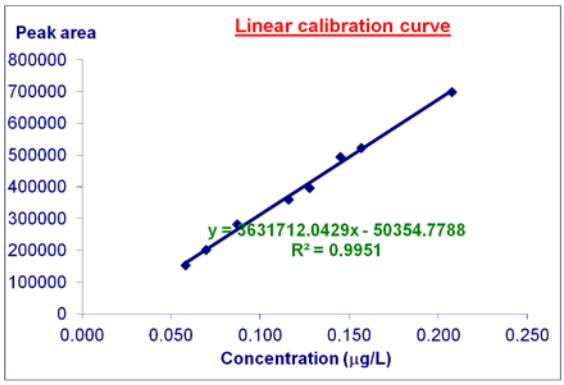
$VT = DS^2 / S^2_{y/x(2^o)}$

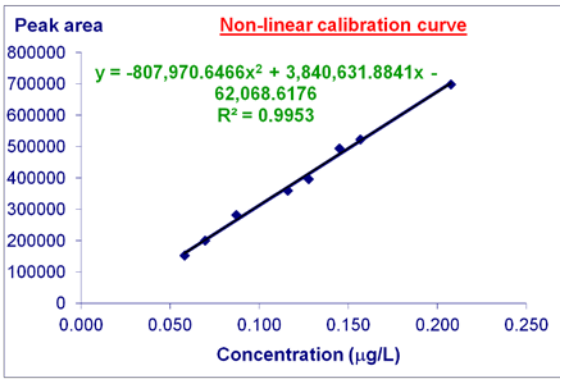
VT= -3.999E+00

$F_{(1,4)95\%}$= 7.71

1,3-diethylbenzene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.058	153472	160285	157970
0.070	201486	202412	201325
0.087	282603	265604	265951
0.116	360539	370924	372573
0.128	396392	413052	414841
0.145	494636	476243	477835
0.157	522873	518371	519560
0.208	698479	703589	700425





Linear calibration function

$(y-y_i)^2$
4.641E+07
8.582E+05
2.890E+08
1.078E+08
2.775E+08
3.383E+08
2.027E+07
2.611E+07

Sum =	1.106E+09
N-2 =	6
$S_{y/x}$ =	1.358E+04

Non-linear calibration function

$(y-y_i)^2$
2.023E+07
2.578E+04
2.773E+08
1.448E+08
3.404E+08
2.823E+08
1.098E+07
3.786E+06

Sum =	1.080E+09
N-3 =	5
$S_{y/x(2^a)}$ =	1.470E+04

$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$$

DS^2= 2.654E+07

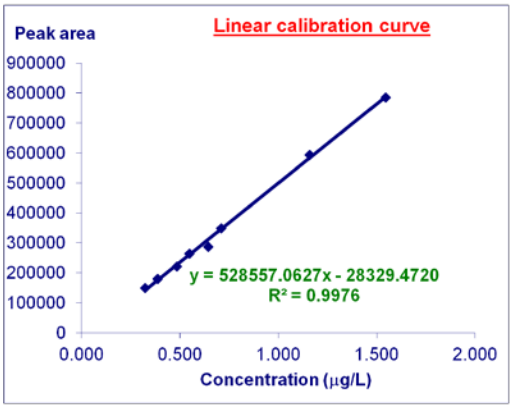
$$VT = DS^2 / S^2_{y/x(2^o)}$$

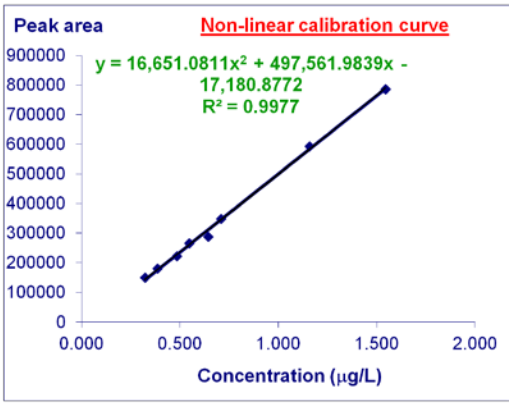
VT= 1.229E-01

$F_{(1.5)95\%}$= 6.61

Indane

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.322	150724	141866	144760
0.386	179980	175905	177563
0.483	222316	226964	227026
0.547	265415	261003	260174
0.644	288193	312061	310155
0.708	349218	346100	343648
1.158	593761	583740	581324
1.544	785794	787763	790750





Linear calibration function

$(y-y_i)^2$
7.847E+07
1.661E+07
2.160E+07
1.947E+07
5.697E+08
9.720E+06
1.004E+08
3.876E+06

Sum =	8.199E+08
N-2 =	6
$S_{y/x}$ =	1.169E+04

Non-linear calibration function

$(y-y_i)^2$
3.556E+07
5.841E+06
2.218E+07
2.747E+07
4.823E+08
3.103E+07
1.547E+08
2.456E+07

Sum =	7.836E+08
N-3 =	5
$S_{y/x(2^a)}$ =	1.252E+04

$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^a)}$$

DS^2= 3.622E+07

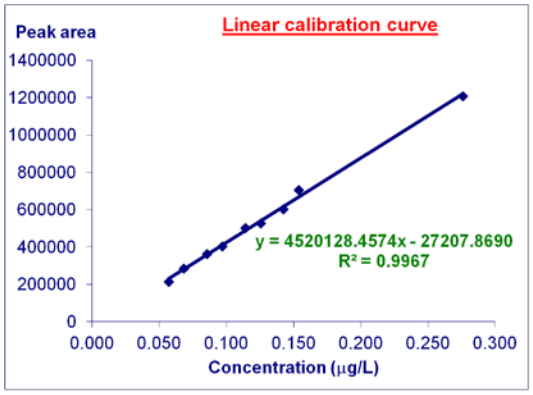
$$VT = DS^2 / S^2_{y/x(2^a)}$$

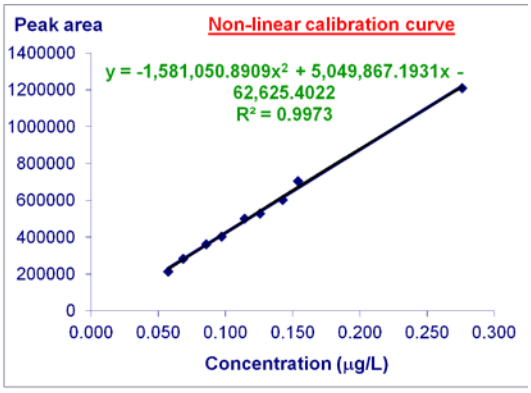
VT= 2.311E-01

$F_{(1.5)95\%}$= 6.61

1,4-diethylbenzene/1,3-dimethyl-5-ethylbenzene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.057	214972	230439	220080
0.068	285176	281969	275388
0.086	362353	359263	357580
0.097	404024	410793	411861
0.114	501707	488087	492512
0.125	528329	539616	545766
0.143	603784	616910	624875
0.154	706040	668440	677102
0.276	1209480	1220348	1210700





Linear calibration function

	$(y-y_i)^2$
	2.392E+08
	1.029E+07
	9.547E+06
	4.581E+07
	1.855E+08
	1.274E+08
	1.723E+08
	1.414E+09
	1.181E+08
Sum =	2.322E+09
N-2 =	7
$S_{y/x}$ =	1.821E+04

Non-linear calibration function

	$(y-y_i)^2$
	2.609E+07
	9.580E+07
	2.278E+07
	6.142E+07
	8.455E+07
	3.040E+08
	4.448E+08
	8.374E+08
	1.488E+06
Sum =	1.878E+09
N-3 =	6
$S_{y/x(2^a)}$ =	1.769E+04

$DS^2 = (N - 2)\ S^2_{y/x} - (N - 3)\ S^2_{y/x(2^o)}$

DS^2= 4.435E+08

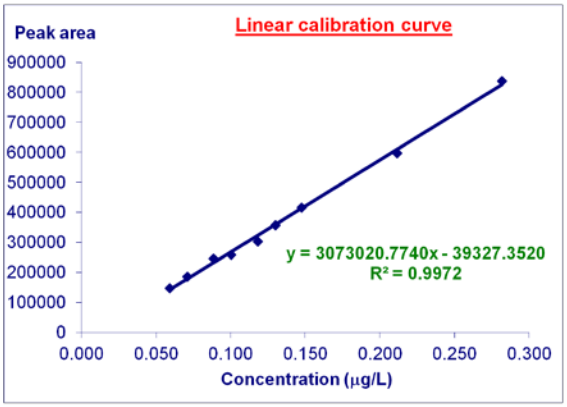
$VT = DS^2 / S^2_{y/x(2^o)}$

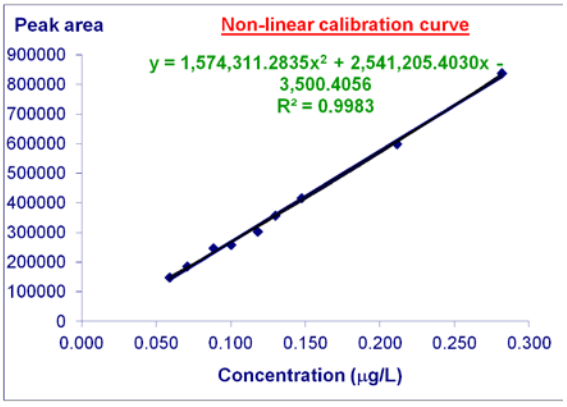
VT= 1.417E+00

$F_{(1,6)95\%}$= 5.99

1,2-diethylbenzene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.059	148437	141981	151911
0.071	186020	178243	184308
0.089	246986	232635	233727
0.100	258927	268897	267220
0.118	303120	323289	318283
0.130	357716	359551	352872
0.148	416227	413943	405579
0.211	598780	609695	603425
0.282	838055	826035	836944





Linear calibration function

$(y-y_i)^2$
4.168E+07
6.049E+07
2.060E+08
9.939E+07
4.068E+08
3.366E+06
5.216E+06
1.191E+08
1.445E+08

