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Faculdade de Ciências e Tecnologia

Optimization and Validation of a Method for the Analysis of Target Compounds Migrating from Organic Materials Used in Contact with Water Intended for Human Consumption by SPME-GC-TOFMS.

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Mestrado em Qualidade em Análises

(European Master in Quality in Analytical Laboratories)

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Abstract

The leaching of toxic organic substances from polymeric materials in distribution systems into drinking water intended for human consumption has led to an urgent need for the continuous development of new analytical methods for their monitoring and evaluation so that high consumer confidence could be established.

An analytical method is developed and validated based on the combination of SPME and GC/TOFMS for the determination of seven target compounds: n-butylacetate, m-xylene, p-xylene, 1,3-dichloroacetone, styrene, o-xylene, cyclohexanone. The chromatographic conditions are optimized so that the analysis is performed in the shortest possible time and a specific mass ion for each compound is targeted in the TOF mass spectrum for quantification.

Three SPME adsorption parameters: mode of extraction, extraction temperature and time are optimized for five fibers to attain the best selectivity and sensitivity for each target compound and based on the highest extraction efficiency, the best fiber and its optimized conditions are used for the validation process.

The LOD and LOQ were lower than the lowest concentrations used in the calibration curves and the determination coefficient (r^2) ranged from 0.995-0.999 within the tested working ranges for all target compounds. The coefficient of variation for repeatability studies was less than 25% for all compounds but it exceeded 25% for some compounds during intermediate precision studies. Recovery studies in both tap and surface water showed that matrix effects play a significant role in the extraction of target compounds from water.

Resumo

A lixiviação de substâncias orgânicas tóxicas provenientes de material polimérico em sistemas de distribuição de água potável destinada para o consumo humano levou a uma necessidade urgente de desenvolver novos métodos analíticos para monitorização e avaliação destes compostos, com objectivo de melhorar a confiança do consumidor.

O método analítico desenvolvido e validado foi baseado na combinação da técnica de SPME com GC/TOFMS para a determinação de sete compostos, sendo estes: n-butilacetato, m-xileno, p-xileno, 1,3-dicloroacetona, estireno, o-xileno e ciclohexanona. As condições cromatográficas foram optimizadas de maneira a reduzir o tempo de análise e ter uma razão massa/carga do ião específica para quantificação dos compostos através do espectro de massas TOF.

O modo de extracção, a temperatura e o tempo foram três dos parâmetros optimizados para a adsorção por SPME. Foram utilizadas cinco fibras de modo a atingir-se melhor selectividade e sensibilidade para cada composto. A validação do método foi feita com a fibra com melhor eficiência e com as condições já optimizadas.

Os valores de LOD e LOQ obtidos foram mais baixos do que a concentração mínima usada nas curvas de calibração e o coeficiente de correlação (r^2) varia entre 0.995 e 0.999 nas gamas de trabalho. O coeficiente de variação para estudos de repetibilidade foi menor que 25% para todos os compostos, mas para alguns compostos excedeu os 25% durante os estudos de precisão intermédia. Nos estudos de recuperação destes compostos em águas da torneira e de superfície verificou-se um efeito significativo de matriz.

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Objective

Develop and validate an analytical method for the analysis of target organic compounds that migrate from organic materials that come into contact with drinking water using SPME-GC/TOFMS.

Symbols

t_R – Retention time

t_m –Dead time

t'_R –Adjusted Retention time

k – Capacity factor

K_C– Distribution constant

α – Selectivity factor

β– Phase ratio

r – Radius of column

d_f– Film thickness of column

R– Resolution

W_h –Peak width at half height of peak

N –Number of theoretical plates

H –Plate height

L –Length of column

A_S –Concentration in stationary phase

A_M– Concentration in mobile phase

n –number of moles

K_{fs}– Partition coefficient

V_f– Volume of polymeric phase

V_s –Volume of aqueous phase

C –Concentration

$S_{y/x}$ – Residual standard deviation of the calibration curve

b– Slope of calibration curve

r^2– Determination coefficient

Abbreviations

DEHP– Bis (2ethylhexyl) phthalate

DVB–Divinylbenzene

ECD– Electron capture detector

FID –Flame ionization detector

GC –Gas chromatography

HDPE– High density polyethylene

HPLC –High performance liquid chromatography

HS– Headspace

LLE– Liquid liquid extraction

LOD– Limit of detection

LOQ– Limit of quantification

MCL –Maximum concentration level

NPD –Nitrogen phosphorous detector

PG– Test value

PDMS– Polydimethylsiloxane

PVC –Polyvinylchloride

SPE– Solid phase extraction

SPME– Solid phase microextraction

Oa-TOF– Orthogonal acceleration-time of flight

TON– Threshold odor number

USEPA– United states environmental protection agency

WHO– World Health Organization

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1.1 Introduction.

The definition of water quality is usually associated with a set of upper and lower limits on selected performance parameters. Therefore, drinking water could be considered unfit for consumption if one or more parameters exceed from specific regulations, or if these regulations do not exist at all, they exceed guidelines or self-imposed limits set by consumer service needs[1].

Any typical modern water supply is a complex system composed of: water source, treatment plant, transmission mains, and a water distribution network, which is comprised of pipes, pumps and storage tanks. Since water is usually in contact with these components that could compromise quality, the distribution network is usually the most critical because it is nearest to the delivery point and if filter devices are absent at the consumer level, there would be no safe barriers before the water is consumed[1].

Older pipes used for the supply of drinking water were manufactured from metallic materials. With these pipes, water contamination was prevalent because corrosion and devices used in plumbing caused an increase in the concentration of metal content in water. Although different metals are affected by different corrosion processes, the contributing factors that increase corrosion rates are low water pH, dissolved oxygen, high temperature and dissolved solids. The leaching of heavy metals (lead and cadmium) and secondary metals (Copper from home plumbing, Iron from distribution pipes and Zinc from galvanized pipes) into drinking water causes not only taste, odor and color problems but also serious health risks to humans[1].

Nowadays, most of the pipes used in the supply of drinking water are manufactured from polymer materials. These polymer materials contain certain organic and inorganic additives that enhance the durability of the material, its manufacturing, the handling throughout installation, and modification of color. These additives include: antioxidants and some stabilizers, lubricants, softeners and coloring agents. High-density polyethylene (HDPE) and polyvinylchloride (PVC) polymers are mostly used in producing pipes for water supply though the former is usually preferred to the latter[2].

All pipes made of polymer materials are expected to have a lifetime of 100 years in the ground. This implies that these pipes have to comply with stringent quality requirement pertaining to good mechanical strength. Though good mechanical properties are fundamental, there is always a possibility of toxic organic compounds being leached from these pipes into drinking water[2].

Diffusion is a common process by which organic compounds can be leached from pipes made of polymeric materials into drinking water[2]. For example, 2,4-di-tert-butyl-phenol is a known degradation product from antioxidants that easily migrates from HDPE pipes into drinking water. Also, a variety of esters, aldehydes, ketones, aromatic hydrocarbons and terpenoids are also believed to migrate from HDPE pipes into drinking water by diffusion[3].

The formation of biofilm on the interior surface of a pipe also causes the leaching of metabolites into drinking water[2]. A biofilm is a deposition of microorganisms, products of microbial activities or detritus at the surface of the pipe. When any injured bacteria pass from the treatment plant into distribution network, the presence of a biofilm encourages bacteria regrowth. This regrowth of bacteria in the distribution system leads to an increase in demand of chlorine in the system while reducing the amount of free chlorine and this hinders the ability of the system to cope with minute occurrences of contamination[1].

Drinking water intended for human consumption should have no significant taste or odor. A quantitative parameter called threshold odor number (TON) can be used to assess odor, thus providing information about the presence of organic compounds that migrate from pipes of polymeric materials into drinking water. It has been established that water transported through pipes made of HDPE has a TON above four indicating the presence of organic compounds in water transported through these pipes[3].

Organic compounds that migrate from pipes made of polymer materials into drinking water have an adverse impact on the health of humans. These leached compounds could be identified as endocrine disruptors (Bisphenol A, DEHP), suspected as carcinogens (styrene), and teratogens. By having a clear understanding of the interaction and the effect of the distribution system materials, limits could be established for the supply of high quality water to consumers[13].

Analysis of organic compounds in water is usually an important domain in environmental monitoring and evaluation for establishing high consumer confidence. Today, most of the powerful instrumentation used for the quantitative and qualitative analysis of most target organic compounds in water mainly involves the coupling of gas chromatography (GC) with mass spectrometry (GC-MS). However, extracting some of these target organic compounds is always time-consuming and is also the most difficult task in the analysis due to matrix interferences[4].

Matrix interference occurs in two types: interference from non-target compounds and strong adsorption by the matrix. During the extraction of target compounds from the matrix, there is always a possibility that non- target compounds are also extracted. On performing GC-MS analysis, non-target compounds could co-elute with and mask the target compounds. However, strong adsorption by matrix leads to poor recovery[4].

Since the quality of the sample preparation is a major factor that determines the success of any analysis, there is always a need to develop new methods that are sensitive and selective for extracting and isolating target components from matrices. A sample preparation method is considered ideal if it is fast, accurate, precise and consumes very small amount of solvent. Other factors that should be considered for any modern extraction method include sample integrity, high throughput and compatibility with subsequent techniques for analysis. Also, it should be adaptable to the field of work and should consist of low cost materials[5].

A rapidly growing area in analytical sample preparation is solid-phase microextraction (SPME) [6]. The development of this method was simply based on the attempt to redress the limitations in solid-phase extraction (SPE) and liquid-liquid extraction (LLE). With this method, sampling, extraction, concentration and sample introduction are integrated into a single solvent-free step. It involves the direct extraction and concentration of analytes in the sample to an extraction fiber. As a result, there is a great reduction in preparation time and disposal time and significant improvement in detection limits. Also, this method is easily used in combination with GC/MS and could be applied to wide range of organic compounds in environmental, biological and food samples. The method tends to combine good analytical performance with simplicity at low cost and can be easily automated. The extracts obtained are relatively clean and concentrated implying that the method is ideal for MS applications[7].

The main purpose of this project is to develop an analytical method to obtain the desired limit of detection with the required linear range in the shortest possible analysis time for the determination of target organic compounds that migrate from pipes made of polymer materials in drinking water. This mainly involves the usage of SPME for extraction of organic components together with GC/TOFMS for qualitative and quantitative determinations. Several factors considered when developing this SPME method include: selection of the stationary phase on the fiber and cryofocusing temperature that affect sensitivity whereas the SPME adsorption conditions influence efficiency. After optimizing the method, the limit of detection (LOD), limit of quantification (LOQ), linear range and precision are being established. This work therefore discusses the influence of stationary phase, time, temperature and stirring on method development for target organic compounds that migrate from pipes made of polymer materials. A brief description of the European Directive on the Quality of Drinking Water Intended for Human Consumption is discussed below including general information of the target organic compounds used for this study.

1.2 European Legislation[13].

In an attempt to address issues relating to the quality of drinking water intended for human consumption in the European Union, the Council Directive 98/83/EC of 3 November 1998 was adopted. It establishes the quality standards and monitoring programs for water supplied from a distribution network and that substances or materials in contact with potable water do not influence the quality of drinking water. Other issues that are addressed in this directive include the quality assurance of products and materials used in contact with drinking water.

In order to set the basis for the approval of materials that come in contact with drinking water in the European Union, several standards relating to these materials have been approved during the last years in the CEN. These include:

- EN 1420-1 (1999): Determination of odor and flavor assessment of water in piping systems on the influence of organic materials on drinking water.

- EN 13052-1 (2001): Determination of color and turbidity on the influence of organic materials on drinking water.

- EN 14395-1 (2004): Organoleptic assessment in storage systems on the influence of organic materials on drinking water.

- EN 14728 (2006): Determination of chlorine demand on the influence of organic materials on drinking water.

- EN 12873-1 (2003) and EN 12873-2 (2005): Effect of migration on the influence of organic materials on drinking water.

- EN 14944-1 (2006) and EN 14944-3 (2007): Effect of cement products on drinking water.

- prEN 15768 (2009): Identification of water leachable organic substances from materials in contact with water intended for human consumption using GC-MS.

Since Portugal is a member of the European Union, these directives have been adopted and the Portuguese Environment Ministry has established a national level decree for drinking water in Decreto-lei nº 306/2007 de 27 de Agosto.

Though there are no approved systems for materials that come in contact with water at both European and Portuguese level, there is an urgent need to ensure that materials which come in contact with drinking water meet the required quality. It is for this reason that EPAL (Epresa Portuguesa das Aguas Livres, S.A.) has developed an internal approval system for organic and cement materials used in its supply system based on tests defined in European Standards. Other European countries have developed their own national approval systems based on these European standards.

1.3 Target Organic Compounds.

Butylacetate (*1-acetoxybutane, acetic acid butyl ester).*

It is a colorless liquid with a mild odor that is usually used in the manufacture of paints, coatings and adhesive. It is also used as an extraction solvent in the pharmaceutical industries because of its low solubility in water[15].

Since it is applied to plastic coatings, there is a tendency for it migrating from the plastic product to the environmental matrix. Ingestion of this compound is harmful to the lungs and nervous system[15].

Determination of this compound has been done by GC coupled with FID[16]. The Drinking Water Directive has no parametric value for this compound and the WHO guidelines and US EPA have not established any limit yet.

Cyclohexanone

It is a colorless liquid that is produced by the oxidation of cyclohexane in air or partial hydrolysis of phenol. They are usually applied to paints, vanish removers, natural and synthetic resins. This compound degrades rapidly by reaction to sunlight and it is biodegradable in water[17].

This compound could be analyzed by HPLC and GC coupled with FID[18]. There is no parametric value for this compound in the Drinking Water Directive and the WHO guidelines and USEPA have not set any limits for it.

Styrene (*Vinylbenzene, Phenethylene, Ethenylbenzene, Styrol).*

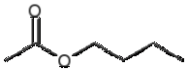
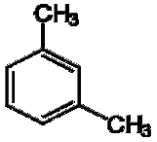
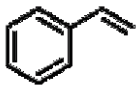
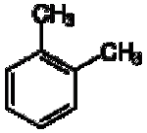
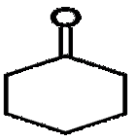
This compound is produced for the catalytic dehydrogenation of ethyl benzene and also used for the production of polystyrene and styrene polymers[19].

Exposure to small quantities of styrene could cause neurotoxic, hematological and carcinogenic effects[20]. This compound has been determined with HPLC coupled with FID, ECD, NPD and MS[21]. There is no parametric value for this compound in the Drinking Water Directive but the WHO guidelines have set a limit of 0.02mg/L and the US EPA has established a limit of 0.1mg/L.

Xylenes (*m-,o-,p-xylene or xylol or dimethylbenzene).*

These compounds occur naturally in petroleum, coal tar and could be formed during forest fires[22]. They are used as solvents in paints, rubber and leather and are also used in the manufacture of plastics[23]. Exposure to these compounds could lead to damaging effects in the liver, heart, kidneys, lungs and nervous system[24].

These compounds are usually analyzed in water samples by GC/MS with the use of sample preparation techniques like Purge and Trap, HS, SPME and LLE. There are no parametric values for these substances in the Drinking Water Directive and the WHO guidelines have not set any value for them. However, the US EPA has set an MCL of 1mg/L for these compounds.

Compound	Structure	Nominal Mass(Da)	Boiling Point (°C)	Melting Point (° C)	Water Solubility	Vapour Pressure at 20°C
n-Butylacetate ($C_6H_{12}O_2$)		116	126	-74	0.7g/100ml	1.3KPa
m-Xylene(C_8H_{10})		106	139	-48	Insoluble	1.12KPa
p-Xylene(C_8H_{10})		106	138.35	13.2	Insoluble	1.11KPa
Styrene(C_8H_8)		104	145	-30	<1%	5mmHg
o-Xylene		106	144.4	-24	Insoluble	0.19KPa
Cyclohexanone ($C_6H_{10}O$)		98	155.65	-16.4	10g/100ml	2mmHg

2. Gas Chromatography-Mass Spectrometry Time of Flight (GC/TOFMS).

The coupling of gas chromatography to mass spectrometry is an advanced technique that is commonly used in the identification and quantification of organic pollutants in environmental samples. This technique offers a wide range of applications because while gas chromatography results in high separation efficiency, mass spectrometry provides good qualitative information and high sensitivity[12].

2.1 Gas Chromatography.

It is one of the most common and powerful instrumental techniques used in the identification if coupled to mass spectrometry and measurement of individual components in a sample based on differences in their volatilities and structures[8]. This technique is capable of achieving high efficiencies of separation with capillary columns, provides high sensitivity of detection for very small amounts of separated components and gives precise and accurate data for quantitative analysis of complex samples[9].

2.2 Gas Chromatographic Parameters[8].

- *Retention Time (t_R).*

It is a measure of how long it takes a compound to travel down the column and could be considered as the time a compound spends in the column. It is the total time that the compound spends in the stationary and mobile phases. This time is dependent on the column type, column temperature and carrier gas linear velocity.