Sum =	1.086E+09
N-2 =	7
$S_{y/x}$ =	1.246E+04

Non-linear calibration function

$(y-y_i)^2$
1.207E+07
2.930E+06
1.758E+08
6.878E+07
2.299E+08
2.346E+07
1.134E+08
2.158E+07
1.235E+06

Sum =	6.492E+08
N-3 =	6
$S_{y/x(2^a)}$ =	1.040E+04

$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$

DS^2= 4.373E+08

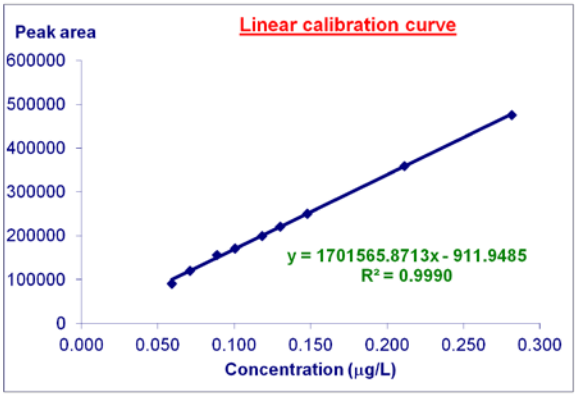
$VT = DS^2 / S^2_{y/x(2^o)}$

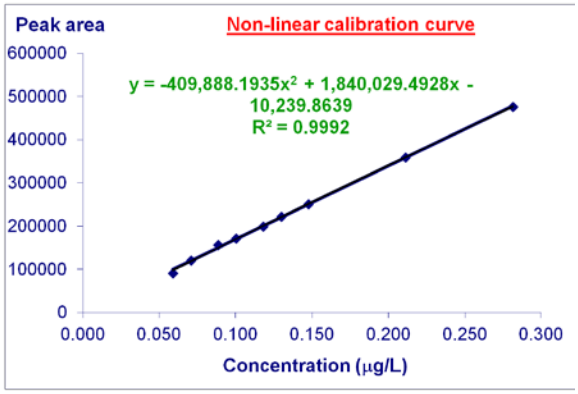
VT= 4.042E+00

$F_{(1,6)95\%}$= 5.99

1,4-dimethyl-2-ethylbenzene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.059	91131	99480	96895
0.071	120275	119559	117980
0.089	156052	149677	149392
0.100	170908	169755	170192
0.118	199170	199873	201176
0.130	221631	219951	221690
0.148	250664	250069	252247
0.211	359137	358459	360091
0.282	476104	478249	475409





Linear calibration function

$(y-y_i)^2$
6.971E+07
5.128E+05
4.065E+07
1.329E+06
4.940E+05
2.821E+06
3.540E+05
4.600E+05
4.601E+06

Sum =	1.209E+08
N-2 =	7
$S_{y/x}$ =	4.156E+03

Non-linear calibration function

$(y-y_i)^2$
3.322E+07
5.269E+06
4.435E+07
5.133E+05
4.025E+06
3.495E+03
2.505E+06
9.103E+05
4.832E+05

Sum =	9.128E+07
N-3 =	6
$S_{y/x(2^a)}$ =	3.901E+03

$DS^2 = (N - 2)\ S^2_{y/x} - (N - 3)\ S^2_{y/x(2^o)}$

DS^2= 2.965E+07

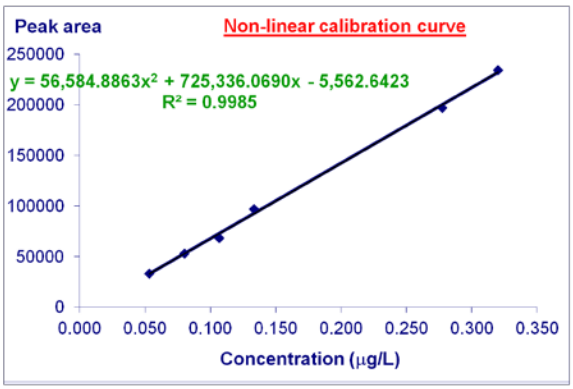
$VT = DS^2 / S^2_{y/x(2^o)}$

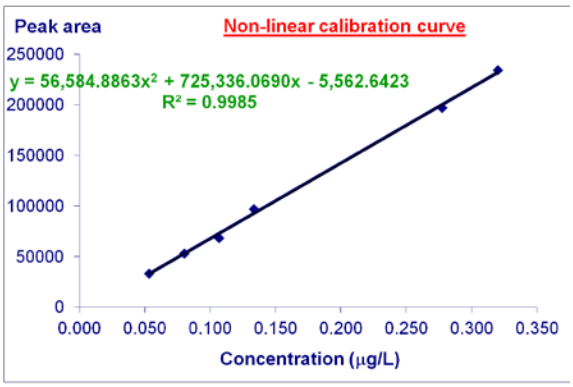
VT= 1.949E+00

$F_{(1,6)95\%}$= 5.99

1,3-dimethyl-4-ethylbenzene

Concentration (µg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.053	33339	32837	33283
0.080	53077	52754	52826
0.107	68467	72671	72450
0.133	96984	92589	92155
0.277	196978	200142	199949
0.320	234158	232010	232339





Linear calibration function

$(y-y_i)^2$
2.523E+05
1.043E+05
1.768E+07
1.932E+07
1.001E+07
4.614E+06

Sum =	5.198E+07
N-2 =	4
$S_{y/x}$ =	3.605E+03

Non-linear calibration function

$(y-y_i)^2$
3.142E+03
6.278E+04
1.587E+07
2.332E+07
8.829E+06
3.308E+06

Sum =	5.139E+07
N-3 =	3
$S_{y/x(2^a)}$ =	4.139E+03

$DS^2 = (N - 2) S^2_{y/x} - (N - 3) S^2_{y/x(2^a)}$

DS^2= 5.870E+05

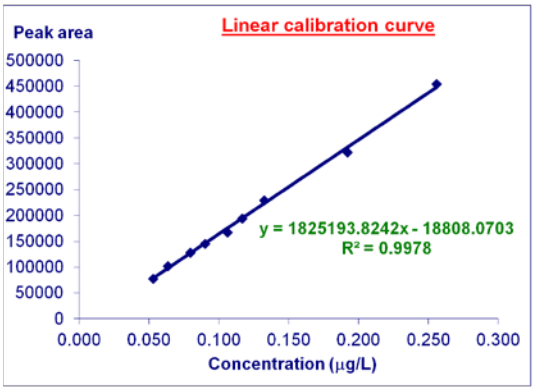
$VT = DS^2 / S^2_{y/x(2^a)}$

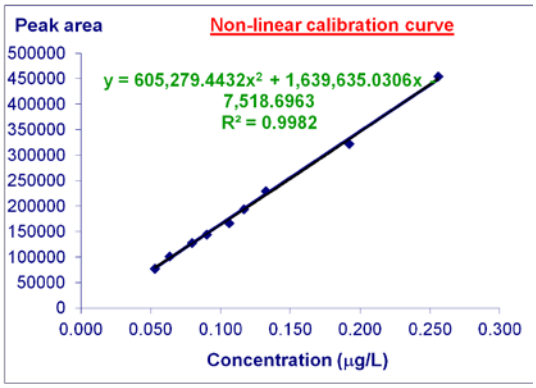
VT= 3.427E-02

$F_{(1.3)95\%}$= 10.13

1,2-dimethyl-4-ethylbenzene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.053	77746	77927	81082
0.064	101772	97274	99210
0.080	128089	126295	126658
0.090	144810	145642	145126
0.106	167002	174662	173084
0.117	193819	194010	191892
0.133	229338	223030	220359
0.192	322088	331629	329604
0.256	454247	448442	451895





Linear calibration function

$(y-y_i)^2$
3.283E+04
2.023E+07
3.219E+06
6.920E+05
5.868E+07
3.630E+04
3.979E+07
9.103E+07
3.370E+07

Sum =	2.474E+08
N-2 =	7
$S_{y/x}$ =	5.945E+03

Non-linear calibration function

$(y-y_i)^2$
1.113E+07
6.562E+06
2.048E+06
9.991E+04
3.698E+07
3.714E+06
8.062E+07
5.649E+07
5.530E+06

Sum =	2.032E+08
N-3 =	6
$S_{y/x(2^a)}$ =	5.819E+03

$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^a)}$$

DS^2= 4.424E+07

$$VT = DS^2 / S^2_{y/x(2^a)}$$

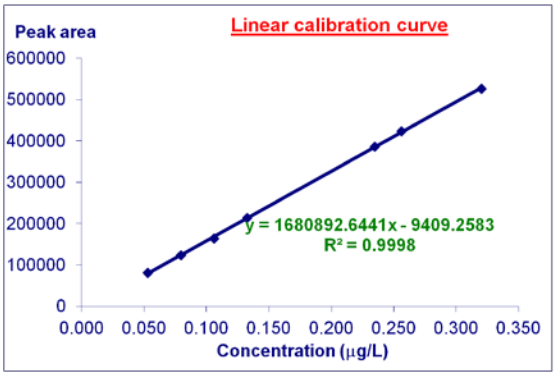
VT= 1.306E+00

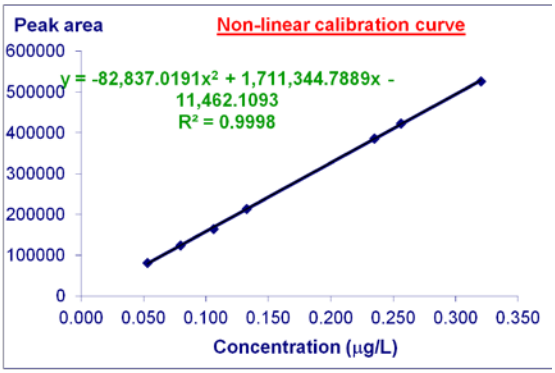
$F_{(1,6)95\%}$= 5.99

xxx

1,2-dimethyl-3-ethylbenzene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.053	81195	79678	79006
0.080	124118	124222	124066
0.106	164884	168765	169010
0.133	214289	213309	213837
0.235	386201	385040	385572
0.256	423520	420899	421213
0.320	526183	528476	527686





Linear calibration function

$(y-y_i)^2$
2.301E+06
1.076E+04
1.506E+07
9.604E+05
1.347E+06
6.868E+06
5.260E+06

Sum =	3.181E+07
N-2 =	5
$S_{y/x}$ =	2.522E+03

Non-linear calibration function

$(y-y_i)^2$
4.790E+06
2.677E+03
1.702E+07
2.045E+05
3.959E+05
5.321E+06
2.258E+06

Sum =	2.999E+07
N-3 =	4
$S_{y/x(2^a)}$ =	2.738E+03

$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^o)}$

DS^2= 1.820E+06

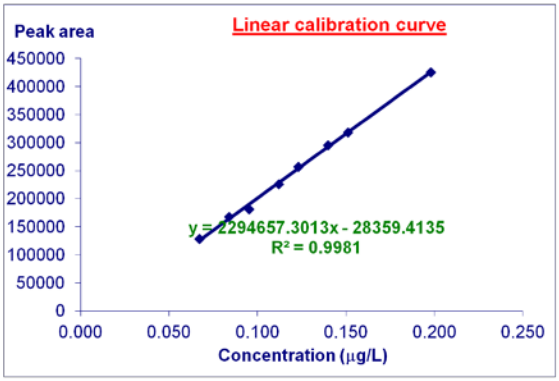
$VT = DS^2 / S^2_{y/x(2^o)}$

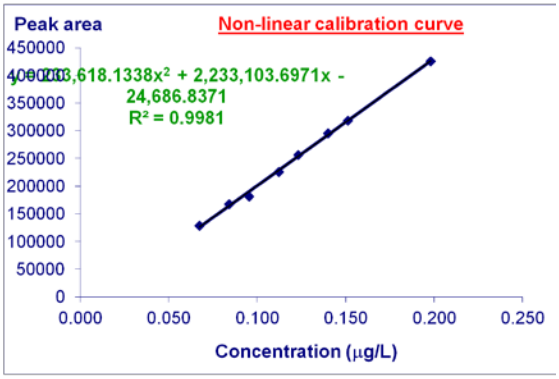
VT= 2.427E-01

$F_{(1,4)95\%}$= 7.71

Hexachlorobutadiene

Concentration (μg/L)	Peak Area	Estimated Area Linear calibration function	Estimated Area Non-linear calibration function
0.067	128571	125842	126433
0.084	168042	164392	164542
0.095	181293	190092	190022
0.112	225898	228642	228351
0.123	256828	254342	253977
0.140	295985	292893	292527
0.151	318638	318593	318299
0.198	425523	425983	426626





Linear calibration function

$(y-y_i)^2$
7.450E+06
1.332E+07
7.742E+07
7.531E+06
6.178E+06
9.563E+06
2.046E+03
2.114E+05

Sum =	1.217E+08
N-2 =	6
$S_{y/x}$ =	4.503E+03

Non-linear calibration function

$(y-y_i)^2$
4.572E+06
1.225E+07
7.619E+07
6.019E+06
8.125E+06
1.196E+07
1.147E+05
1.218E+06

Sum =	1.205E+08
N-3 =	5
$S_{y/x(2^a)}$ =	4.908E+03

$$DS^2 = (N - 2)\, S^2_{y/x} - (N - 3)\, S^2_{y/x(2^a)}$$

DS^2= 1.229E+06

$$VT = DS^2 / S^2_{y/x(2^a)}$$

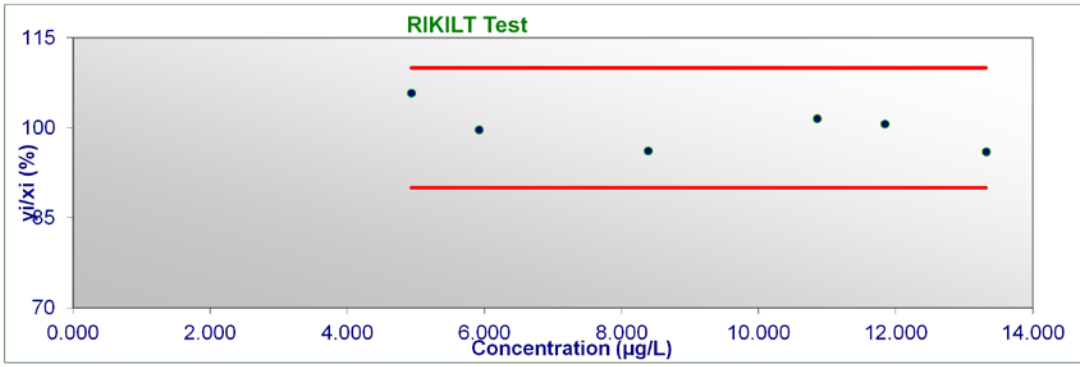
VT= 5.103E-02

$F_{(1,5)95\%}$= 6.61

ANNEX III RIKILT TEST

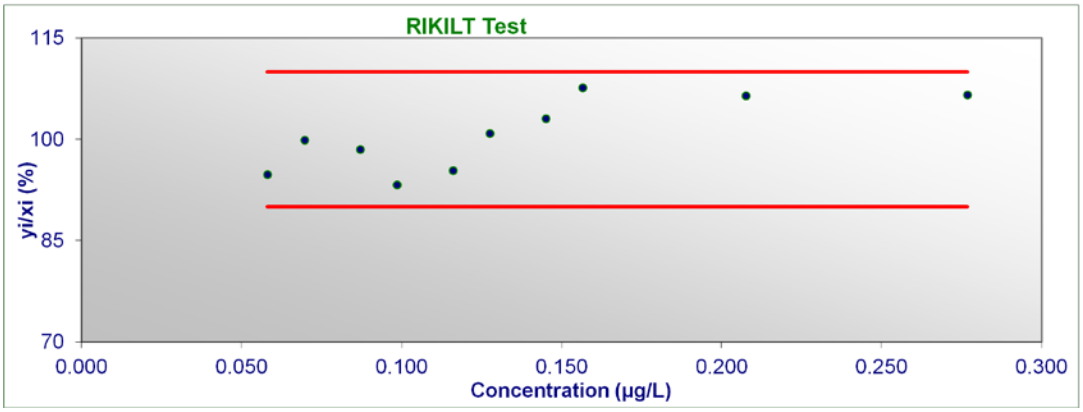
MTBE

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
4.933	161614	32761.8	106	110	90
5.920	182793	30879.3	100	110	90
8.386	249883	29797.3	96	110	90
10.853	341446	31462.1	102	110	90
11.840	369215	31183.7	101	110	90
13.319	396240	29749.8	96	110	90



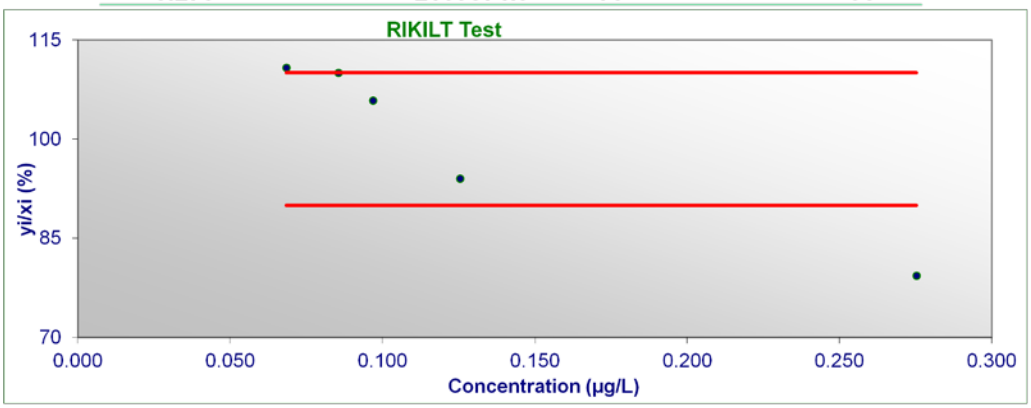
3-ethyltoluene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.058	94139	1623086.2	95	110	90
0.070	119049	1710474.1	100	110	90
0.087	146757	1686862.1	99	110	90
0.099	157504	1597403.7	93	110	90
0.116	189488	1633517.2	95	110	90
0.128	220397	1727249.2	101	110	90
0.145	255875	1764655.2	103	110	90
0.157	288627	1843084.3	108	110	90
0.208	378514	1823285.2	106	110	90
0.277	505037	1824555.6	107	110	90



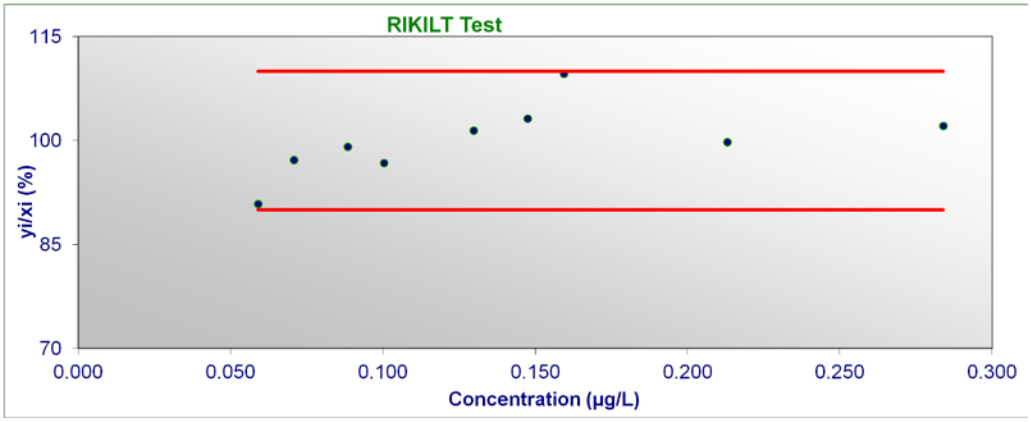
4-ethyltoluene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.068	207342	3031315.8	111	110	90
0.086	257246	3008725.1	110	110	90
0.097	280436	2894076.4	106	110	90
0.125	322511	2571858.1	94	110	90
0.275	597067	2169574.9	79	110	90