- *Adjusted Retention Time (t^{l}_{R}).*

It is simply the difference between the retention time (t_R) and the column dead time (t_m). The column dead time is usually determined by injecting a non-retained compound and measuring its retention time.

$$t^{l}_{R} = t_R - t_m \qquad \textbf{Equation 2.2.1}$$

- *Retention Factor (k).*

It is the ratio of the time a compound spends in the stationary phase (t^{l}_{R}) to the time it spends in the mobile phase(t_m).

$$k = t^{l}_{R}/t_m \qquad \textbf{Equation 2.2.2}$$

- *Phase Ratio (β).*

It is a dimensionless value relating the diameter (2r) and film thickness (d_f) of the column. It is used to assess the effect of column diameter and thickness on retention.

$$\beta = r/2d_f \qquad \textbf{Equation 2.2.3}$$

- *Distribution Constant (K_c).*

It is the ratio of the concentration of a compound in the stationary and mobile phase. It can also be expressed as a product of the phase ratio (β) and capacity factor (*k).*

$$K_c = (A)_S/(A)_M = k\beta \qquad \textbf{Equation 2.2.4}$$

- *Selectivity or Separation Factor (α).*

It is the ratio of the capacity factors of two peaks. If α = 1, it means that the two peaks have the same retention and co-elute.

$$\alpha = k_2/k_1 \qquad \textbf{Equation 2.2.5}$$

k_1 = partition ratio of the earlier eluting peak.

k_2 = partition ratio of the later eluting peak.

- *Resolution (R).*

It is a measure of the amount of separation between two peaks by considering the widths of the peaks. A resolution of 0.3% overlap means that two peaks are fully resolved without any baseline or space between them. Resolution less than 1.5 means that two peaks are partially resolved or have some degree of overlap. Resolution of more than 1.5 means that peaks have a baseline between them.

$$R = 1.18(t_{R_2}-t_{R_1})/(w_{h_1}+w_{h_2}) \qquad \textbf{Equation 2.2.6}$$

t_{R_1} = retention time of peak 1.

t_{R_2} = retention time of peak 2.

w_{h_1} =peak width at half height of peak 1.

w_{h_2} = peak width at half height of peak 2.

- *Column Efficiency.*

Column efficiency enables us to account for the occurrence of broader peaks at longer retention times. Two main parameters can be used to express column efficiency:

- *Number of Theoretical Plates (N).*

This is a simple relationship between the retention time of a peak and its width. This is a better parameter that could be used in comparing chromatographic columns.

$$N = 5.545(t_R)^2/(W_h)^2 \qquad \textbf{Equation 2.2.7}$$

t_R = retention time of peak.

W_h = peak width at half height.

- *Height Equivalent to a Theoretical Plate (H).*

This is a measure of efficiency that is independent of the total column height. If each theoretical plate is shorter, there is a greater number that can fit into a unit length of the column leading to a greater number of theoretical plates per meter.

$$H = L/N \qquad \textbf{Equation 2.2.8}$$

L = column length (mm)

2.3 Gas Chromatographic Instrumentation[8].

A basic gas chromatographic system (**Figure 2.1**) is composed of the following components:

- *Gas Supply and Flow Controllers.*

Pressurized cylinders and gas generators are usually used to supply high purity gases. Pressure regulators and flow controllers control the flow and amount of gas delivered into the gas chromatograph.

- *Injector.*

This is a hollow, metal cylinder containing a glass liner or insert. It introduces the sample into the open tubular column. The column is inserted into the bottom of the injector so that the column end resides inside of the glass liner. Samples are always introduced into the injector through a resealable septum using a small syringe. An injector is usually kept at a 100-300°C so that volatile components can be easily transformed into vapor. Injection techniques used in gas chromatography include:

- Split Injection: A sample of 1ul is injected into the injection port followed by rapid vaporization. About 0.1-10% of the vapor enters the column and the rest of the vaporized sample and large flow of carrier gas are transported through a split or purge valve.

- Splitless Injection: A sample of 1-5ul is injected into the heated injection port and it is vaporized. The vaporized sample is slowly carried on a cold column and after a few seconds,

the split valve is opened so that any residual vapor at the injection port is taken out of the system.

- Cold on-column Injection: The sample is injected directly on-column and it is vaporized. A special syringe is usually used with the needle made of silicone or steel and has a diameter of about 0.15mm. It is kept at a temperature of 4^{o} C when it penetrates the column or pre-column before raising the temperature to normal operating temperatures.

- Direct Vaporization injection: This is mostly used for injecting samples in packed columns. An important feature of this injector is that it has a metal tube with a glass sleeve or insert.

- On-column Injection: The sample is introduced by inserting a precisely aligned needle into a capillary column and making injections inside the column.

➢ *Column and Oven*.

The column is placed in the oven where the temperature is accurately controlled. The interior walls of the column are coated with a thin film of polymeric material called the stationary phase that affects the extent at which a compound is retained in the column. The retention of a compound in the column is affected by the length and diameter of the column, chemical structure and amount of the stationary phase and the column temperature. Two main types of columns are used in gas chromatography:

- Packed Columns: These are made of stainless steel or glass, have diameters of 1.18 to 6.35mm and lengths ranging from 1 to 3m. The stationary phase is impregnated or bound on an inert and stable porous solid support made of spheres of approximately 0.2mm in diameter. Silanol groups are present on supports in packed columns.

- Open Tubular Capillary Columns: These columns are usually smaller in diameter and longer in length (3 to 100m). The stationary phases used for these columns are polysiloxanes. The major types of open tubular columns include: wall-coated (WCOT), support-coated (SCOT) and porous-layer (PLOT). Among these types, the WCOT columns are mostly used.

- *Detector.*

There are various types of GC detectors and they usually operate by interacting with a physical or chemical property of the analyzed compound. This interaction leads to an electric signal that corresponds to the amount of compound in a sample.

- *Recording Devices.*

In the past, these included strip chart recorders and integrators. Nowadays, computer data systems which usually vary in degree of complexity, features and price are used. They plot the size of the detector signal versus the time elapsed since sample introduction into injector. The plot yields a chromatogram that appears as a series of peaks.

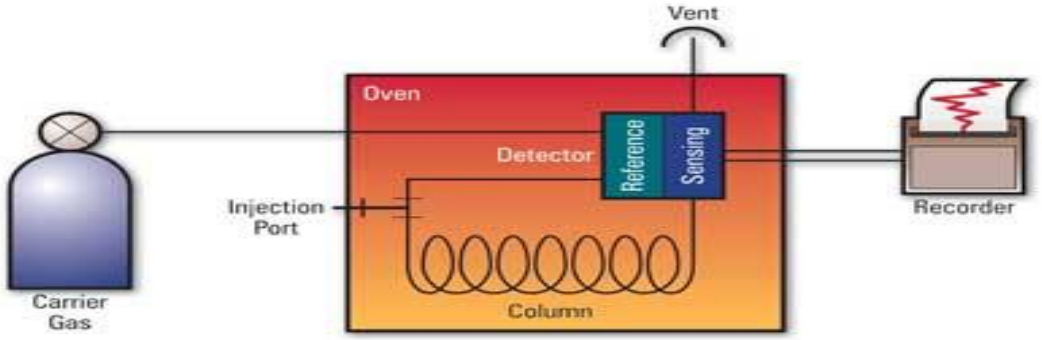


Figure 2.1: Schematic digram of gas chromatograph.

2.4. Mass Spectrometry-Time of Flight.

This is an analytical technique that enables the determination of a compound in a sample based on mass-to- charge ratio (m/z) of charged particles. The technique uses electric and magnetic fields to measure the m/z of ions when they pass through[10].

2.5 Components of a Mass Spectrometer-Time of Flight.

The basic components of the MS-TOF are:

- *Pumping Systems*[10].

These pumps are mainly used to establish vacuums for the mass spectrometer in two stages: a fore-pump leads to a drop in vacuum from 10^{-1} to 10^{-3} torr, and either an oil diffusion pump or turbomolecular pump drops the analyzer pressure from 10^{-5} to 10^{-7} torr.

Usually, the vacuum pressures are always measured by two types of gauges: a thermoconductivity gauge such as a Pirani gauge is used to measure the medium- level vacuum of the fore pump while a hot cathode gauge is used to measure high vacuums produced by oil diffusion or turbomolecular pumps.

- *The Interface to the Gas Chromatograph*[10].

The interface enables the transfer of sample from the GC into the source without the mixing of separated bands. It can be adapted as a separator so that the sample is concentrated to about 50-fold and reduces the source pressure by removing much of the carrier gas. A basic interface that is commonly used is a direct connection of the capillary column end into the sample inlet port on the ionized source.

- *The Ionization Chamber and Electron Source*[10].

Though a number of sources have been designed for sample ionization in mass spectrometers, only two sources are commonly used in GC/MS systems. These include:

- *Electron Impact (EI) Source:* The sample leaving the GC interface is exposed to a beam of 70-eV electrons from the filaments. As a result, the sample is ionized and could also fragment producing a fragmentation pattern that is characteristic of the ionized sample.

- *Chemical Ionization (CI) Source:* The sample exiting the GC interface interacts with ionization gas in the ionization chamber. Gases which are normally used to absorb the initial ionization electron include CH_4, C_4H_{10} and CO_2. Usually, the collision process generates an

uncharged gas molecule and a molecular ion with very little fragmentation. This ionization process occurs at a much lower energy than EI ionization.

- *Time of Flight (TOF) Analyzer*[12]

All the ionized fragments leaving the source are accelerated into a field- free flight tube within a linear electric field. The movement of ions in this field leads to the generation of kinetic energy which is related to the velocity. As the velocity is inversely proportional to the square root of the mass to charge ratio of the ions, separation of ions occurs such that lighter ions travel faster through the acceleration and drift regions and reach the detector before the heavier ions.

The resolving power of this analyzer is dependent for a given uncertainty of time and square of mass to charge ratio. This mass resolving power can be improved by:

- Increasing the flight time which is achieved by decreasing the acceleration voltages or using longer flight distance.
- Decreasing the uncertainty of time.

There are several factors that have an effect on the time of flight. These include:

- Spatial Spread: Difference in initial positions at which ions are formed.
- Temporal Spread: Difference in time over which ions are formed.
- Kinetic Energy Spread: Difference in initial velocity of the ions.
- Angular Spread: Difference in direction of motion of ions.

In order to correct for dispersion in arrival time of ions at the detector due to differences in ionization positions, spatial focusing is employed. This involves the generation of ions in an electric field such that ions formed further from the detector would experience a force much longer so that they achieve higher velocities. This leads to time-focusing where ions of the same mass reach the detector at the same time.

Also, the analyzer has a device called the reflectron which enables velocity focusing. This device is situated at the end of the drift region and it acts as a retarding field due to the presence of a series of lens plates with different voltages. Ions of the same mass with higher kinetic energy will penetrate more deeply into the retarding field and take more time to energize. As a result, they reach the detector at the same time with ions of lower kinetic energy.

- *Microchannel Plate Detector*[10].

This is an assembly of several point detectors that are connected to act as a single detector. Secondary electrons are produced on collision with the wall from electrons that cascade through individual electron multiplier. This results in signal amplification which is received by the data system.

3. Sample Preparation

3.1. Solid Phase Microextraction.

This technique, usually combined with GC uses an immobilized liquid phase (i.e. polydimethylsiloxane or polyacrylate polymers) as a stationary phase and enables the direct extraction of target organic substances from water by dipping the fiber into the aqueous sample or headspace[11]. Solid phase microextraction technique consists of two steps:

- Adsorption of target compounds from the aqueous matrix by dipping the SPME fiber into the matrix or headspace.
- Desorption of target compounds from the polymeric layer of the fiber into the carrier gas of the heated GC injector.

3.2. Theory of Solid Phase Microextraction[11].

The principle of SPME is based on the partitioning of components between an aqueous sample and the polymeric film on the fiber. A simple mathematic model has been developed which relates a direct proportionality between the amount of component adsorbed on the polymeric film at equilibrium (infinite volume) and its concentration in the aqueous solution and is determined by the partition coefficient.

$$n = K_{fs}V_fC_oV_s/(K_{fs}V_f + V_s) \qquad \text{Equation 3.2.1}$$

n= number of moles of component adsorbed on polymeric film.

K_{fs}= partition coefficient of component between polymeric film and aqueous phase.

V_f = volume of the polymeric film.

V_s= volume of the aqueous phase.

C_o= initial concentration of component in the aqueous phase.

Since $V_s >>> K_{fs}V_f$, the amount of component extracted on the polymeric film is:

$$n=K_{fs}V_fC_o \qquad \text{Equation 3.2.2}$$

This implies that the amount of component adsorbed on the polymeric fiber is not dependent on the volume of the aqueous phase (V_s) but on the amount of component in the aqueous phase (C_o). After extracting the organic components, the fiber is transferred to the hot GC injector where the components are thermally desorbed.

Since there is a linear relationship between the concentration of component in the sample and the amount adsorbed on the fiber, a linear GC detector response is obtained if adsorption conditions in the sample and desorption conditions at the GC injector are reproducible.

In order to extract organic components from aqueous samples using SPME, two approaches are mainly used:

- *Headspace Sampling:* This approach is mostly used for the extraction of volatile compounds in aqueous samples and it is advantageous because of faster extraction times and improved selectivity. It mainly involves the exposure of the fiber above the liquid sample.

- *Liquid Sampling:* This approach is used for the extraction of semi-volatile compounds from the aqueous matrix. In this approach, the fiber is completely immersed in the liquid sample.

4. Method Validation[14].

Method validation is the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires.

Possible performance parameters that are usually evaluated in a method validation study include:

➢ *Accuracy:* It is a measure of how close the measured value is to the true value. Usually, accuracy is determined by three different ways:

- Comparison to a reference material.
- Analyte recovery.
- Standard addition method.

➢ *Precision:* It is defined as the degree of agreement among individual test values when the procedure is applied repeatedly to multiple samplings of a homogenous sample. It is often expressed by standard deviation (SD) or relative standard deviation (RSD) According to the International Conference on Harmonization (ICH), precision is divided into:

- Repeatability: It involves using the same conditions over a short period of time to measure precision of the method.
- Intermediate precision: It is performed when the same method is applied many times in the same laboratory varying time of analysis and operator.
- Reproducibility: It mainly examines the precision between laboratories and it is usually estimated in collaborative studies or method transfer experiments.

➢ *Linearity:* It simply measures how well a calibration plot of response against concentration approximates a straight line. The least square method is regularly used to determine the correlation coefficient which is a measure of the linearity of an analytical method. A linearity coefficient of 0.999 is considered acceptable for most analytical methods. However, this coefficient can be influenced by low and high concentration values. Another

method that is usually being used in determining linearity is by plotting the response factor (sensitivity) against the concentration. The response factor of each concentration is obtained as a quotient of the detector response by the analyte concentration.

- *Range:* It is the lower and upper concentration for which a method has adequate accuracy, precision and linearity.

- *Limit of Detection:* It is the smallest amount of analyte that gives a measurable response. Mathematically, it can be calculated as:

$$LOD = 3 * S_{y/x}/b \quad \text{or} \quad LOD = 3 * \sigma$$

$S_{y/x}$ and b are the residual standard deviation of the calibration curve and slope respectively of the calibration curve.

Also, σ is the standard deviation of a series of measurements of the lowest concentration under repeatability conditions.

Also the limit of detection is based on a signal to noise ratio which is typically 2 or 3.

- *Limit of Quantification:* It is the smallest amount of analyte that gives a response that can be accurately quantified.

Mathematically, it can be calculated as:

$$LOD = 10 * S_{y/x}/b \quad \text{or} \quad LOD = 10 * \sigma$$

$S_{y/x}$ and b are the residual standard deviation of the calibration curve and slope respectively of the calibration curve.

Also, σ is the standard deviation of a series of measurements of the lowest concentration under repeatability conditions.

5. Experimental Procedure.

5.1 Materials and Equipments.

- Analytical Balance Mettler XS 204.
- Agilent Technologies 6890N-Gas Chromatograph.

  Injector: Split/Splitless.

  Column: HP 5 MS (30m x 0,25mm x 0,25um).

  PAL Combi SPME System Autosampler.

  MassLynx Software Version 4.1
- Waters Micromass GCT Premier Mass Spectrometer.

  Analyzer: Oa –TOF.

  Ion Source: Electronic Ionization (EI).

  Microchannel Plate Detector.
- SPME fiber assembly:

✓ 100um Polydimethylsiloxane coating (Red).

✓ 85um Polyacrylate, Fused Silica (White).

✓ 50/30um DVB/Carboxen/PDMS Stable Flex (Gray).

✓ 65um Polydimethylsiloxane- Divinylbenzene (Blue).

✓ 75um Carboxen-PDMS (Black).

- Vortex, MS 3 Digital, IKA
- System for obtaining Ultrapure water: Ultrapore model Milli-Q® , Advantage A-10® , Millipore
- 20ml SPME vials with Ultra Clean Closure.

5.2 Reagent.

- Methanol (CH_3OH) - Merck KGaA (99.8%).
- Ultrapure Water.

5.3 Samples.

- Tap water from EPAL Laboratory.
- Surface water(Tagus River)

5.4 Analytical Standards.

Table 5.1: Analytical Standards.

Standard	*% Purity*	*Supplier*
n-Butylacetate	99.5	CHEMSERVICE
o+p Xylene	*99.5*	CHEMSERVICE
m-Xylene	*99.5*	Dr.Ehrenstofer GmBH
Styrene	*99.5*	CHEMSERVICE
1,3-Dichloroacetone	*NA*	CHEMSERVICE
Cyclohexanone	*99.5*	CHEMSERVICE

5.5 Preparation of Solutions.

5.5.1 Preparation of Individual Standard Stock Solutions.

These solutions are prepared by weighing a certain mass of each standard into a 25-ml volumetric flask followed by dilution with methanol.

Table 5.2: Concentration of individual standard stock solutions.

Compounds	Concentration(mg/l)
n-Butylacetate	8596
m- Xylene	16060
1,3- Dichloroacetone	20660
Styrene	9344
o-Xylene	26424
p-Xylene	12412
Cyclohexanone	29288

5.5.2 Preparation of Mixed Solutions.

Several mixed solutions are prepared by measuring a specific volume from the individual standard stock solutions into a volumetric flask followed by dilution with methanol. These mixed solutions are used for different purposes.

- *Preparation of Mixed Solution A.*

  It is prepared by measuring a certain volume (V_1) of each individual standard stock solution into a 10-ml volumetric flask followed by dilution with methanol. This solution would be used to pre-establish the chromatographic and SPME conditions using the 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) fiber.

Table 5.3: Concentration of standards in Mixed Solution A

Compound	V_1(ml)	Concentration(mg/l)
n- Butylacetate	0.005	4.3
m-Xylene	0.003	4.8
1,3-Dichloroacetone	0.1	61
Styrene	0.005	4.7
o-Xylene	0.002	5.3
p-Xylene	0.004	5.0
Cyclohexanone	0.1	291

- *Preparation of Mixed Solution B.*

It is prepared by measuring a certain volume (V_2) of each individual standard stock solution into a 10-ml volumetric flask followed by dilution with methanol. This solution would be used for fiber optimization.

Table 5.4: Concentration of standards in Mixed Solution B.

Compound	V_2(ml)	Concentration(mg/l)
n- Butylacetate	0.5	430
m-Xylene	0.01	16
1,3-Dichloroacetone	4	8264
Styrene	0.01	9.3
o-Xylene	0.01	26
p-Xylene	0.01	12
Cyclohexanone	0.2	586

- *Preparation of Mixed Solution C.*

It is prepared by measuring a certain volume (V_b) of Mixed Solution B into a 10-ml volumetric flask followed by dilution with methanol. This solution would be used to prepare the calibration standards.

Table 5.5: Concentration of standards in Mixed Solution C

Compound	V_b(ml)	Concentration(mg/l)
n- Butylacetate	0.4	17
m-Xylene	0.4	0.64
1,3-Dichloroacetone	0.4	330
Styrene	0.4	0.37
o-Xylene	0.4	1.0
p-Xylene	0.4	0.50
Cyclohexanone	0.4	23

- *Preparation of Mixed Solutions for Precision Studies.*

The mixed solutions for the repeatability and intermediate studies were carried out using two levels of concentration for each standard i.e. minimum and medium concentration

Table 5.6: Two levels of Concentration for Precision Studies.

Compound	Min. Conc.(µg/l)	Med. Conc. (µg/l)
n-Butylacetate	34	92
m-Xylene	1.3	3.4
1,3-Dichloroacetone	660	1432
Styrene	1.4	2.6
o-Xylene	2.1	5.6
p-Xylene	1.0	2.6
Cyclohexanone	234	453

5.6 Gas Chromatographic and Mass Spectrometric Conditions.

5.6.1 Equipment.

The equipment used for the analysis is an Agilent Technologies 6890N-Gas Chromatograph coupled to a Waters Micromass GCT Premier Oa -TOF Mass Spectrometer.

5.6.2 Gas Chromatographic Conditions.

- Mode of injection: Split mode.
- Column dimensions: 30m x 0.25mm x 0.25um
- Software for data analysis: MassLynx 4.1
- Carrier gas: Helium (purity 99.999%).
- Flow rate: 1ml/min.
- Pressure: 72kPa

- Temperature of injector: 200°c
- Desorption Time: 1min

5.6.3 Mass Spectrometric Conditions.

- MCP Voltage:2600-2700
- Electron Energy: 70eV
- Source Temperature:180°C
- Trap Current:178μA
- TOF Flight Tube: 4602V
- Reflectron Voltage: 1763
- Push out Voltage: 981
- Mass Range:50-250
- GC Re-entrant Temperature: 250° C
- Re-entrant Temperature: 100° C

6. Discussion of Results.

6.1 Optimization of Chromatographic Parameters.

All gas chromatographic parameters were optimized according to validated methods at the EPAL Central Laboratory.

Table 6.1 shows one of the temperature programs of the column oven that is routinely used at the laboratory for the analysis of volatile and semi-volatile components with an initial oven temperature of 40°C at 1min.

Table 6.1: Column oven program used at EPAL Laboratory.

Temperature(°C)	Rate(°C/min)	Hold(min)	Total(min)
50	2	4	9
70	4	2	7
250	10	10	28

The retention times and areas of the target compounds are obtained by injecting a certain concentration of each standard using a syringe followed by analysis with GC/TOFMS. Since electron ionization mode was used, identification of each compound was made by comparing the obtained fragmentation pattern at that retention time to spectral library stored in the NIST library search. Since several fragmented ions are produced for each compound, the ion mass of each spectrum that produced the largest area in full scan mode of each target compound was used for quantification.

Table 6.2 shows the retention times and the ion masses that are used for the quantification of each compound.

Table 6.2: Retention times and mass ions for target compounds.

Compound	Retention Time(min)	Ion Mass used for Quantification
n-Butylacetate	5,23	56,06
m-Xylene	7,06	91,06
p-Xylene	7,09	91,06
1,3-Dichloroaectone	7,63	76,98
Styrene	8,09	78,05
o-Xylene	8,19	91,06
Cyclohexanone	9,19	98,07

6.1.1 Optimization of Column Oven Temperature Program.

Since all of the compounds are eluted within a very short period, it was necessary to adjust the column oven temperature program so that the analysis could be performed in a very short time. By injecting a mixed solution containing all components in equal concentration with a syringe, it was observed that there was co-elution between m-xylene and p- xylene and also between styrene and o-xylene. Several column oven temperature programs were tried so as to obtain good resolution between the compounds that co-eluted but all proved to be unsuccessful. As a result, the best option was selected based on the shortest length of time for the analysis (20mins). **Table 6.3** is the column oven temperature program used for all the analysis with an initial oven temperature of 40 °C at 1min.

Table 6.3: Optimized column oven program for analysis.

Temperature(°C)	Rate(°C/min)	Hold(min)	Total(min)
50	2	4	9
70	4	2	7
100	0	10	3

With this column oven temperature program, the identification and quantification of styrene and o-xylene were done using the ion masses of 78,05 and 91,06 respectively. However, since m-xylene and p-xylene could both be identified and quantified using 91,06, it was assumed

that the resulting peak and area was a combination of both compounds. This meant that the peak area at 7,09 min was the addition of the concentrations of m-xylene and p-xylene which was considered as m+p xylene. This column oven temperature program was now used for the optimization of SPME.

Figure 6.0 is a representation of the chromatogram obtained using the optimized column oven temperature program.

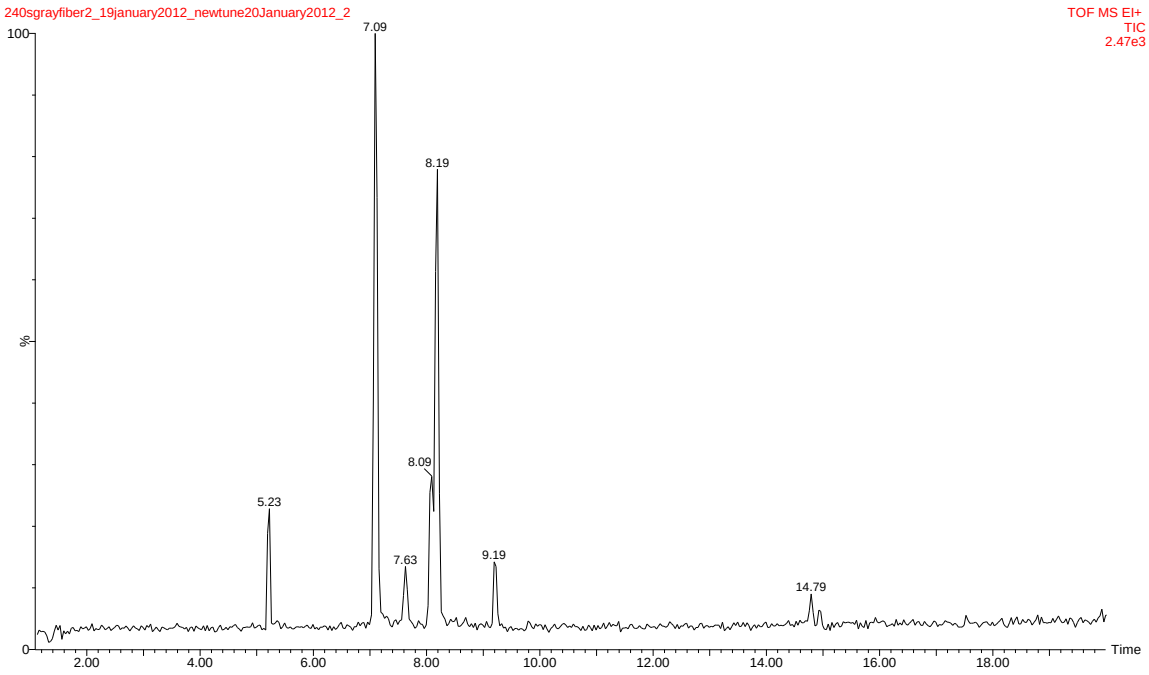


Figure 6.0: Chromatogram of target compounds. The peak at 7.09 is because of the co elution of m+p Xylene.

6.2 Optimization of SPME Method.

After investigating several mixed solutions containing different concentrations of the target compounds with the 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) fiber, it was observed that 400ul of Mixed Solution A in 18ml of water (immersion mode for SPME) produced detectable peaks that are characteristic for all target compounds. **Table 6.4** shows the concentrations used in the immersion mode for the optimization of the SPME method.

Table 6.4: Concentration of components in immersion mode (18ml water).

Compound	Concentration(mg/l) in 18ml of Water
n-Butylacetate	0.10
m-Xylene	0.11
1,3-Dichloroacetone	1.36
Styrene	0.10
o-Xylene	0.12
p-Xylene	0.11
Cyclohexanone	6.51

6.2.1 Optimization of Split Ratio.

Since all target compounds were considered to be volatile, split injection mode was used for the analysis and several split ratios were investigated. **Figure 6.1** shows the areas obtained for each compound at different split ratios.

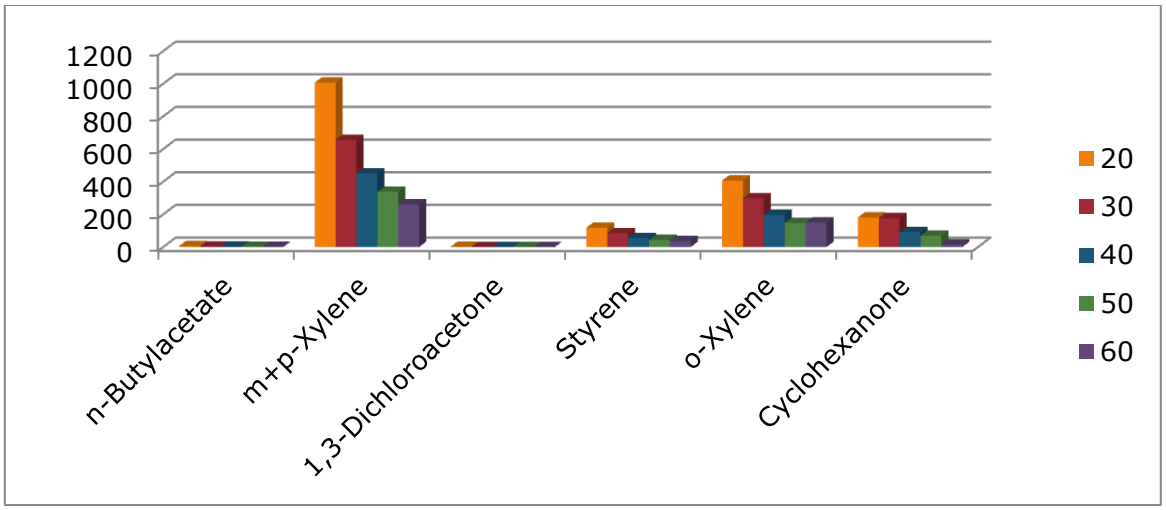


Figure 6.1: Optimisation of split ratio using 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) fiber.

It could be observed that a split ratio of 20 produced highest areas for most compounds. The usage of lower split ratios other 20 and Splitless injection produced broad peaks. A split ratio of 20 was chosen for further analysis since it produced thin peaks.

6.2.2 Optimization of Extraction Temperature.

With a split ratio of 20, several extraction temperatures were investigated. From **Figure 6.2**, it could be seen that an extraction temperature of 50°C produced largest areas for most compounds.

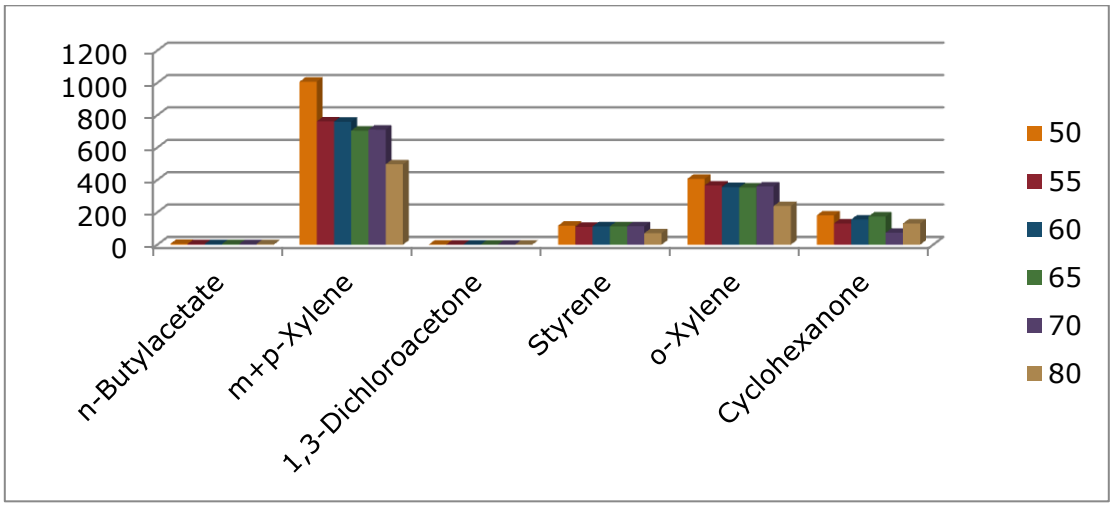


Figure 6.2: Optimisation of extraction temperature using 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) fiber.

6.2.3 Optimization of Stirring Speed.

With a split ratio of 20 and an extraction temperature of 50°C, three rotational speeds were investigated and **Figure 6.3** shows that a speed of 500rpm produced highest areas for each compound.

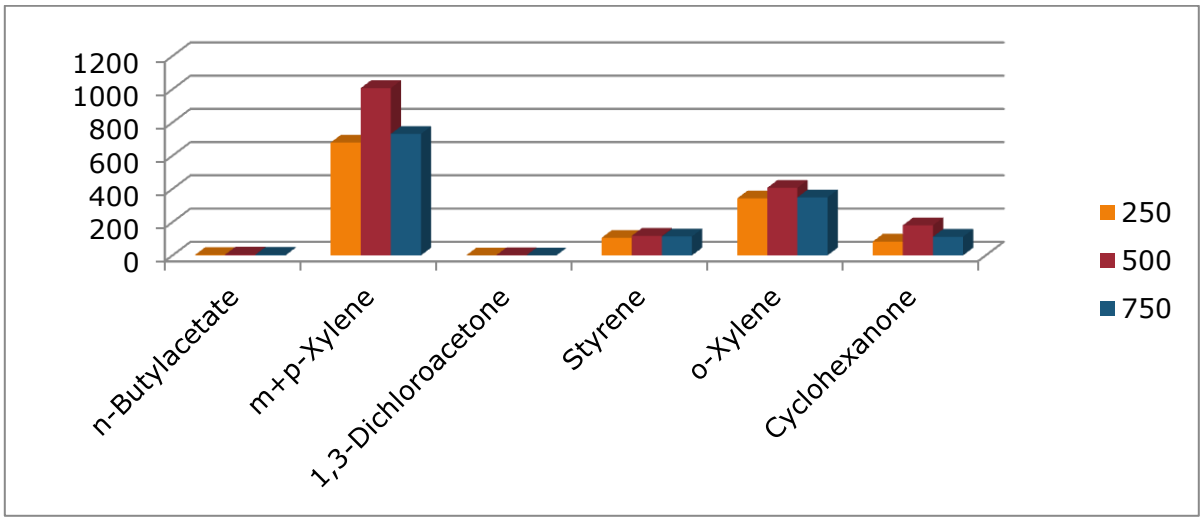


Figure 6.3: Optimization of stirring speed using 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) fiber.

6.2.4 Optimization of Extraction Time.

With a split ratio of 20, extraction temperature of 50°C and rotational speed of 500rpm, the areas of each compound were obtained at several extraction times. **Figure 6.4** shows that 120seconds was the best extraction time for most compounds.