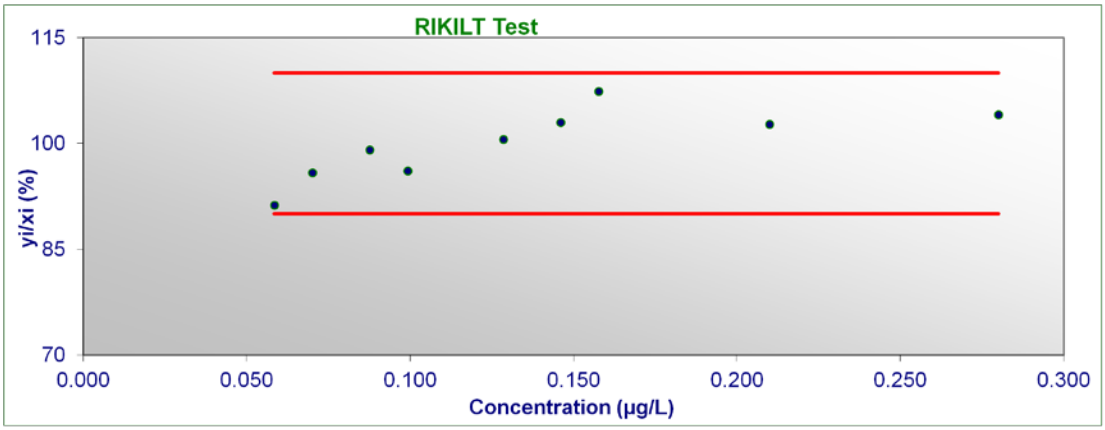
2-ethyltoluene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.059	117046	1983830.5	91	110	90
0.071	150234	2121949.2	97	110	90
0.089	191517	2164033.9	99	110	90
0.100	211924	2112901.3	97	110	90
0.130	287436	2214453.0	101	110	90
0.148	332128	2251715.3	103	110	90
0.159	381173	2392799.7	110	110	90
0.213	463980	2178309.9	100	110	90
0.284	633294	2229908.5	102	110	90



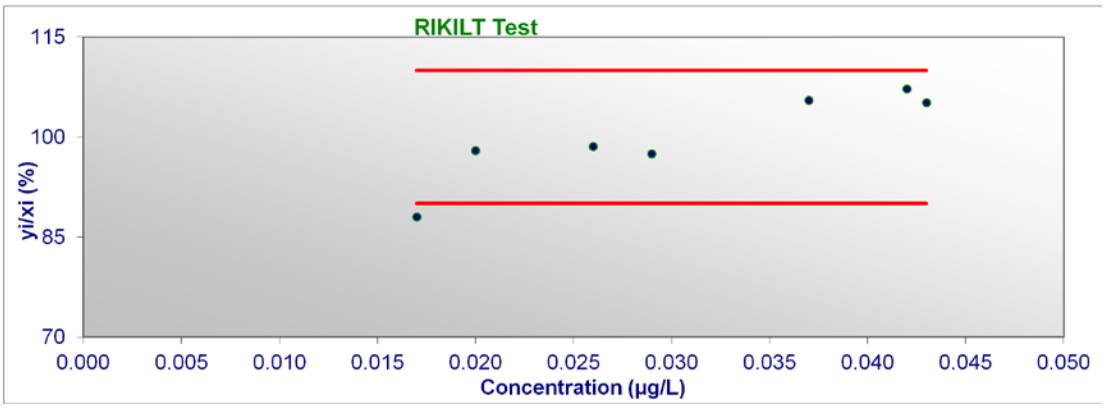
1,2,4-trimethylbenzene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.058	106586	1825102.7	91	110	90
0.070	134369	1917365.9	96	110	90
0.088	173641	1982203.2	99	110	90
0.099	190850	1922340.9	96	110	90
0.128	258488	2011892.9	101	110	90
0.146	300636	2059150.7	103	110	90
0.158	338676	2147869.1	107	110	90
0.210	431452	2054533.3	103	110	90
0.280	582848	2081600.0	104	110	90



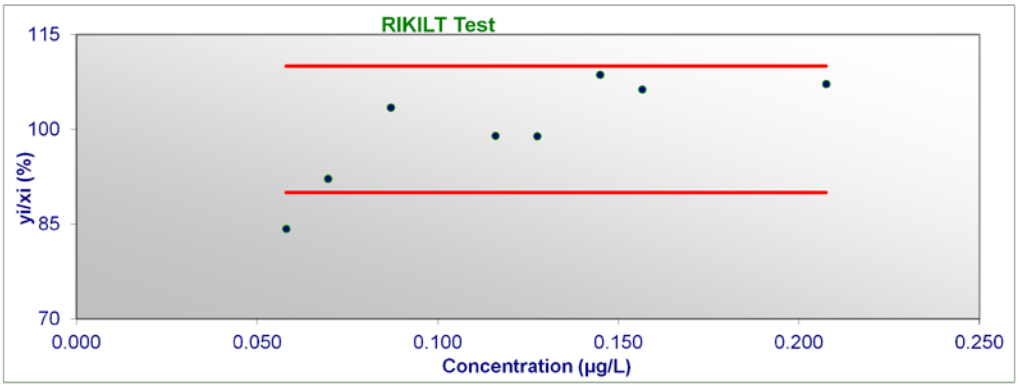
4-isopropyltoluene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.017	55964	3292000.0	88	110	90
0.020	73270	3663500.0	98	110	90
0.026	95852	3686615.4	99	110	90
0.029	105766	3647103.4	98	110	90
0.037	146061	3947594.6	106	110	90
0.043	169182	3934465.1	105	110	90
0.042	168414	4009857.1	107	110	90



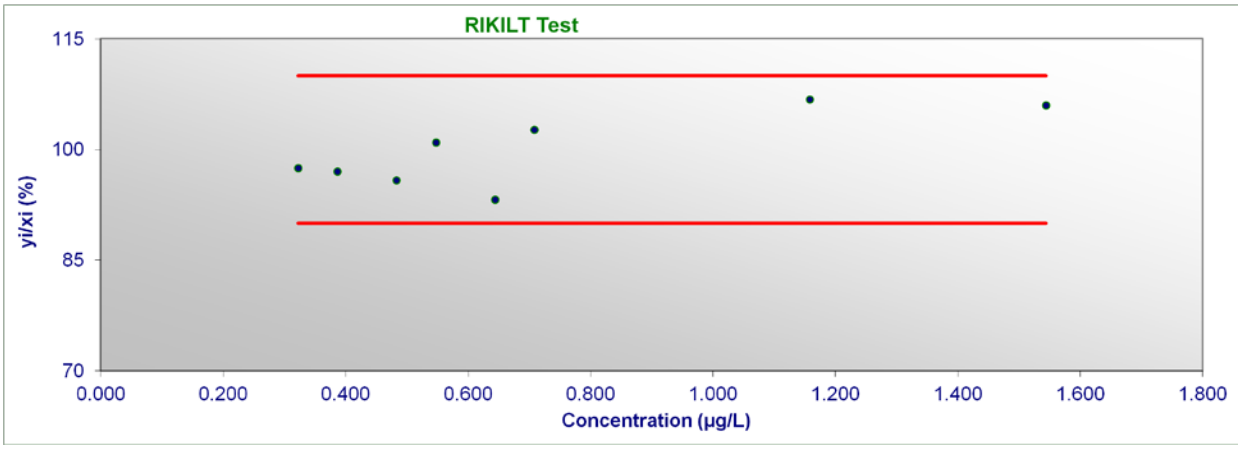
1,3-diethylbenzene

Concentration (μg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.058	153472	2646069.0	84	110	90
0.070	201486	2894913.8	92	110	90
0.087	282603	3248310.3	103	110	90
0.116	360539	3108094.8	99	110	90
0.128	396392	3106520.4	99	110	90
0.145	494636	3411282.8	109	110	90
0.157	522873	3338908.0	106	110	90
0.208	698479	3364542.4	107	110	90



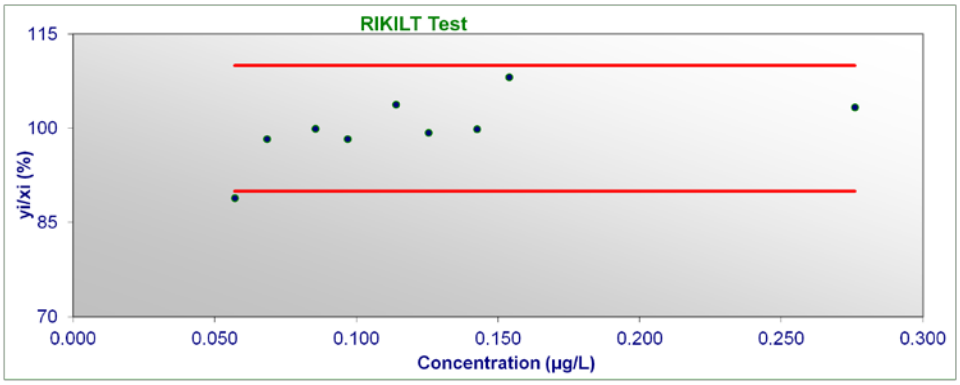
Indane

Concentration (μg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.322	150724	468087.0	97	110	90
0.386	179980	465786.7	97	110	90
0.483	222316	460281.6	96	110	90
0.547	265415	484864.8	101	110	90
0.644	288193	447504.7	93	110	90
0.708	349218	492967.3	103	110	90
1.158	593761	512747.0	107	110	90
1.544	785794	508933.9	106	110	90