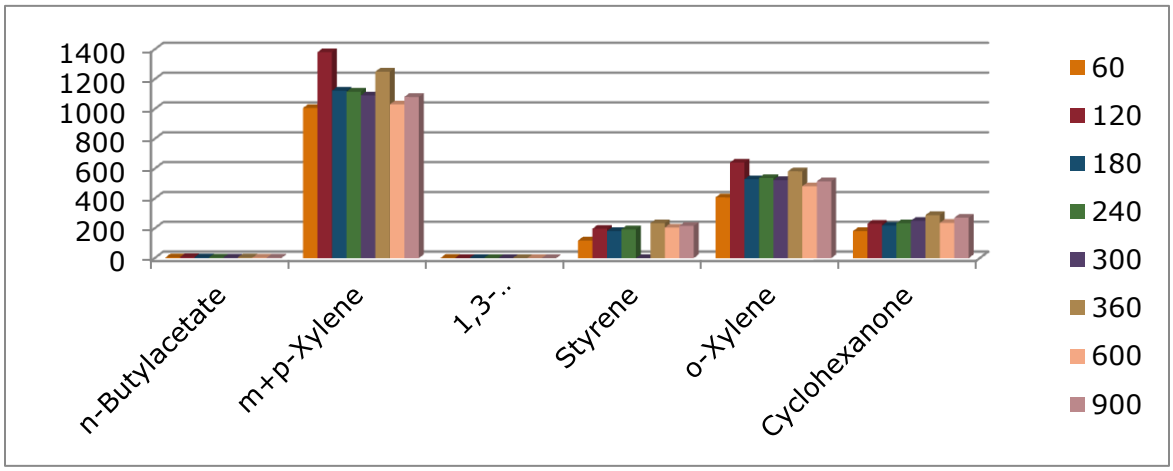


Figure 6.4: Optimisation of extraction time using 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) fiber.

6.2.5 Effect of Sodium Chloride (Salting-out) on Extraction of Components.

With a split ratio of 20, extraction temperature of 50°C, rotational speed of 500rpm and extraction time of 120seconds, all the compounds are extracted from a solution containing 5,4g of NaCl and the areas of the extracted components were compared to those of a solution with no NaCl (**Figure 6.5**). It was observed that though the addition NaCl increased the amount of extracted components; the effect was not so significant compared to when no NaCl is used. This implied that it was preferable to extract components without salting-out since there was not any significant difference in areas for all compounds with and without salting out.

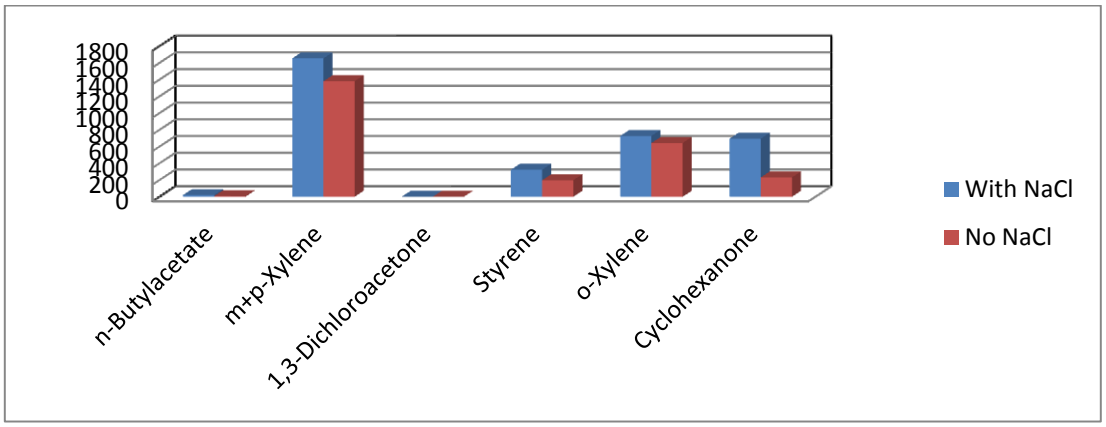


Figure 6.5: Salting-out effect using 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) fiber.

It was decided that in order to optimize the SPME adsorption conditions for each fiber, a split ratio of 20 would be used and the rotational speed of the autosampler would kept constant at 500rpm because investigation of higher speeds could damage the fiber the SPME fiber assembly.

6.3 Optimization of Fibers for SPME.

In order to obtain the best fiber and its optimal conditions that could be used for validation studies, 400ul of Mixed Solution B was placed in 15ml of water and three SPME adsorption parameters (mode of extraction, temperature of extraction and extraction time) were optimized of all five fibers using a constant split ratio of 20 and rotational speed of 500rpm. **Table 6.5** shows the concentration of each compound in the vial of 15ml of water.

Table 6.5: Concentration of standards in Headspace mode (15ml water).

Compound	Concentration(mg/l) in 15ml of Water
n-Butylacetate	11.46
m-Xylene	0.43
1,3-Dichloroacetone	220.37
Styrene	0.25
o-Xylene	0.70
p-Xylene	0.33
Cyclohexanone	15.62

6.3.1 Optimization of 65um Polydimethylsiloxane-Divinylbenzene (Blue) Fiber.

This fiber is coated in a cross-linked form and it is bipolar in nature. It is mostly used for the extraction of polar volatiles and adsorption is the main principle of extraction[7].

- *Optimization of Mode of Extraction for 65um Polydimethylsiloxane-Divinylbenzene (Blue) Fiber.*

Figure 6.6 is a comparison of the extraction efficiency at headspace and immersion mode. It was observed that the extraction efficiency for all compounds was higher when using headspace as compared to immersion mode.

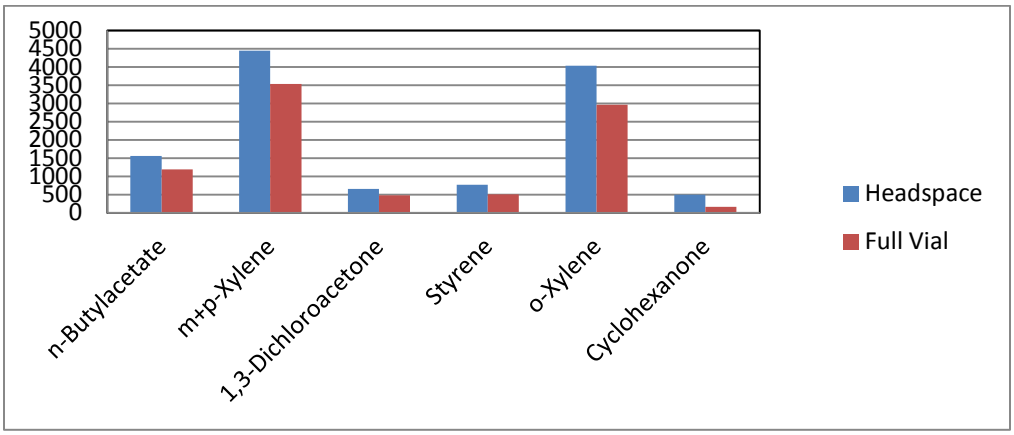


Figure 6.6: Comparison of extraction efficiency at different extraction modes with 65um Polydimethylsiloxane- Divinylbenzene (Blue) fiber.

- *Optimization of Temperature in Headspace Mode for 65um Polydimethylsiloxane-Divinylbenzene (Blue) Fiber.*

With headspace mode, a comparison of the extraction efficiency is made at different temperatures (**Figure 6.7**). It was considered that 60 °C could provide better extraction efficiency than at other temperatures.

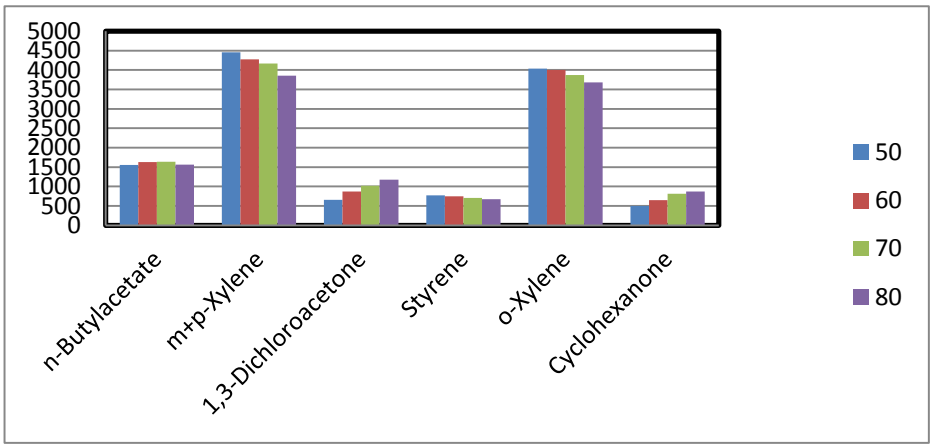


Figure 6.7: Comparison of extraction efficiency at different temperatures with 65um Polydimethylsiloxane- Divinylbenzene (Blue) fiber.

• *Optimization of Extraction Time in Headspace Mode at 60° C for 65um Polydimethylsiloxane-Divinylbenzene (Blue) Fiber.*

With these conditions, a comparison of extraction efficiency is made at different times (**Figure 6.8**). The extraction efficiency was highest at 240s for most of the compounds. This implied that after this time, there could be no significant change in extraction efficiency. It is therefore assumed that all components would be extracted after 240s

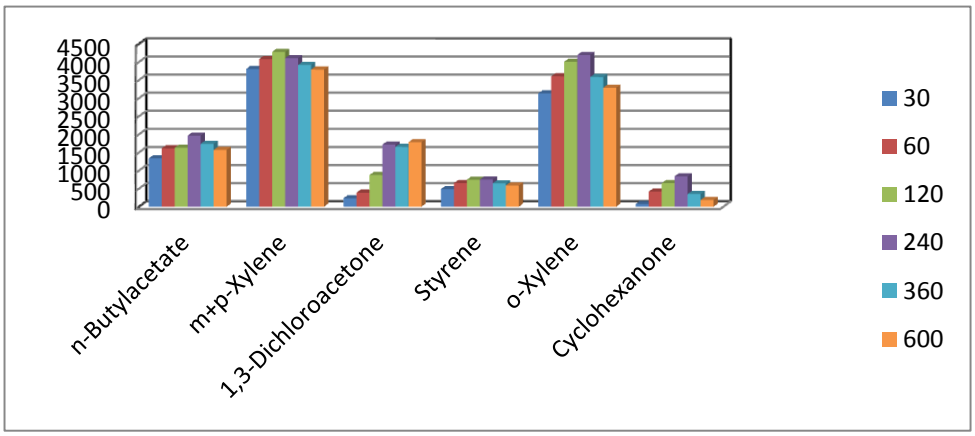


Figure 6.8: Comparison of extraction efficiency at different times (in seconds) with 65um Polydimethylsiloxane- Divinylbenzene (Blue) fiber.

The optimal conditions for SPME adsorption with the 65um Polydimethylsiloxane-Divinylbenzene (Blue) fiber were an extraction time of 240s at 60° C in headspace mode.

6.3.2 Optimization of 100um Polydimethylsiloxane Coating (Red) Fiber.

This fiber is coated in a non-bonded form and it is non-polar in nature. It is mostly used for the extraction of volatiles and absorption is the main principle of extraction[7].

• *Optimization of Mode of Extraction for 100um Polydimethylsiloxane Coating (Red) Fiber.*

Figure 6.9 represents a comparison of extraction efficiency for all compounds at different extraction modes. It was observed that the extraction efficiencies were significantly higher in headspace than in immersion mode for most of the compounds.

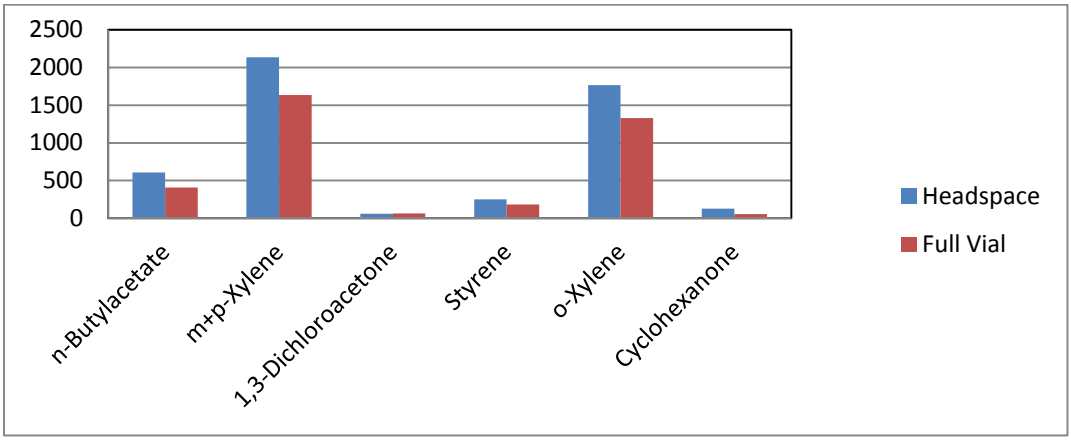


Figure 6.9: Comparison of extraction efficiency at different extraction modes with *100um Polydimethylsiloxane Coating (Red)* fiber.

• *Optimization of Temperature in Headspace Mode for 100um Polydimethylsiloxane Coating (Red) Fiber.*

With headspace, a comparison of extraction efficiency at different temperatures (**Figure 6.10**) was made. It was consider that 60°C provided the highest efficiency for most compounds than at other temperatures.

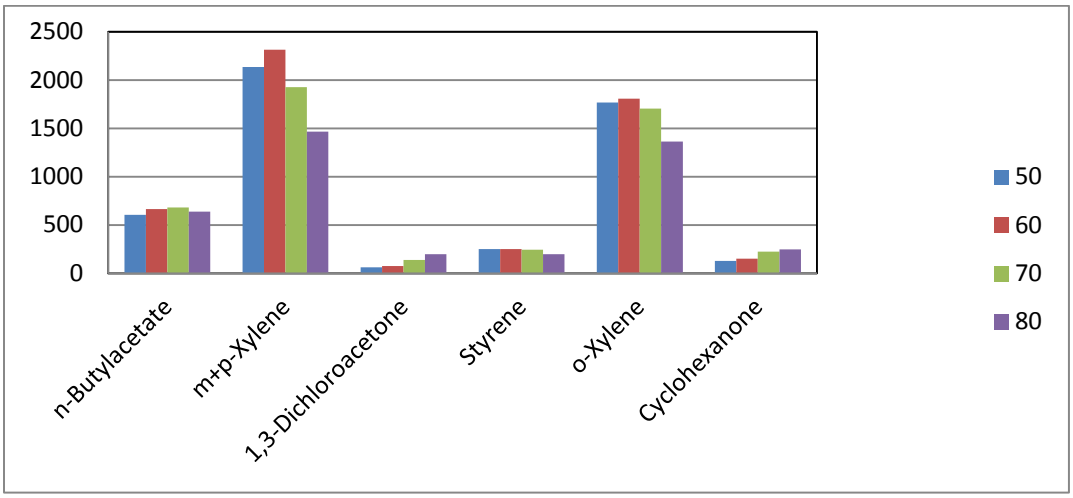


Figure 6.10: Comparison of extraction efficiency at different temperatures with *100um Polydimethylsiloxane Coating (Red)* fiber.

- *Optimization of Extraction Time in Headspace Mode at 60° C for 100um Polydimethylsiloxane Coating (Red) Fiber.*

A comparison of extraction efficiency at different times (**Figure 6.11**) is made in headspace mode at 60° C. In this case, it was considered that 240s provided the highest extraction efficiency for most of the compounds than at other times.

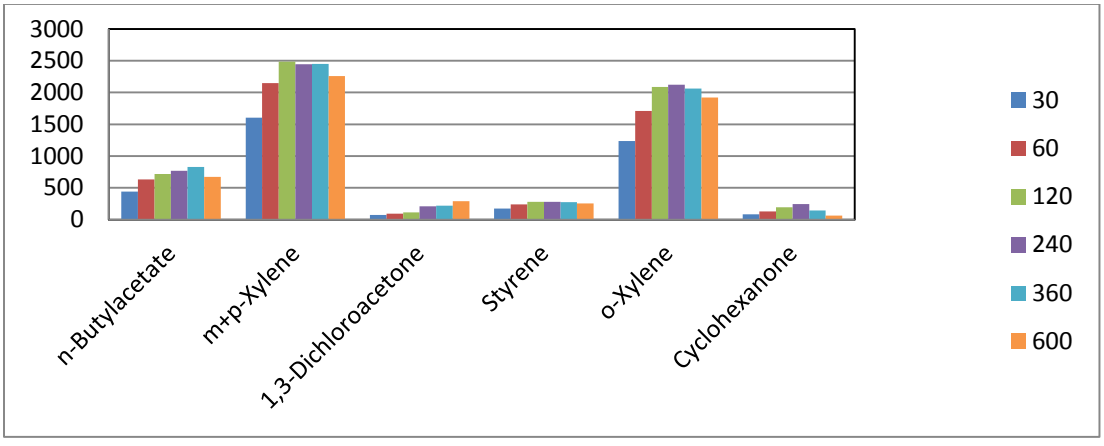


Figure 6.11: Comparison of extraction efficiency at different times (in seconds) with *100um Polydimethylsiloxane Coating (Red)* fiber.

The optimal conditions for SPME adsorption with the *100um Polydimethylsiloxane Coating (Red)* fiber were an extraction time of 240s at 60° C in headspace mode.

6.3.3 Optimization of 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) Fiber.

This fiber is coated in a cross-linked form and it is bipolar in nature. It is mostly used for the extraction of odours and flavours and absorption is the main principle of extraction[7].

- *Optimization of Mode of Extraction for 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) Fiber.*

A comparison of the extraction efficiency at different extraction modes (**Figure 6.12**) indicates significantly higher extraction efficiency in headspace than immersion mode.

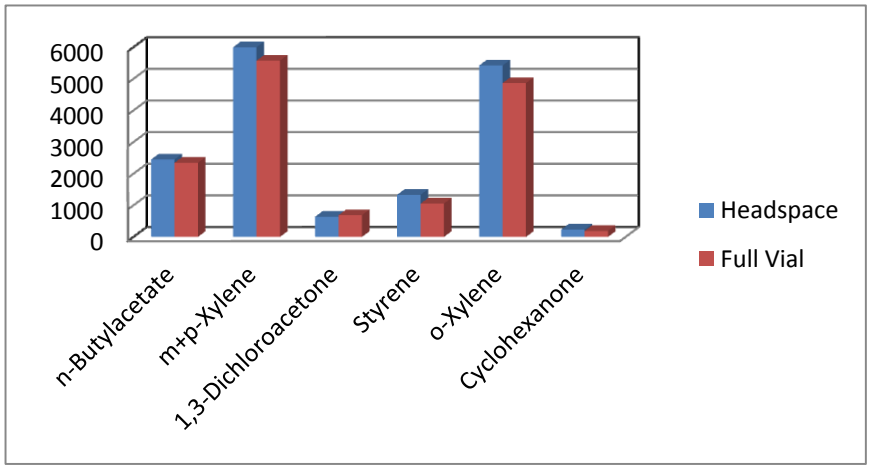


Figure 6.12: Comparison of extraction efficiency at different extraction modes with *50/30um DVB/Carboxen/PDMS Stable Flex (Gray)* fiber.

- *Optimization of Temperature in Headspace Mode for 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) Fiber.*

In headspace mode, a comparison of extraction efficiency at different temperatures (**Figure 6.13**) is performed. It was also considered that the extraction efficiency was higher at 70°C for most of the compounds than at other temperatures.

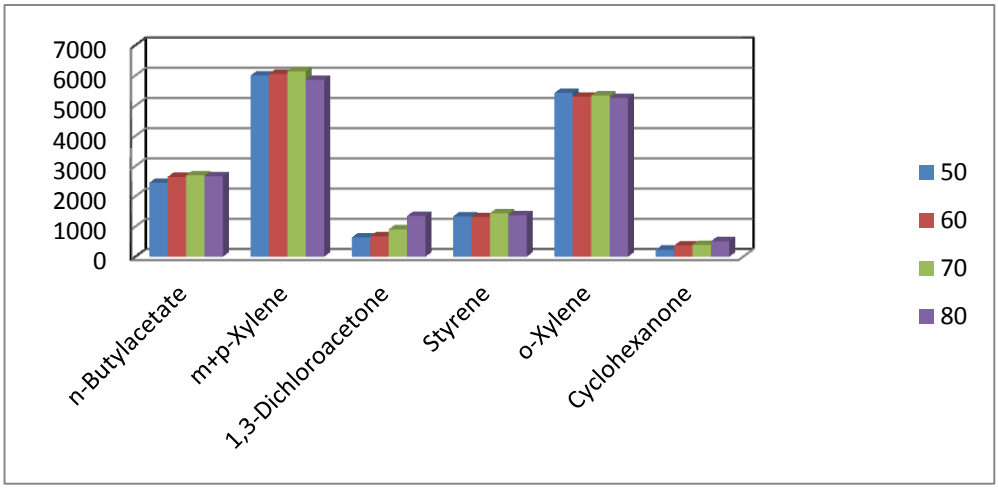


Figure 6.13:Comparison of extraction efficiency at different temperatures with *50/30um DVB/Carboxen/PDMS Stable Flex (Gray)* fiber.

- *Optimization of Extraction Time in Headspace Mode at 70° C for 50/30um DVB/Carboxen/PDMS Stable Flex (Gray) Fiber.*

Under these conditions, a comparison of extraction efficiency at different times (**Figure 6.14**) shows that the extraction efficiency was highest at 240s for most of the compounds.

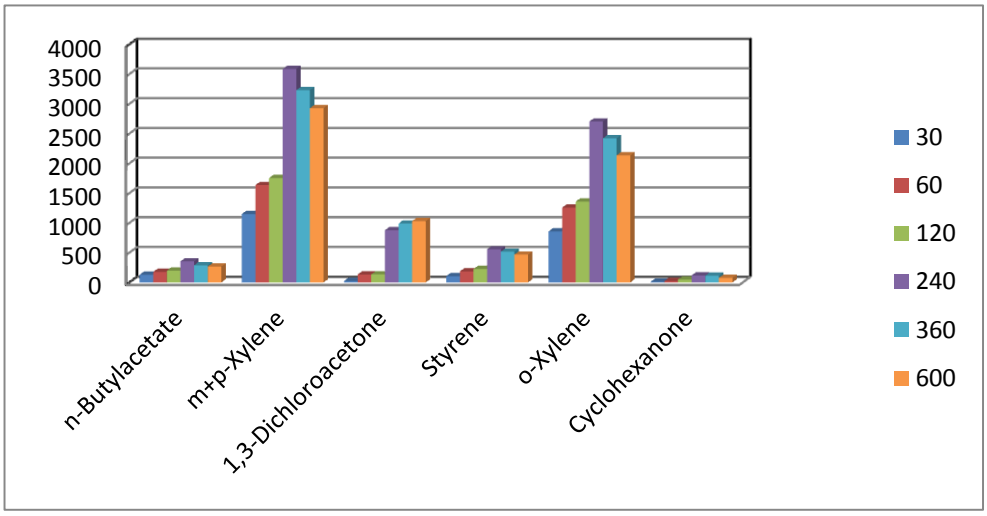


Figure 6.14: Comparison of extraction efficiency at different times (in seconds) with *50/30um DVB/Carboxen/PDMS Stable Flex (Gray)* fiber.

The optimal conditions for SPME adsorption with*50/30um DVB/Carboxen/PDMS Stable Flex (Gray)* fiber were an extraction time of 240s at 70° C in headspace mode.

6.3.4 Optimization of 85um Polyacrylate, Fused Silica (White) Fiber.

This fiber is coated in a cross-linked form and it is polar in nature. It is mostly used for the extraction of polar semi volatiles (phenols) and absorption is the main principle of extraction[7].

- *Optimization of Mode of Extraction for 85um Polyacrylate, Fused Silica (White) Fiber.*

Figure 6.15 is a comparison of extraction efficiency at different extraction modes. Higher extraction efficiencies were observed in headspace than immersion mode.

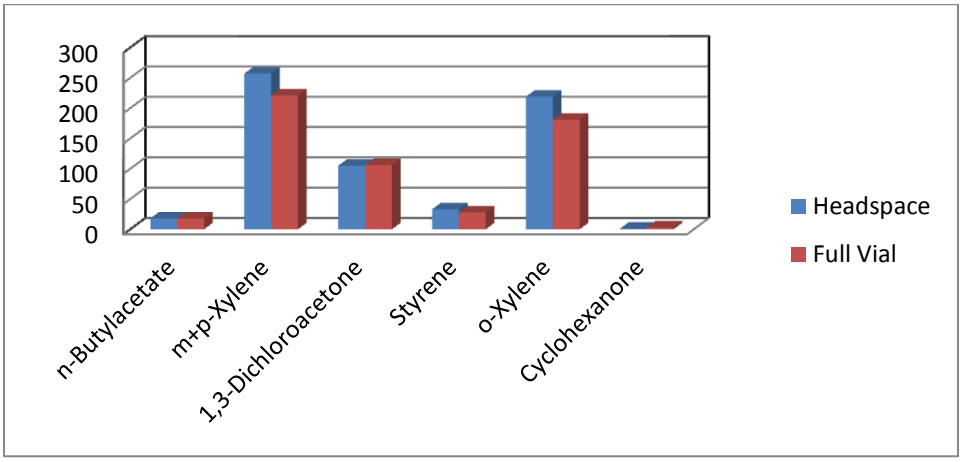


Figure 6.15: Comparison of extraction efficiency at different extraction modes with *85um Polyacrylate, Fused Silica (White)* fiber.

- *Optimization of Temperature in Headspace Mode for 85um Polyacrylate, Fused Silica (White).*

In headspace, a comparison of extraction efficiency at different temperatures (**Figure 6.16**) shows that 80 °C provided the highest extraction efficiency than at other temperatures in headspace mode.

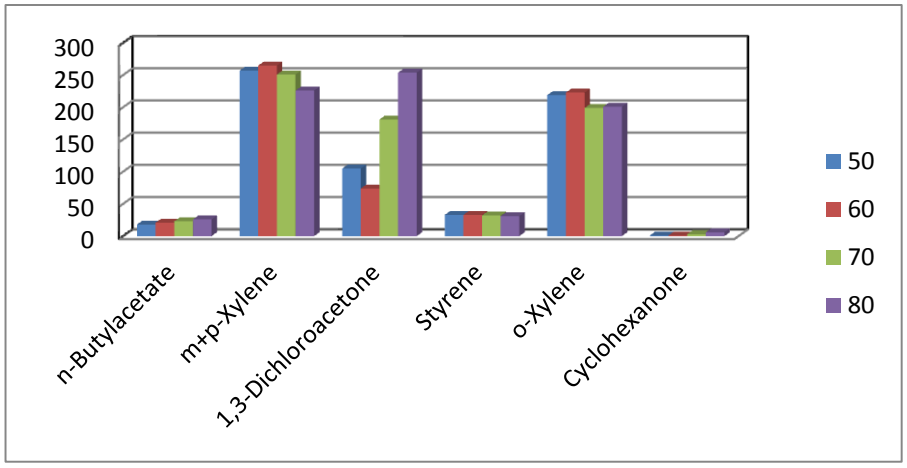


Figure 6.16: Comparison of extraction efficiency at different temperatures with 85um *Polyacrylate, Fused Silica (White)* fiber.

- *Optimization of Extraction Time in Headspace Mode at 80 °C 85um Polyacrylate, Fused Silica (White) Fiber.*

A comparison of extraction efficiency at different times (**Figure 6.17**) showed that extraction efficiency was highest at 240s for most of the compounds under these conditions.

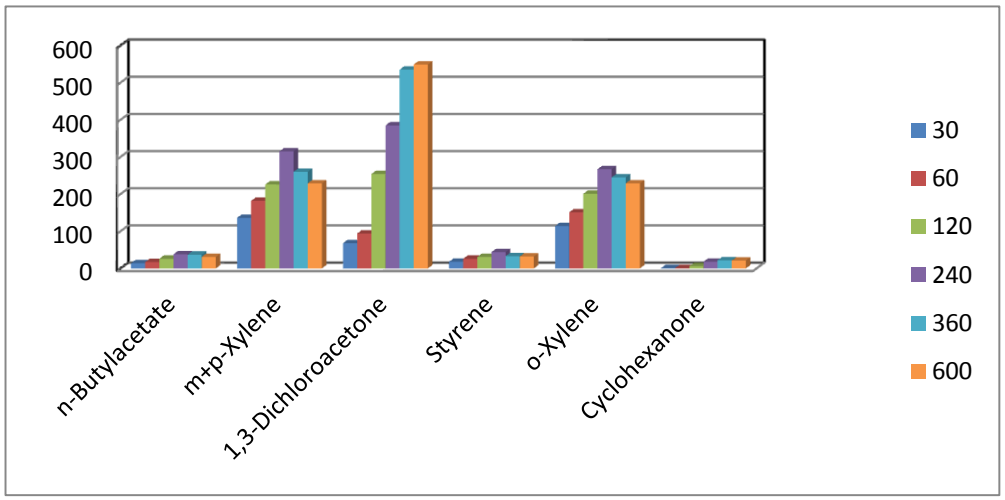


Figure 6.17: Comparison of extraction efficiency at different times (in seconds) with *85um Polyacrylate, Fused Silica (White)* fiber.

The optimal conditions for SPME adsorption with *85um Polyacrylate, Fused Silica (White)* fiber were an extraction time of 240s at 80° C in headspace mode.

6.3.5 Optimization of 75um Carboxen-PDMS (Black) Fiber.

This fiber is coated in a cross-linked form and it is bipolar in nature. It is usually used for the extraction of gases and volatiles and adsorption is the operating principle of extraction[7].

- *Optimization of Mode of Extraction for 75um Carboxen-PDMS (Black) Fiber.*

Figure 6.18 is a comparison of extraction efficiency at different extraction modes. This fiber showed higher extraction efficiency at headspace than at immersion mode.

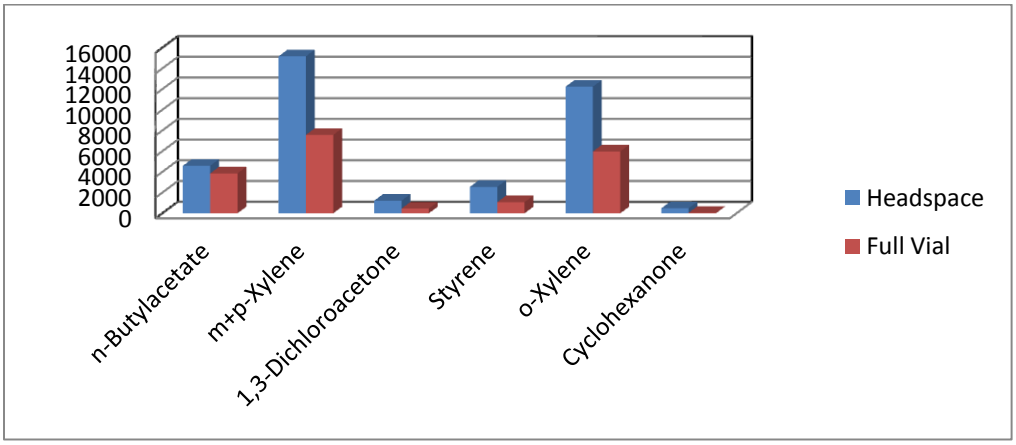


Figure 6.18: Comparison of extraction efficiency at different extraction modes with *75um Carboxen-PDMS (Black)* fiber.

- *Optimization of Temperature at Headspace Mode for 75um Carboxen-PDMS (Black) Fiber.*

In headspace mode, a comparison of extraction efficiency at different temperatures (**Figure 6.19**) is performed. It was considered that extraction efficiency was highest at 80 °C than other temperatures in headspace mode.

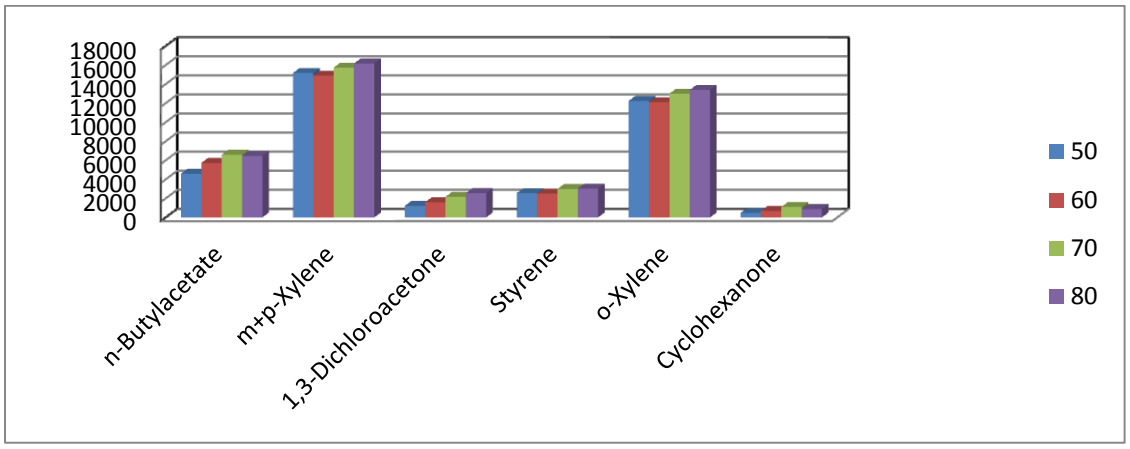


Figure 6.19: Comparison of extraction efficiency at different temperatures with *75um Carboxen-PDMS (Black)* fiber.

- *Optimization of Extraction Time in Headspace at 80° C 75um Carboxen-PDMS (Black) Fiber.*

Under these conditions, a comparison of extraction efficiency at different times (**Figure 6.20**) is made. It was considered that the extraction efficiency was highest at 360s for most of the compounds.

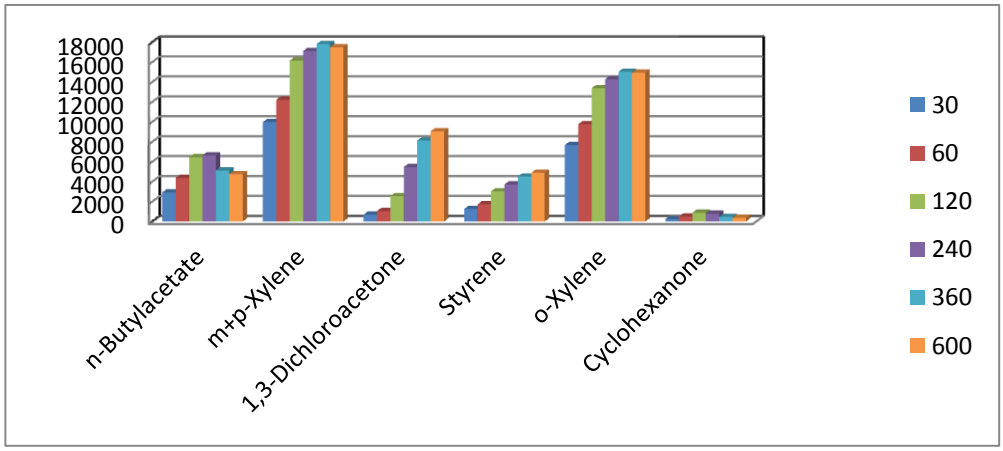


Figure 6.20: Comparison of extraction efficiency at different times (in seconds) with *75um Carboxen-PDMS (Black)* fiber.