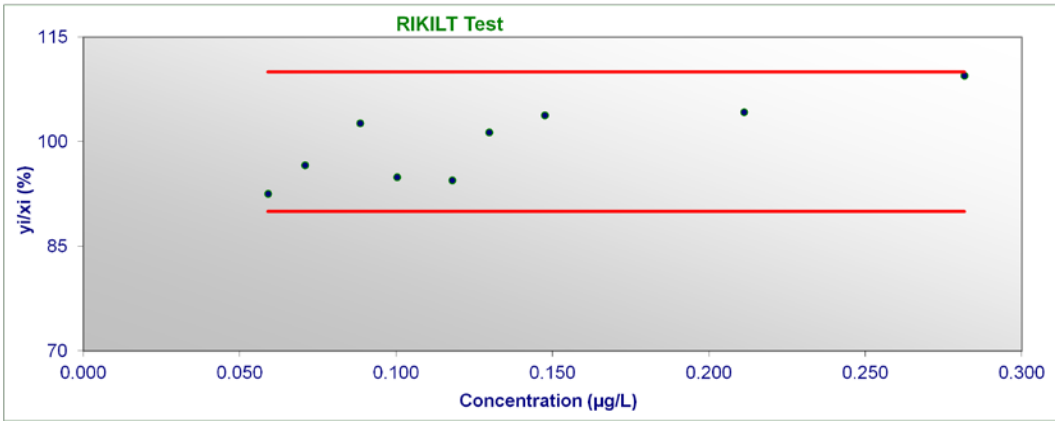
1,4-diethylbenzene/1,3-dimethyl-5-ethylbenzene

Concentration (μg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.057	214972	3771438.6	89	110	90
0.068	285176	4169239.8	98	110	90
0.086	362353	4238046.8	100	110	90
0.097	404024	4169494.3	98	110	90
0.114	501707	4400938.6	104	110	90
0.125	528329	4213149.9	99	110	90
0.143	603784	4237080.7	100	110	90
0.154	706040	4587654.3	108	110	90
0.276	1209480	4382173.9	103	110	90



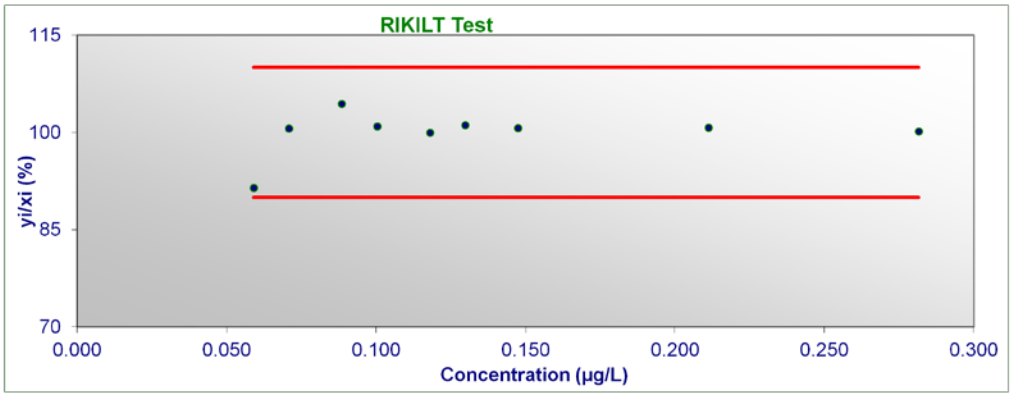
1,2-diethylbenzene

Concentration (μg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.059	148437	2515881.4	93	110	90
0.071	186020	2627401.1	97	110	90
0.089	246986	2790802.3	103	110	90
0.100	258927	2581525.4	95	110	90
0.118	303120	2568813.6	94	110	90
0.130	357716	2755901.4	101	110	90
0.148	416227	2821878.0	104	110	90
0.211	598780	2835132.6	104	110	90
0.282	838055	2976047.6	109	110	90



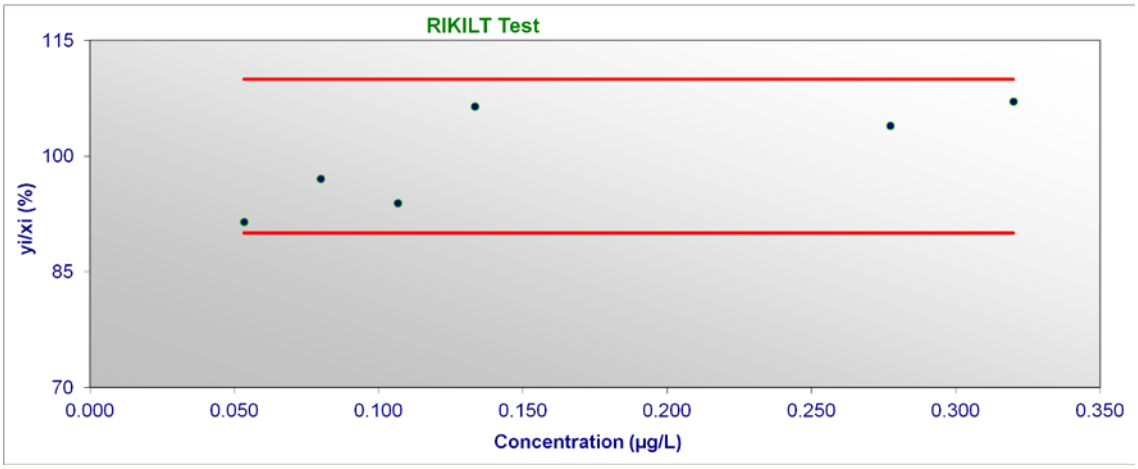
1,4-dimethyl-2-ethylbenzene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.059	91131	1544593.2	91	110	90
0.071	120275	1698799.4	101	110	90
0.089	156052	1763299.4	104	110	90
0.100	170908	1703968.1	101	110	90
0.118	199170	1687881.4	100	110	90
0.130	221631	1707480.7	101	110	90
0.148	250664	1699416.9	101	110	90
0.211	359137	1700459.3	101	110	90
0.282	476104	1690710.2	100	110	90



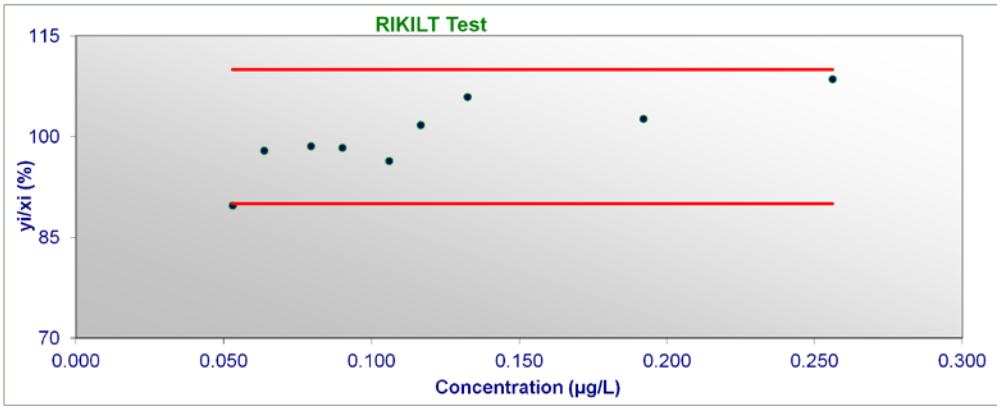
1,3-dimethyl-4-ethylbenzene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.053	33339	625106.3	91	110	90
0.080	53077	663462.5	97	110	90
0.107	68467	641878.1	94	110	90
0.133	96984	727380.0	106	110	90
0.277	196978	710257.2	104	110	90
0.320	234158	731743.8	107	110	90



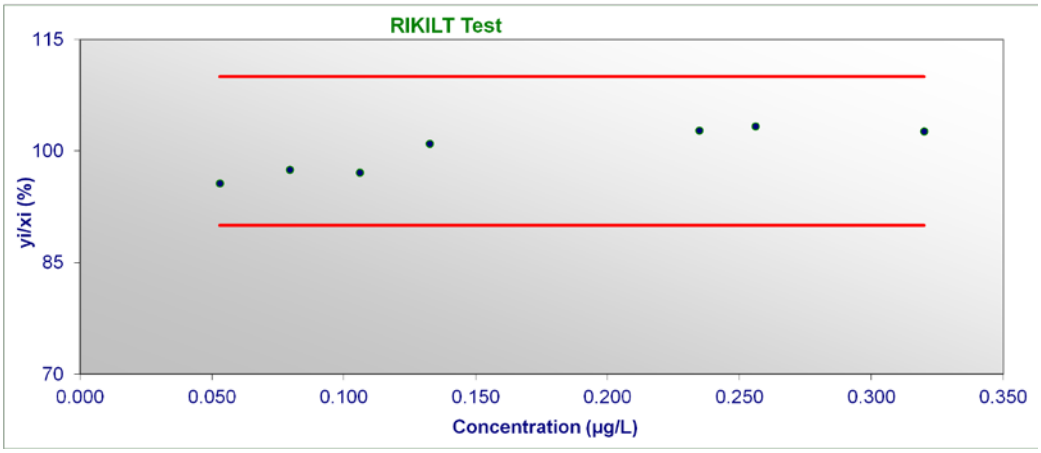
1,2-dimethyl-4-ethylbenzene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.053	77746	1466905.7	90	110	90
0.064	101772	1600188.7	98	110	90
0.080	128089	1611182.4	99	110	90
0.090	144810	1607214.2	98	110	90
0.106	167002	1575490.6	96	110	90
0.117	193819	1662255.6	102	110	90
0.133	229338	1730852.8	106	110	90
0.192	322088	1677541.7	103	110	90
0.256	454247	1774402.3	109	110	90