The optimal conditions for SPME adsorption with *75um Carboxen-PDMS (Black)* fiber were an extraction time of 360s at 80° C in headspace mode.

6.4 Selection of Fiber and Conditions for Validation Studies.

Figure 6.21 is a representation of all the fibers and the best conditions (time and temperature of extraction) for which each fiber had the highest efficiencies for the extracted components in headspace mode.

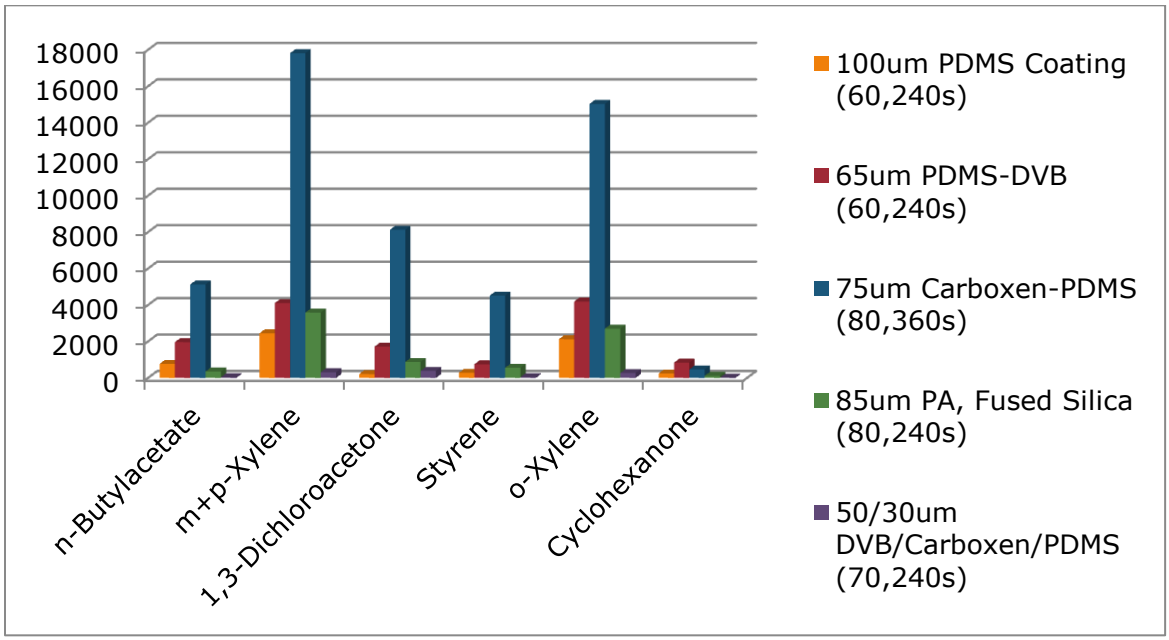


Figure 6.21: Comparison of different fibers at their optimal conditions.

It is clearly seen that *75um Carboxen-PDMS (Black) Fiber* showed the highest extraction efficiencies at 80 °C and 360s in the headspace mode. This is a confirmation that the thickness of this fiber and its chemical properties exert an influence on its extraction efficiency. This fiber and these conditions were used for carrying out the validation studies.

6.5 Method Validation Studies.

The calibration standards for these studies were prepared by measuring specific volumes ranging from 30ul to 450ul of Mixed Solution C into vials containing 15ml of water. Calibration curves were obtained by plotting the areas of the target ion mass against concentration. From these plots, a number of parameters are evaluated. These include:

6.5.1 Linearity and Working Range.

The least-square regression method (**Annex 1**) is used in calculating the determination coefficient (r^2) which is a good determinant of the linearity. Two statistical tests methods are used to investigate the linearity within a working range:

- *Mandel Test.*

The aim of this test is to investigate if the linear equation provides a better fit to the calibration curve and could always be used instead of the quadratic equation (**Annex 2**).

Table 6.6 is a summary of the results obtained for each compound using the Mandel test.

Table 6.6: Determination of linearity and linear range using Mandel Test

Compound	Number of Standards(N)	Determination Coefficient(r^2)	Working Range (µg/l)	Mandel Test	
				VT	F
n-Butylacetate	10	0.999	34–160	3.8	5.6
m+p Xylene	8	0.999	2.3–9.9	3.3	6.6
1,3-Dichloroacetone	8	0.996	660–2644	0.67	6.6
Styrene	8	0.998	0.87–3.4	-4.8	6.6
o-Xylene	9	0.999	2.1–9.5	0.80	5.6
Cyclohexanone	6	0.995	234–687	-0.32	10

Since VT is less than F for all components, it implies that the linear equation is a better approximation for the calibration curve than the quadratic equation.

- *Rikilt Test*.

The purpose of this test is to investigate if instrument calibration could be done with response factor instead of a calibration curve (**Annex 3**). If the calibration points fall within a

specified percentage range (90-110) for this test, it could be assumed that instrument calibration could be done using response factor.

Based on the calculations obtained using this test, it was observed that response factor could not be used for 1,3-dichloroacetone, styrene and cylcohexanone when instrument calibration is performed since some points did not fall within the specified percentage range.

However, response factor could be used for instrument calibration for n-butylacetate, m+p xylene and o-xylene in the working ranges shown in **Table 6.7**.

Table 6.7: Determination of linearity and linear range using Rikilt Test.

Compounds	Number of Standards (N)	Determination Coefficient (r^2)	Working Range (µg/l)	Rikilt Test (%)
n-Butylacetate	7	0.999	80–160	96-104
m+p Xylene	6	0.999	4.6–9.9	91-106
o-Xylene	7	0.999	4.2–9.5	94-103

6.5.2 Limit of Quantification and Limit of Detection.

These parameters are calculated using the residual standard deviation and slope of the calibration curve.

Table 6.8 is a representation of the LOD and LOQ of compounds used in this study. It can be observed that all LOD values for all compounds are lower than the lowest concentration for the calibration curves. This is a clear indication that LOD and LOQ are dependent of the amount of component extracted.

Table 6.8: LOD and LOQ values for target compounds using calibration curves.

Compound	LOD(µg/l)	LOQ(µg/l)	Lowest Concentration used for Calibration Curve (µg/l).
n-Butylacetate	3.8	13	34
m+p Xylene	0.20	0.80	2.3
1,3-Dichloroacetone	138	458	660
Styrene	0.10	0.50	0.87
o-Xylene	0.20	0.70	2.1
Cyclohexanone	37	123	234

6.5.3 Precision Studies.

These studies were mainly carried out at two levels of concentration (minimum and medium) for all compounds.

6.5.3.1 *Repeatability Studies.*

Ten standards of each compound at both concentration levels were measured within the same day and the coefficient of variation was calculated using the areas of target ions.

Table 6.9: Precision for SPME-GC/TOFMS method under repeatability conditions (n=10)

Compound	CV(%) for Minimum Level	CV(%) for Medium Level
n-Butylacetate	13	4.5
m+p Xylene	20	4.6
1,3-Dichloroacetone	10	9.9
Styrene	13	4.1
o-Xylene	18	3.3
Cyclohexanone	8.2	6.0

It was observed from **Table 6.9** that the coefficient of variation was lower for all compounds at the medium level than at the minimum concentration. However, all CV values for both minimum and medium level fell within the recommended limit of less than 25%.

6.5.3.2 *Intermediate Precision Studies*.

These studies were carried out by measuring six standards of each compound at both levels of concentration for a period of six days. **Table 6.10** represents the coefficient of variation of the compounds at intermediate conditions.

Table 6.10: Precision for SPME-GC/TOFMS method under intermediate precision conditions (n=6)

Compound	CV(%) for Minimum Level	CV(%) for Medium Level
n-Butylacetate	24	27
m+p Xylene	38	23
1,3-Dichloroacetone	27	27
Styrene	26	18
o-Xylene	34	25
Cyclohexanone	14	20

By comparing the coefficient of variation at repeatability conditions and intermediate conditions for minimum level (**Figure 6.22**) and medium level (**Figure 6.23**), it can be seen that CV values are higher at intermediate conditions than repeatability conditions.

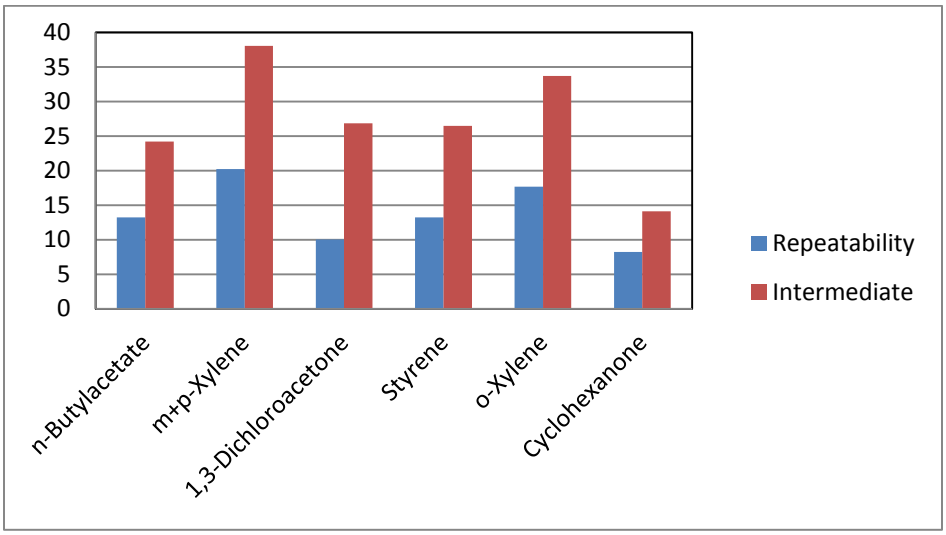


Figure 6.22: Comparison of repeatability and intermediate precision at minimum level concentration.

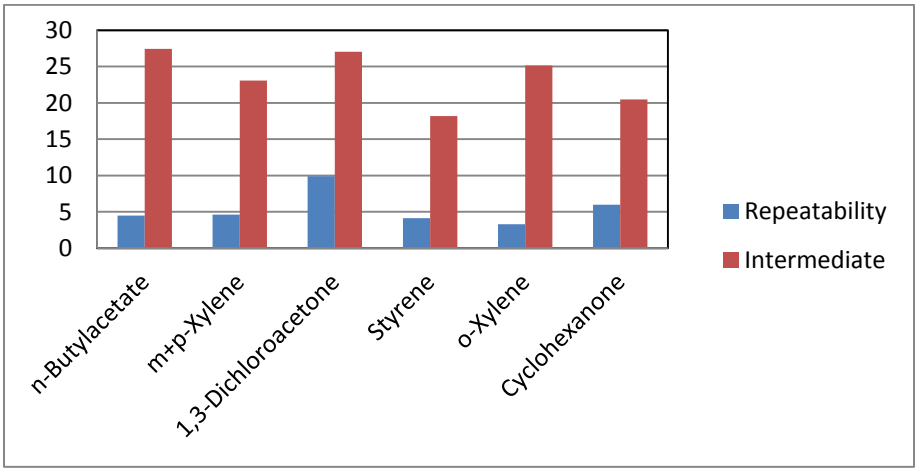


Figure 6.23: Comparison of repeatability and intermediate precision at medium level concentration.

6.5.4 Analyte Recovery.

Just like the precision studies, the recovery studies were performed using the same levels of concentration (minimum and medium) for all compounds. Calibration curves were prepared for the compounds in ultrapure water and two different water matrices were spiked with compounds at the two levels of concentration.

6.5.4.1 *Analyte Recovery in Tap Water.*

Table 6.11 represents the recoveries for the components at both levels of concentration in tap water

Table 6.11: Recovery studies for tap water.

Compound	Minimum Level Concentration		Medium Level Concentration	
	Recovery (%)	RSD (%) (n=6)	Recovery (%)	RSD (%) (n=6)
n-Butylacetate	53	48	69	3.7
m+p Xylene	145	70	78	20
1,3-Dichloroacetone	0		0	
Styrene	44	64	64	12
o-Xylene	161	63	83	17
Cyclohexanone	31	62	71	10

Except for the xylenes, it is observed that the recoveries at the medium level concentrations are significantly higher than for minimum level concentration. However, there is no recovery for 1,3-Dichloroacetone at both levels. This could be explained by the reaction of 1,3-Dichloroacetone with chlorine which is an active component of treated tap water. Therefore, other target compounds originating from the reaction of 1,3-Dichloroacetone with chlorine should be investigated and targeted in tap water instead of identifying and targeting 1,3-Dichloroacetone in tap water.

6.5.4.2 *Analyte Recovery in Surface Water*.

Table 6.12 represents the recoveries for the components at both levels of concentration in surface water.

Table 6.12: Recovery studies for surface water

Compound	Minimum Level Concentration		Medium Level Concentration	
	Recovery (%)	RSD (%) (n=6)	Recovery (%)	RSD (%) (n=6)
n-Butylacetate	12	50	80	7.8
m+p Xylene	32	65	44	6.6
1,3-Dichloroacetone	0		0	
Styrene	24	49	17	8.3
o-Xylene	54	52	47	4.5
Cyclohexanone	30	24	47	25

Apart of styrene, o-xylene, and cyclohexanone, the recoveries were higher for the medium level concentrations for n-butylacetate and m+p xylene. Also, there was no recovery for 1,3-dichloroacetone at both levels of concentration.

By comparing the recovery in both tap and surface water for all compounds at both minimum (**Figure 6.24**) and medium (**Figure 6.25**) levels, it can be clearly seen that recovery in tap water is higher than surface water. This would indicate that matrix effect is more significant in surface water than tap water.

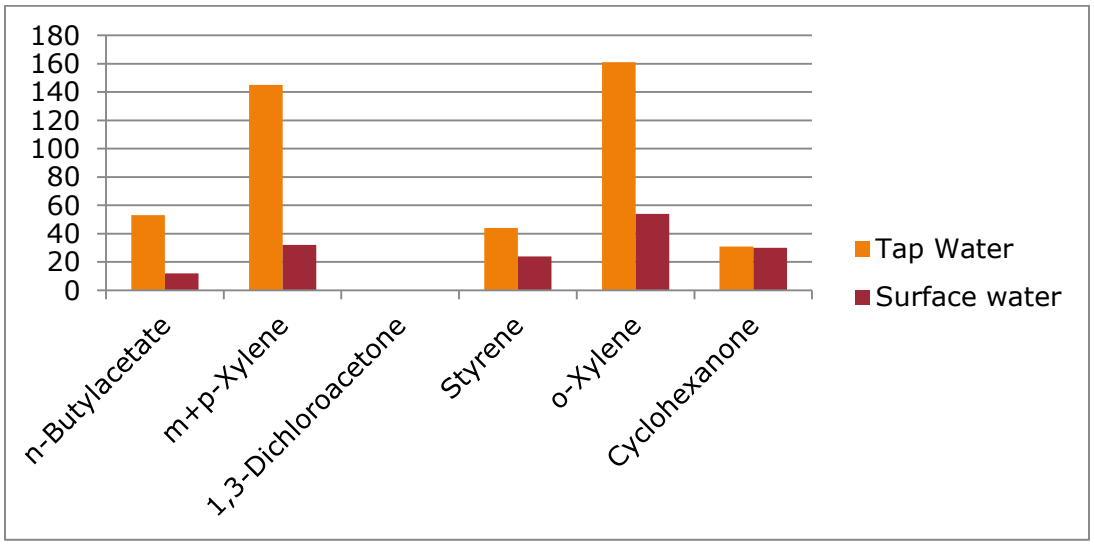


Figure 6.24: Comparison of recovery(%) in tap and surface water at minimum level concentration.

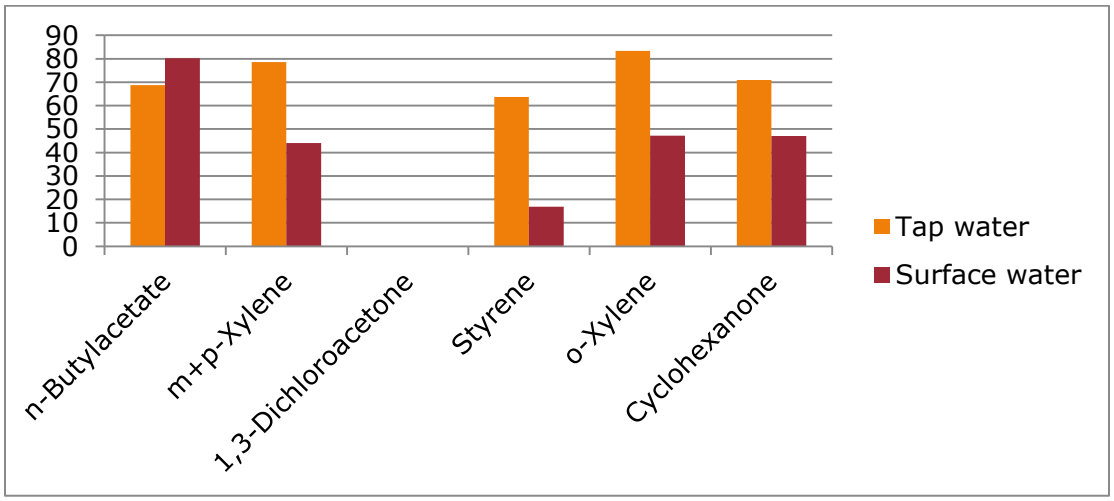


Figure 6.25: Comparison of recovery(%) in tap and surface water at medium level concentration.