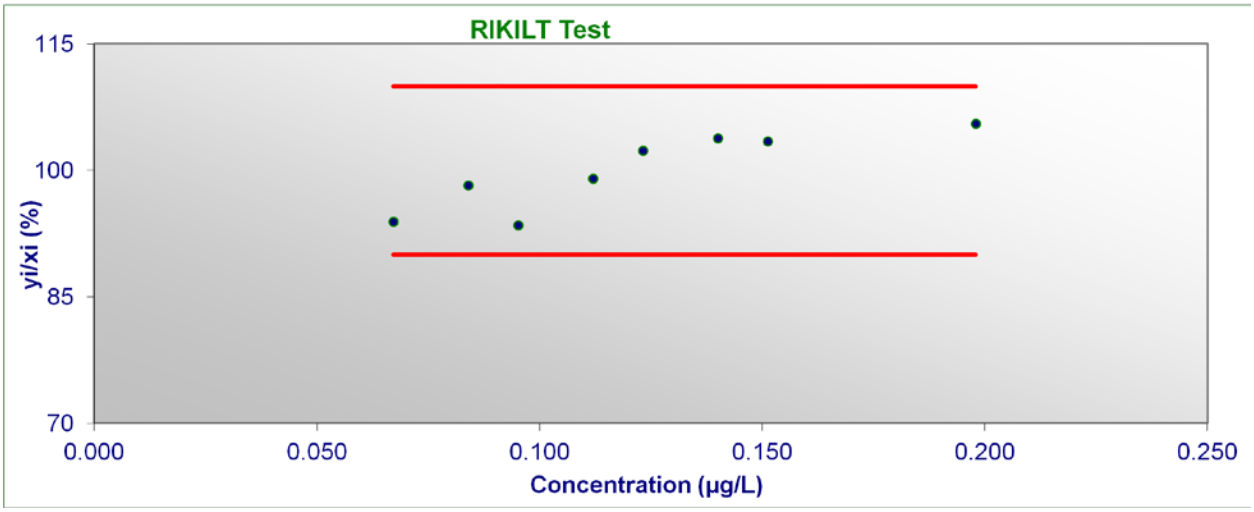
1,2-dimethyl-3-ethylbenzene

Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.053	81195	1531981.1	96	110	90
0.080	124118	1561232.7	97	110	90
0.106	164884	1555509.4	97	110	90
0.133	214289	1617275.5	101	110	90
0.235	386201	1645742.9	103	110	90
0.256	423520	1654375.0	103	110	90
0.320	526183	1644321.9	103	110	90



Hexachlorobutadiene

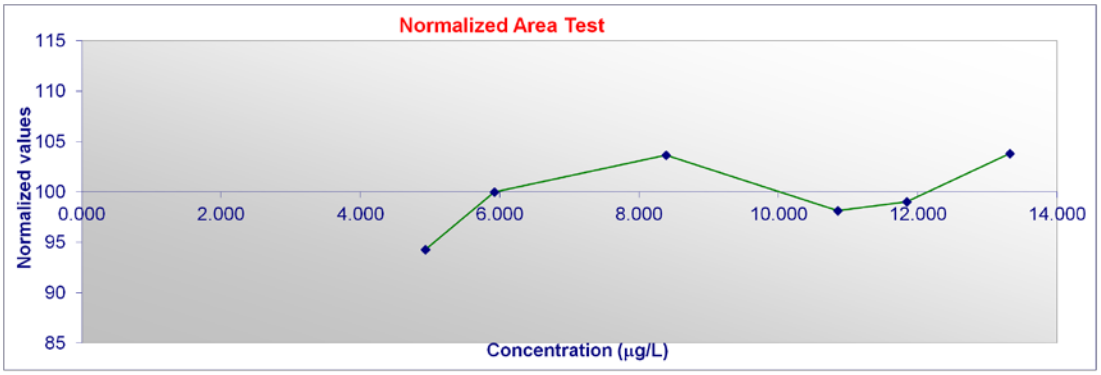
Concentration (µg/L) = xi	Peak area = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
0.067	128571	1913258.9	94	110	90
0.084	168042	2000500.0	98	110	90
0.095	181293	1904338.2	94	110	90
0.112	225898	2016946.4	99	110	90
0.123	256828	2084642.9	102	110	90
0.140	295985	2114178.6	104	110	90
0.151	318638	2107394.2	103	110	90
0.198	425523	2149106.1	106	110	90



ANNEX IV STANDARDIZED AREA TEST

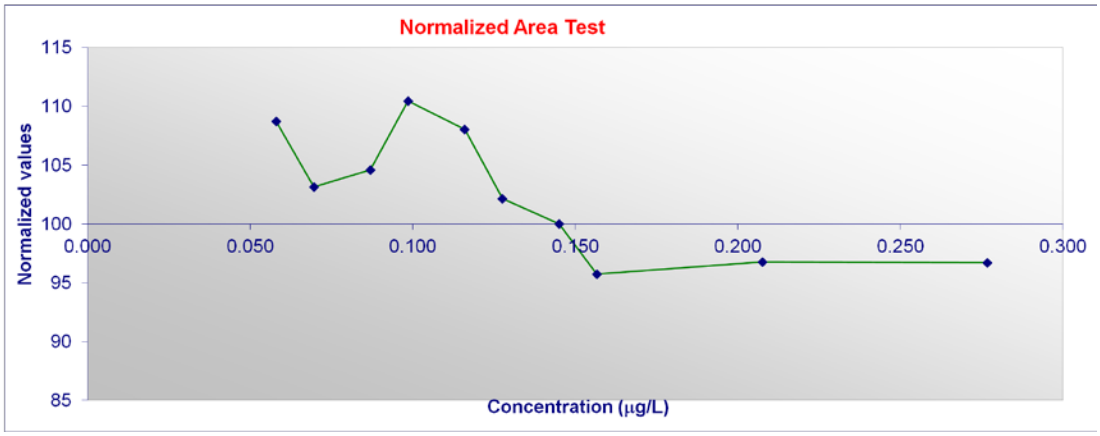
MTBE

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Normalized Values
157145.4	1.028	4.933	94.3
186310.6	0.981	5.920	100.0
259223.6	0.964	8.386	103.6
332136.5	1.028	10.853	98.1
361325.4	1.022	11.840	99.0
405049.5	0.978	13.319	103.8



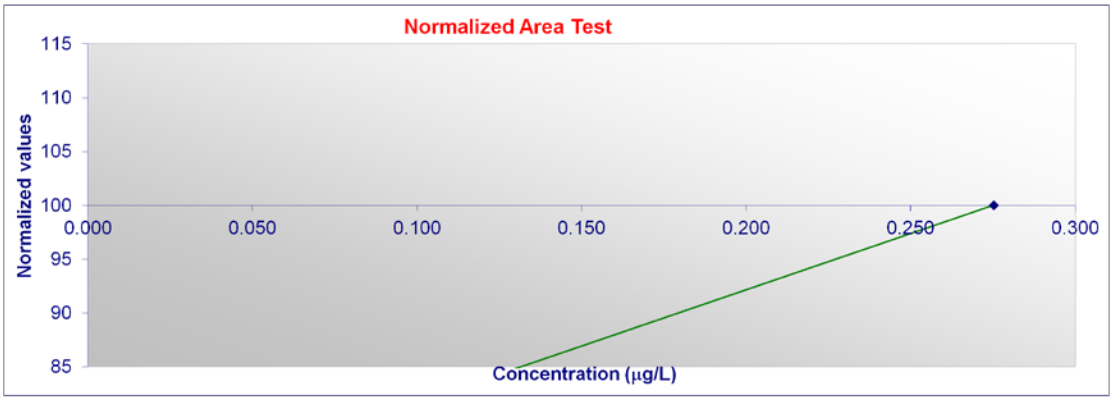
3-ethyltoluene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Normalized Values
90267.6	1.043	0.058	108.7
112359.2	1.060	0.070	103.2
145496.5	1.009	0.087	104.6
167588.1	0.940	0.099	110.5
200725.4	0.944	0.116	108.0
222817.0	0.989	0.128	102.2
255954.4	1.000	0.145	100.0
278045.9	1.038	0.157	95.7
375172.6	1.009	0.208	96.8
506960.21	0.996	0.277	96.7



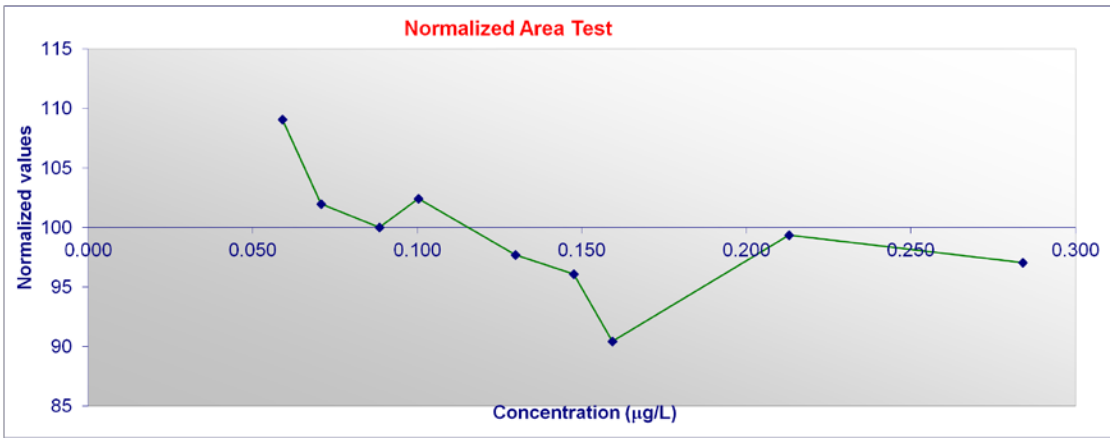
4-ethyltolunene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
219570.5	0.944	0.068	71.6
250893.8	1.025	0.086	72.1
271775.9	1.032	0.097	75.0
323981.4	0.995	0.125	84.4
598380.5	0.998	0.275	100.0



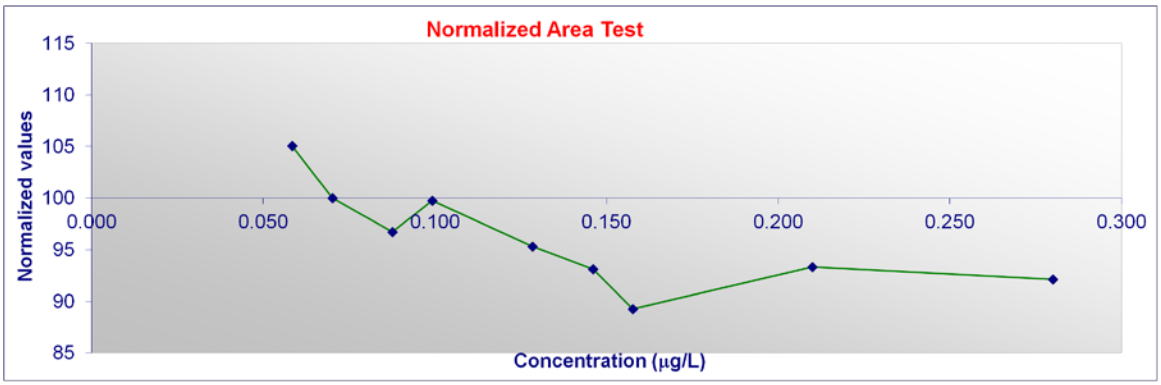
2-ethyltoluene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
124778.1	0.938	0.059	109.1
151704.9	0.990	0.071	102.0
192095.1	0.997	0.089	100.0
219021.9	0.968	0.100	102.4
286338.9	1.004	0.130	97.7
326729.0	1.017	0.148	96.1
353655.8	1.078	0.159	90.4
476195.6	0.974	0.213	99.3
638212.7	0.992	0.284	97.0



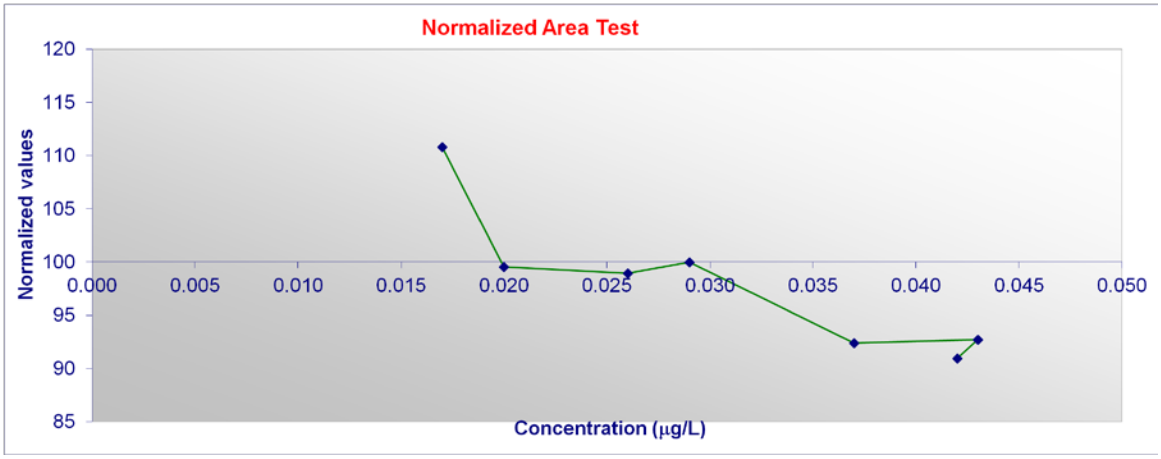
1,2,4-trimethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Normalized Values
109550.1	0.973	0.058	105.1
134678.0	0.998	0.070	100.0
172369.8	1.007	0.088	96.7
197497.6	0.966	0.099	99.7
260317.3	0.993	0.128	95.3
298009.1	1.009	0.146	93.1
323137.0	1.048	0.158	89.3
435696.0	0.990	0.210	93.3
586291.1	0.994	0.280	92.1



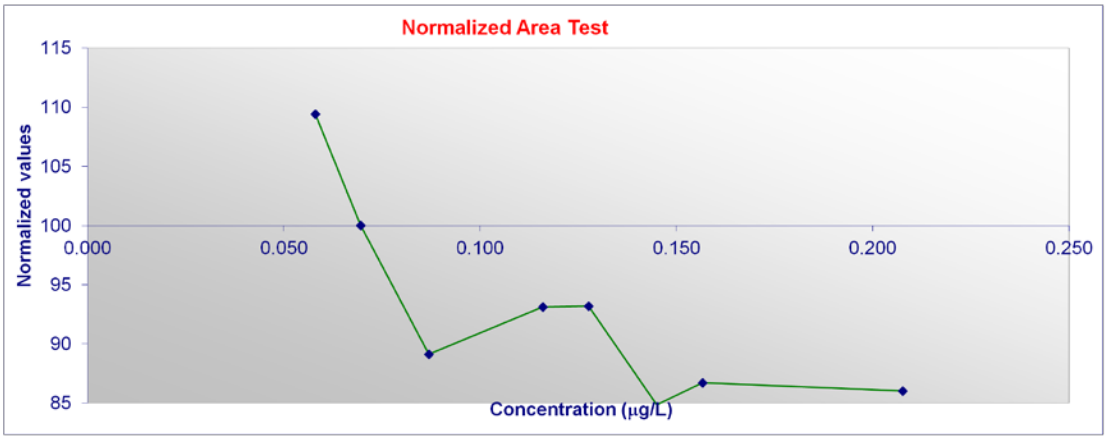
4-isopropyltoluene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Normalized Values
56922.0	0.983	0.017	110.8
70060.6	1.046	0.020	99.6
96337.7	0.995	0.026	98.9
109476.3	0.966	0.029	100.0
144512.5	1.011	0.037	92.4
170789.7	0.991	0.043	92.7
166410.1	1.012	0.042	91.0



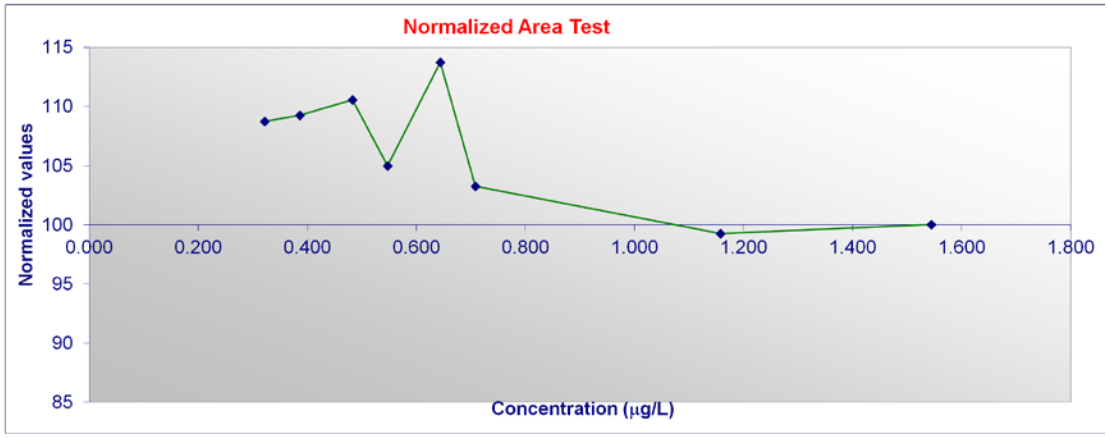
1,3-diethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Normalized Values
160284.5	0.957	0.058	109.4
202412.4	0.995	0.070	100.0
265604.2	1.064	0.087	89.1
370923.8	0.972	0.116	93.1
413051.7	0.960	0.128	93.2
476243.5	1.039	0.145	84.9
518371.3	1.009	0.157	86.7
703588.6	0.993	0.208	86.0



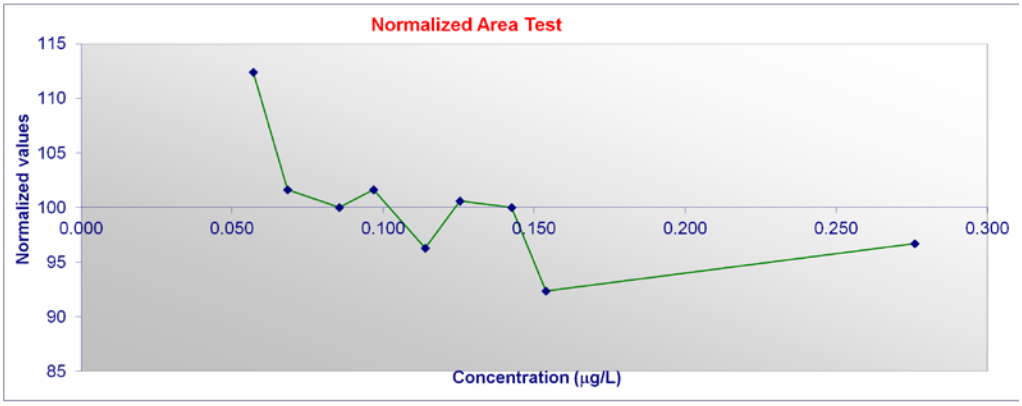
Indane

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Normalized Values
141865.9	1.062	0.322	108.7
175905.0	1.023	0.386	109.3
226963.6	0.980	0.483	110.6
261002.7	1.017	0.547	105.0
312061.3	0.924	0.644	113.7
346100.4	1.009	0.708	103.2
583739.6	1.017	1.158	99.3
787762.6	0.998	1.544	100.0