7. Conclusions.

The combination of SPME together with GC/TOFMS is an analytical method that could be routinely used for the determination of target compounds that migrate from organic materials into drinking water because it combines good analytical performance with simplicity and easy automation.

The gas chromatographic parameters are optimized using the gray fiber (*50/30um DVB/Carboxen/PDMS Stable Flex*) to achieve a GC run time of 20mins and target mass ions are used to quantify each organic compound in the full scan mode for the TOF/MS.

The optimization of SPME adsorption conditions (mode of extraction, temperature and time) for five fibers (black, white, gray, blue and red) is performed to achieve high extraction efficiency for each target compound. The black fiber (*75um Carboxen-PDMS*) showed the highest extraction efficiency for all target compounds at 80° C and an extraction time of 360s in the headspace mode. This clearly indicates that extraction efficiency is greatly influenced by the thickness and chemical composition of the stationary phase of the fiber.

The black fiber (*75um Carboxen-PDMS*) is selected for method validation and based on the calibration curves for each target compound, the LOD ranged from 0.2µg/l for the xylenes to 137.5µg/l for 1,3-dichloroacetone. The regression coefficients for the various calibration curves fell within the accepted limit but further statistical tests on this coefficient showed that response factors could only be used for instrument calibration with n-butylacetate and the xylenes within specific working ranges. For the other compounds (styrene, 1,3-dichloroacetone and cyclohexanone), calibration curves have to be prepared each day for instrument calibration.

The coefficient of variation (CV) was less than 25% during repeatability studies for all compounds but this value was higher than 25% for compounds during intermediate precision studies. This clearly indicates that certain factors could affect instrument stability in the long-term.

Recovery studies in both tap and surface water indicated that the matrix composition contributed significantly in the extraction of target compounds from water. This is clearly explained by the fact that Cl_2 which is usually used as a treatment agent of tap water could react with 1,3-dichloroacetone to form other products leading to the non-identification and

quantification of 1,3-dichloroacetone. This means that other target compounds should be searched for in water since there is always the possibility of a reaction occurring between our interested target compounds and matrix components.

Since the extraction of target components is dependent on the thickness and chemical composition of the stationary phase of the fiber, it is recommended that other fibers in different SPME adsorption conditions be used to investigate other target compounds that migrate into drinking water and also to obtain higher extraction.

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Annex 1 Least Square Regression Method.

The equation for the calibration curve is represented in the linear form as:

$y = a + bx$

Where:

a = ordinate intercept

b= gradient of the line

y= instrumental signal

The ordinate intercept could be calculated as:

$a = y - bx$

The slope is calculated from the equation below:

$b = \Sigma(x_i - x)(y_i - y)/\Sigma(x_i - x)^2$

Where:

x= concentration

y= instrumental signal

Σ= summation of N- number of concentration levels.

The standard deviation of the regression line ($S_{y/x}$) is calculated as:

$S_{y/x} = \sqrt{\Sigma(y_i - y)^2/N-2}$

Where:

y_i= instrumental signal

y = predicted instrumental signal

Σ= summation for N– number of concentration levels.

N–2= number of degrees of freedom

The coefficient of determination (r^2) is calculated as:

$r^2 = (y - y_{GM})^2 / (y_i - y_{GM})$

Where:

Y_{GM} = mean of y/s distribution.

The standard deviation (S_{xo}) is calculated by:

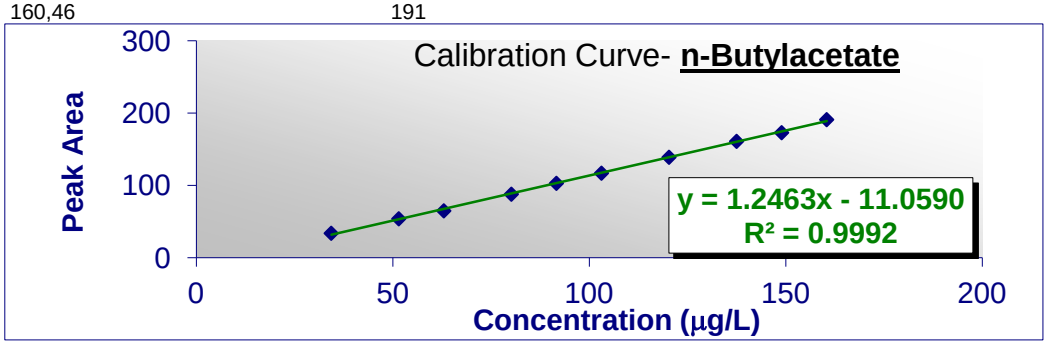
$S_{xo} = S_y / b$

The concentration (x_i) which is obtained by interpolation of the linear equation is calculated as:

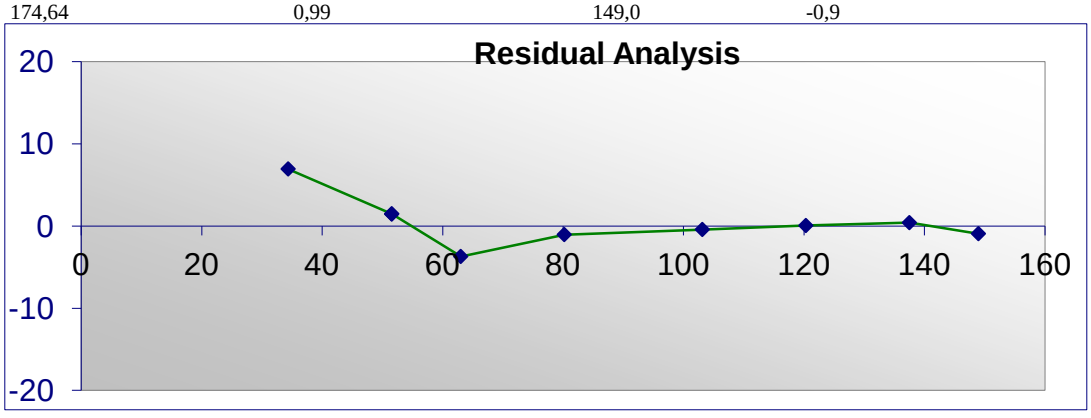
$x_i = (y_i - a)/b$

n-Butylacetate.

Concentration (µg/L)	Peak Area
34,38	34
51,58	54
63,04	65
80,23	88
91,69	103
103,15	117
120,34	139
137,54	161
149,00	173
160,46	191

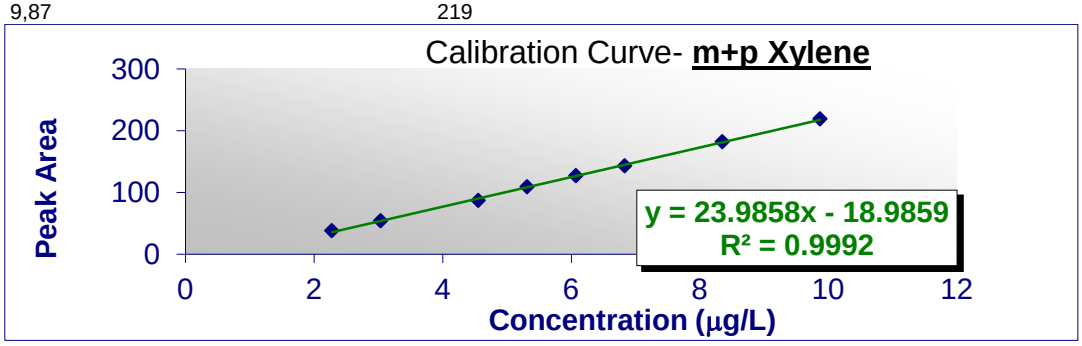


Estimated Peak Area	Experimental Area/ Estimated Area	Concentration (µg/L)	Deviation (%)
31,79	1,07	34,4	6,9
53,22	1,01	51,6	1,5
53,22	1,01	51,6	1,5
67,50	0,96	63,0	-3,7
88,93	0,99	80,2	-1,0
117,50	1,00	103,2	-0,4
138,93	1,00	120,3	0,1
160,35	1,00	137,5	0,4
174,64	0,99	149,0	-0,9

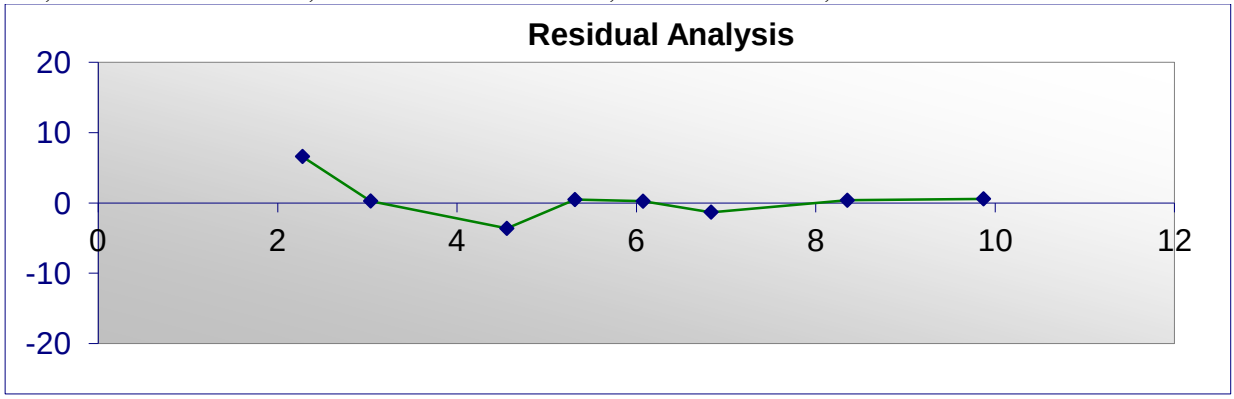


m+p Xylene

Concentration (µg/L)	Peak Area
2,28	38
3,04	54
4,56	87
5,31	109
6,07	127
6,83	143
8,35	182
9,87	219

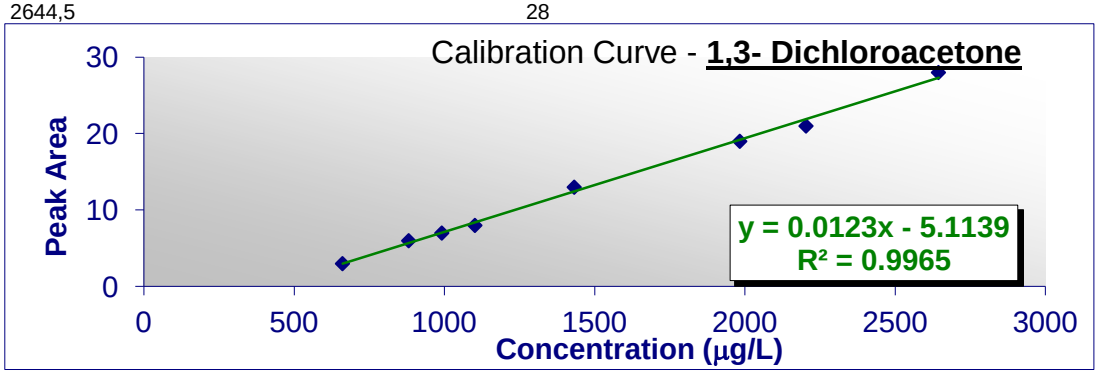


Estimated Peak Area	Experimental Area/ Estimated Area	Concentration (µg/L)	Deviation (%)
35,65	1,07	2,3	6,6
53,86	1,00	3,0	0,3
90,28	0,96	4,6	-3,6
108,49	1,00	5,3	0,5
126,70	1,00	6,1	0,2
144,92	0,99	6,8	-1,3
181,34	1,00	8,4	0,4
217,76	1,01	9,9	0,6

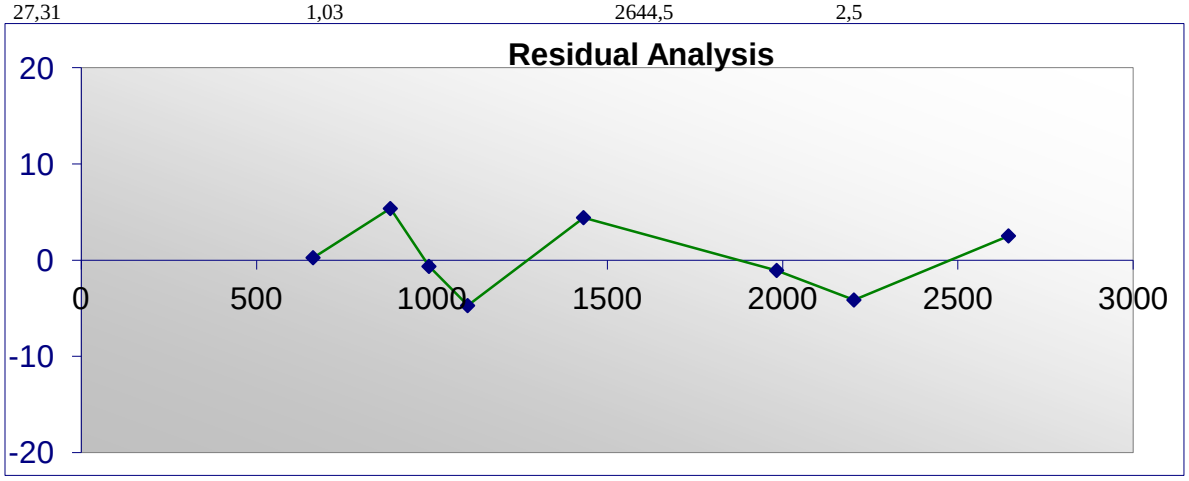


1,3-Dichloroacetone

Concentration (µg/L)	Peak Area
661,1	3
881,5	6
991,7	7
1101,9	8
1432,4	13
1983,4	19
2203,7	21
2644,5	28

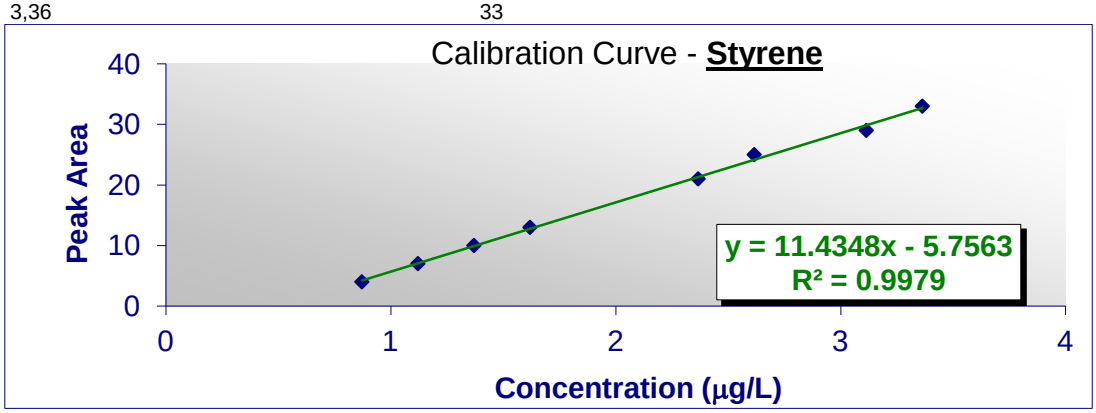


Estimated Peak Area	Experimental Area/ Estimated Area	Concentration (µg/L)	Deviation (%)
2,99	1,00	661,1	0,3
5,69	1,05	881,5	5,4
7,05	0,99	991,7	-0,6
8,40	0,95	1101,9	-4,7
12,45	1,04	1432,4	4,4
19,20	0,99	1983,4	-1,1
21,91	0,96	2203,7	-4,1
27,31	1,03	2644,5	2,5

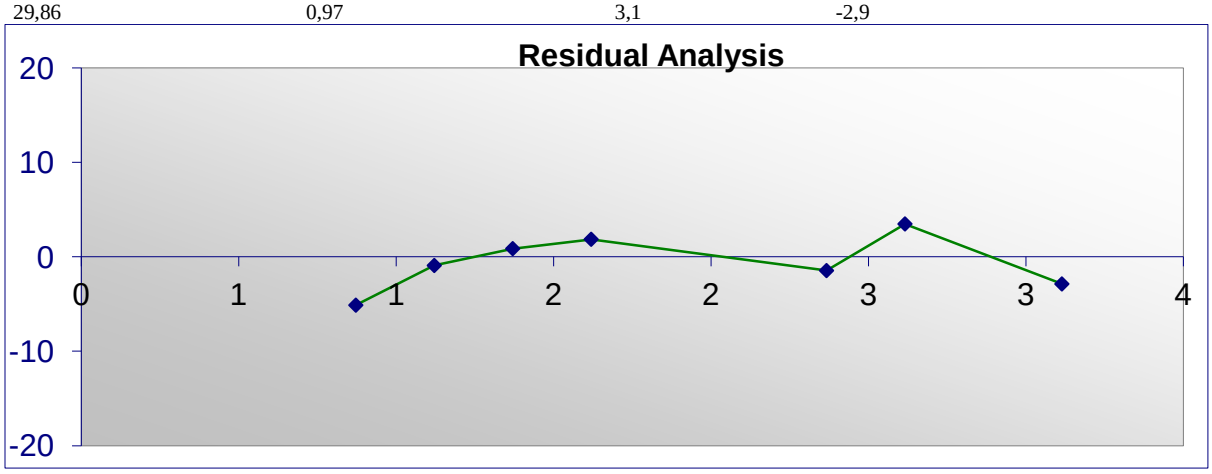


Styrene

Concentration (µg/L)	Peak Area
0,87	4
1,12	7
1,37	10
1,62	13
2,37	21
2,62	25
3,11	29
3,36	33