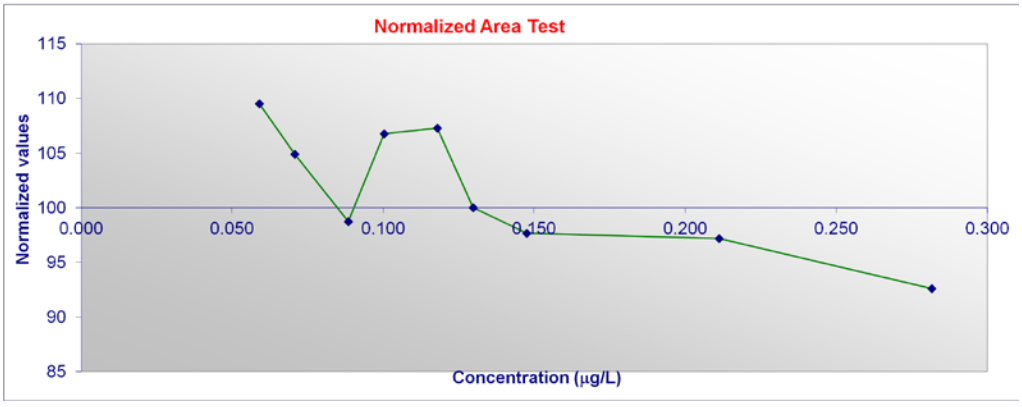
1,4-dethylbenzene/1,3-dimethyl-5-ethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
230439.5	0.933	0.057	112.4
281968.9	1.011	0.068	101.7
359263.1	1.009	0.086	100.0
410792.6	0.984	0.097	101.6
488086.8	1.028	0.114	96.3
539616.2	0.979	0.125	100.6
616910.4	0.979	0.143	100.0
668439.9	1.056	0.154	92.4
1220347.6	0.991	0.276	96.7



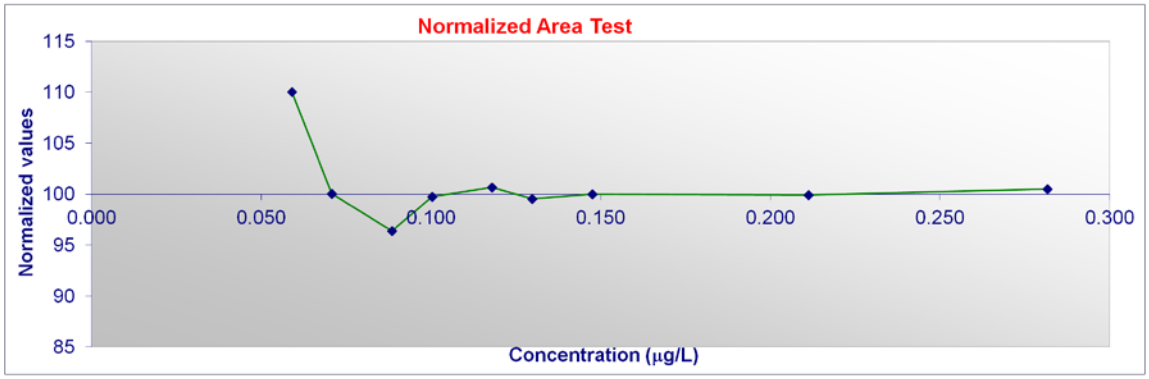
1,2-diethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
141980.9	1.045	0.059	109.5
178242.5	1.044	0.071	104.9
232635.0	1.062	0.089	98.7
268896.6	0.963	0.100	106.8
323289.1	0.938	0.118	107.3
359550.7	0.995	0.130	100.0
413943.2	1.006	0.148	97.7
609694.6	0.982	0.211	97.2
826035.3	1.015	0.282	92.6



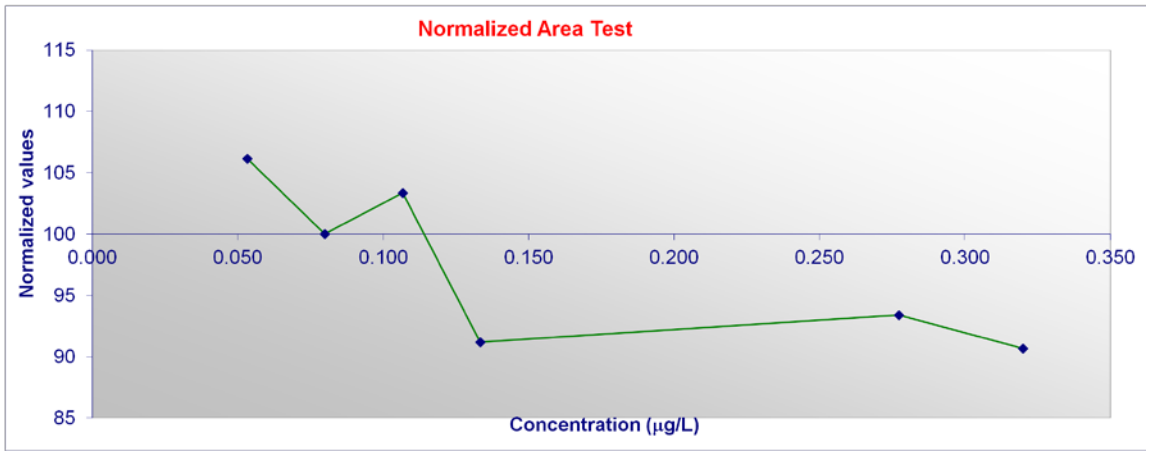
1,4-dimethyl-2-ethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
99480.4	0.916	0.059	110.0
119558.9	1.006	0.071	100.0
149676.6	1.043	0.089	96.4
169755.1	1.007	0.100	99.7
199872.8	0.996	0.118	100.7
219951.3	1.008	0.130	99.5
250069.0	1.002	0.148	100.0
358458.8	1.002	0.211	99.9
478249.0	0.996	0.282	100.5



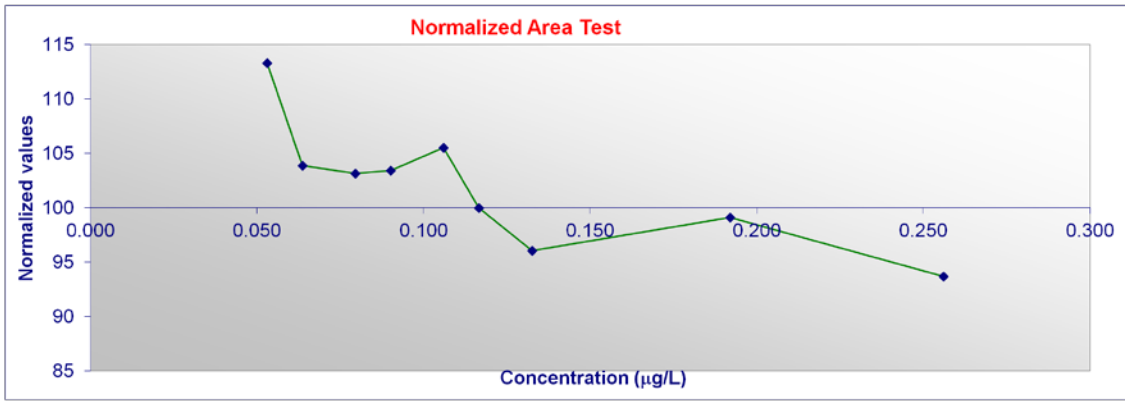
1,3-dimethyl-4-ethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
32836.7	1.015	0.053	106.1
52754.0	1.006	0.080	100.0
72671.3	0.942	0.107	103.4
92588.7	1.047	0.133	91.2
200142.3	0.984	0.277	93.4
232010.0	1.009	0.320	90.7



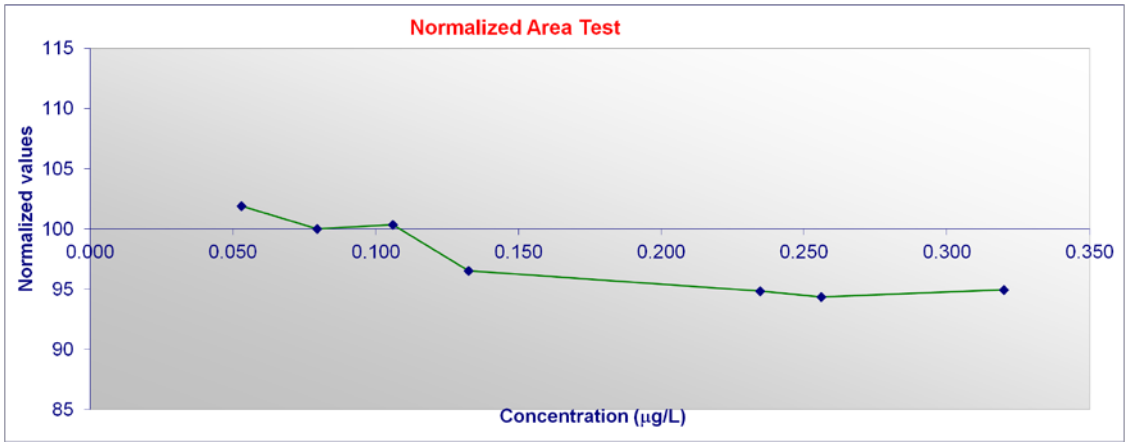
1,2-dimethyl-4-ethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
77927.2	0.998	0.053	113.3
97274.3	1.046	0.064	103.9
126294.8	1.014	0.080	103.2
145641.9	0.994	0.090	103.4
174662.5	0.956	0.106	105.5
194009.5	0.999	0.117	100.0
223030.1	1.028	0.133	96.0
331629.1	0.971	0.192	99.1
448441.5	1.013	0.256	93.7



1,2-dimethyl-3-ethylbenzene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (µg/L)	Normalized Values
79678.1	1.019	0.053	101.9
124221.7	0.999	0.080	100.0
168765.4	0.977	0.106	100.4
213309.0	1.005	0.133	96.5
385040.2	1.003	0.235	94.9
420899.3	1.006	0.256	94.4
528476.4	0.996	0.320	94.9



Hexachlorobutadiene

Estimated peak area	Experimental Peak Area / Estimated Peak Area	Concentration (μg/L)	Normalized Values
125841.6	1.022	0.067	110.1
164391.8	1.022	0.084	105.3
190092.0	0.954	0.095	110.7
228642.2	0.988	0.112	104.5
254342.4	1.010	0.123	101.1
292892.6	1.011	0.140	99.7
318592.8	1.000	0.151	100.0
425982.7	0.999	0.198	98.1