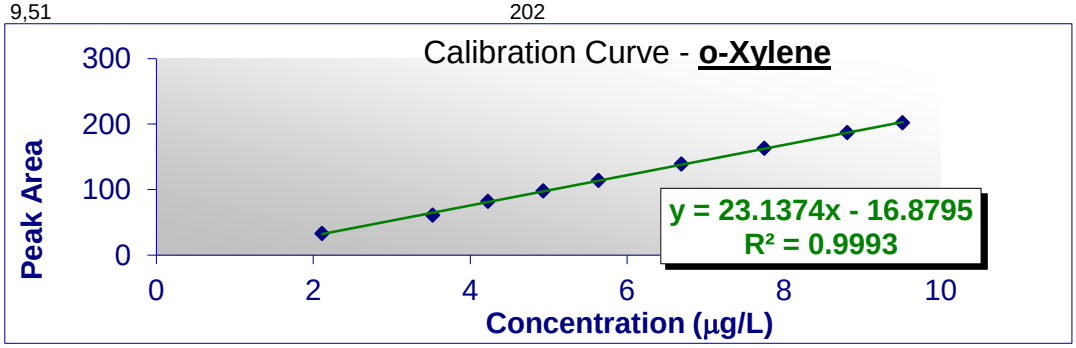


Estimated Peak Area	Experimental Area/ Estimated Area	Concentration (µg/L)	Deviation (%)
4,22	0,95	0,9	-5,1
7,07	0,99	1,1	-0,9
9,91	1,01	1,4	0,9
12,76	1,02	1,6	1,9
21,31	0,99	2,4	-1,5
24,16	1,03	2,6	3,5
29,86	0,97	3,1	-2,9

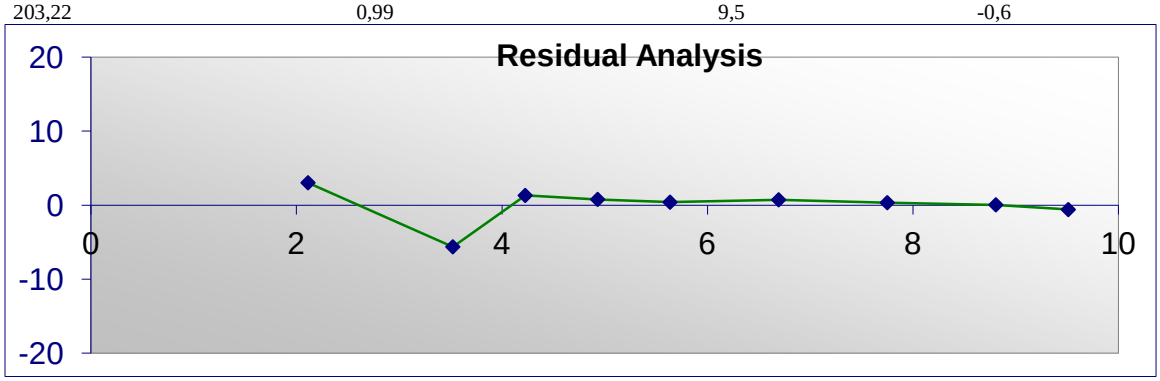


o-Xylene

Concentration (µg/L)	Peak Area
2,11	33
3,52	61
4,23	82
4,93	98
5,64	114
6,69	139
7,75	163
8,81	187
9,51	202

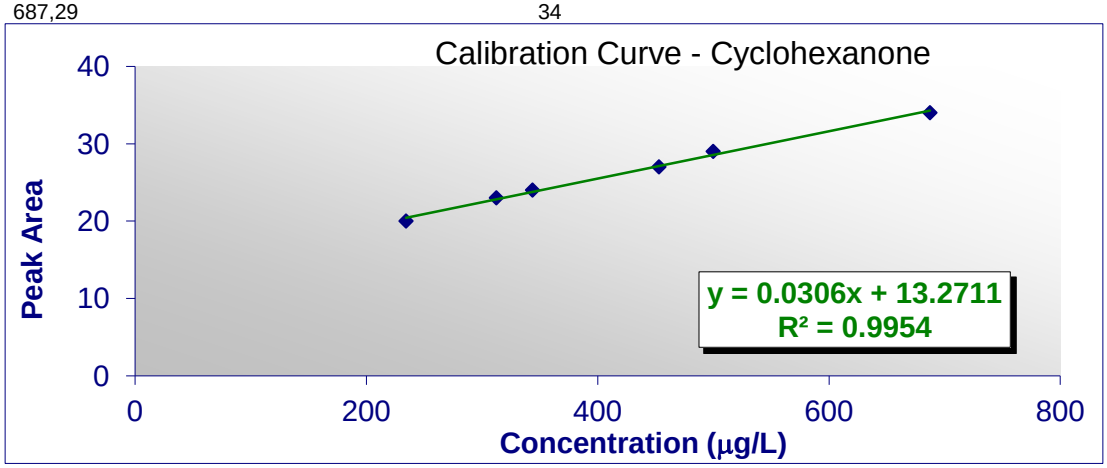


Estimated Peak Area	Experimental Area/ Estimated Area	Concentration (µg/L)	Deviation (%)
32,03	1,03	2,1	3,0
64,64	0,94	3,5	-5,6
80,94	1,01	4,2	1,3
97,25	1,01	4,9	0,8
113,55	1,00	5,6	0,4
138,00	1,01	6,7	0,7
162,46	1,00	7,8	0,3
186,91	1,00	8,8	0,0
203,22	0,99	9,5	-0,6

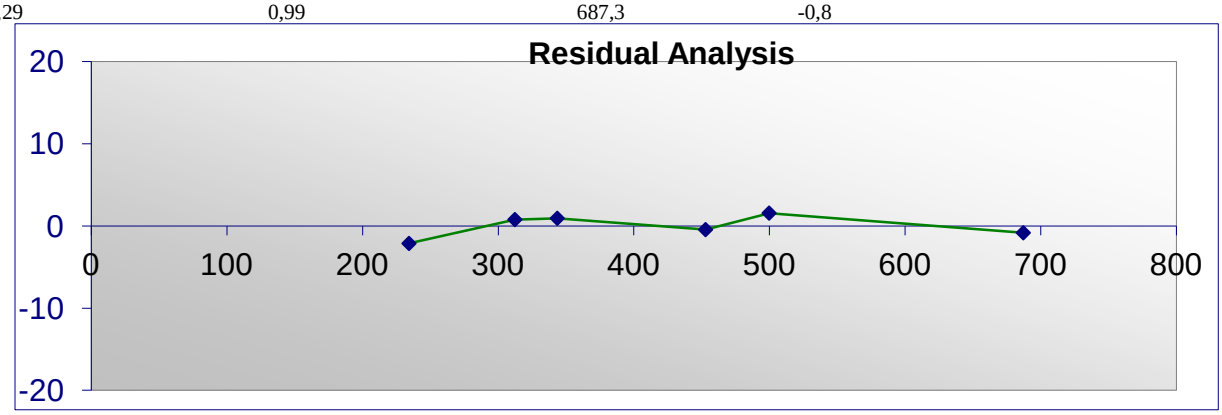


Cyclohexanone

Concentration (µg/L)	Peak Area
234,30	20
312,41	23
343,65	24
452,99	27
499,85	29
687,29	34



Estimated Peak Area	Experimental Area/ Estimated Area	Concentration (µg/L)	Deviation (%)
20,44	0,98	234,3	-2,1
22,82	1,01	312,4	0,8
23,78	1,01	343,6	0,9
27,12	1,00	453,0	-0,4
28,55	1,02	499,8	1,6
34,29	0,99	687,3	-0,8



Annex 2 Mandel Test

The calibration data is used to calculate the residual standard deviations S_{y1} and S_{y2} for the linear and polynomial calibration functions respectively.

$DS^2 = (N-2)S^2_{y1}-(N-3)S^2_{y2}$ Degrees of freedom: f=1

DS^2 and the variance of the polynomial calibration function (S_{y2}) are submitted to the F-test to examine signifant differences.

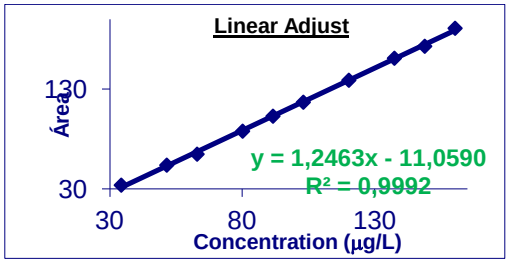
$VT = DS^2/S^2_{y2}$

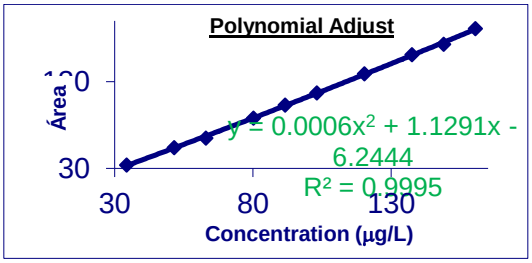
If VT< F: The linear equation leads to a good fit of the experimental points.

If VT> F: The linear equation does not lead to a good fir of the experimenta points.

n-Butylacetate

Concentration (µg/L)	Área	Linear Adjust Area	Polynomial Adjust Area
34.38	34	31.79382	33.28793
51.58	54	53.22024	53.58611
63.04	65	67.50452	67.31528
80.23	88	88.93094	88.20459
91.69	103	103.21522	102.32784
103.15	117	117.49950	116.60872
120.34	139	138.92592	138.32562
137.54	161	160.35234	160.39719
149.00	173	174.63662	175.30861
160.46	191	188.92090	190.37767





Linear Adjust		***Polynomial Adjust***	
	$(y-y_i)^2$		$(y-y_i)^2$
	4.867E+00		5.070E-01
	6.080E-01		1.713E-01
	6.273E+00		5.361E+00
	8.666E-01		4.186E-02
	4.632E-02		4.518E-01
	2.495E-01		1.531E-01
	5.488E-03		4.548E-01
	4.195E-01		3.634E-01
	2.679E+00		5.330E+00
	4.323E+00		3.873E-01
Soma =	2.034E+01	Soma =	1.322E+01
N-2 =	8	N-3 =	7
$S_{y/x}$ =	1.594E+00	$S_{y/x(2^o)}$ =	1.374E+00

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

DS^2= 7.116E+00

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

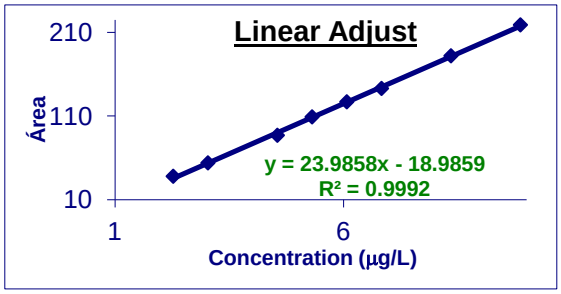
VT= 3.768E+00

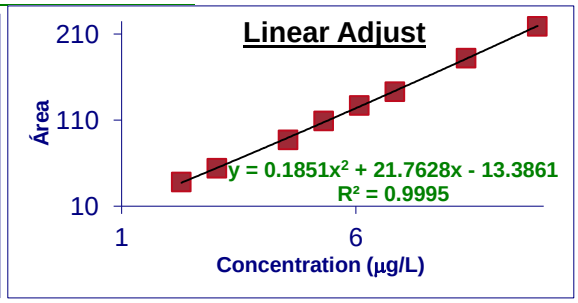
$F_{(1,7)95\%}$= 5.59

If VT<F, a linear calibration function leads to good fit of experimental points.

m+p Xylene

Concentration (µg/L)	Área	Linear Adjust Area	Polynomial Adjust Area
2.28	38	35.64789	37.14467
3.04	54	53.85915	54.41507
4.56	87	90.28169	89.59611
5.31	109	108.49296	107.50673
6.07	127	126.70423	125.63077
6.83	143	144.91549	143.96821
8.35	182	181.33803	181.28332
9.87	219	217.76056	219.45206





Linear Adjust		***Polynomial Adjust***	
	$(y-y_i)^2$		$(y-y_i)^2$
	5.532E+00		7.316E-01
	1.984E-02		1.723E-01
	1.077E+01		6.740E+00
	2.571E-01		2.230E+00
	8.748E-02		1.875E+00
	3.669E+00		9.374E-01
	4.382E-01		5.136E-01
	1.536E+00		2.044E-01
Soma =	2.231E+01	Soma =	1.340E+01
N-2 =	6	N-3 =	5
$S_{y/x}$ =	1.928E+00	$S_{y/x(2^o)}$ =	1.637E+00

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

DS^2= 8.906E+00

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

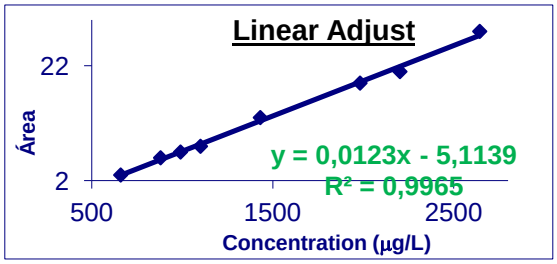
VT= 3.322E+00

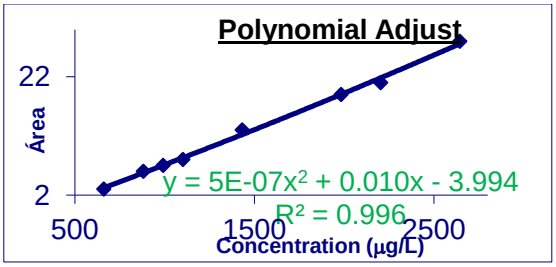
$F_{(1,7)95\%}$= 6.61

If VT<F, a linear calibration function leads to good fit of experimental points.

1,3-Dichloroacetone

Concentration (µg/L)	Área	Linear Adjust Area	Polynomial Adjust Area
661.1	3	2.99229	3.23221
881.5	6	5.69435	5.73814
991.7	7	7.04538	7.00932
1101.9	8	8.39640	8.29264
1432.4	13	12.44949	12.21545
1983.4	19	19.20462	18.99627
2203.7	21	21.90668	21.79359
2644.5	28	27.31079	27.53393





Linear Adjust		***Polynomial Adjust***	
	$(y-y_i)^2$		$(y-y_i)^2$
	5.937E-05		5.392E-02
	9.342E-02		6.857E-02
	2.059E-03		8.691E-05
	1.571E-01		8.564E-02
	3.031E-01		6.155E-01
	4.187E-02		1.388E-05
	8.221E-01		6.298E-01
	4.750E-01		2.172E-01
Soma =	1.895E+00	Soma =	1.671E+00
N-2 =	6	N-3 =	5
$S_{y/x}$ =	5.619E-01	$S_{y/x(2ª)}$ =	5.781E-01

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

DS^2= 2.239E-01

$$VT = DS^2 / S^2_{y/x(2ª)}$$

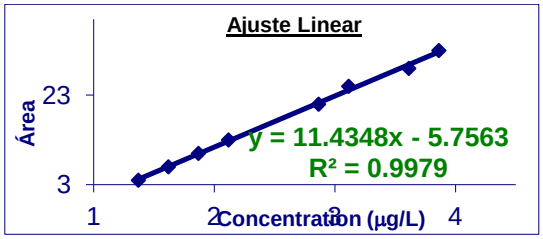
VT= 6.701E-01

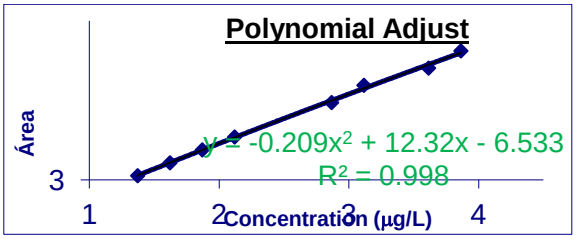
$F_{(1,7)95\%}$= 6.61

If VT<F, a linear calibration function leads to good fit of experimental points.

Styrene

Concentration (µg/L)	Área	Linear Adjust Area	Polynomial Adjust Area
0.87	4	4.21608	4.37326
1.12	7	7.06533	7.54798
1.37	10	9.91457	10.74874
1.62	13	12.76382	13.97554
2.37	21	21.31156	23.81218
2.62	25	24.16080	27.14314
3.11	29	29.85930	33.88317
3.36	33	32.70854	37.29224





Linear Adjust		***Polynomial Adjust***	
	$(y-y_i)^2$		$(y-y_i)^2$
	4.669E-02		1.393E-01
	4.268E-03		3.003E-01
	7.298E-03		5.606E-01
	5.578E-02		9.517E-01
	9.707E-02		7.908E+00
	7.042E-01		4.593E+00
	7.384E-01		2.385E+01
	8.495E-02		1.842E+01
Soma =	1.739E+00	Soma =	5.672E+01
N-2 =	6	N-3 =	5
$S_{y/x}$ =	5.383E-01	$S_{y/x(2ª)}$ =	3.368E+00

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

DS^2= -5.498E+01

$$VT = DS^2 / S^2_{y/x(2ª)}$$

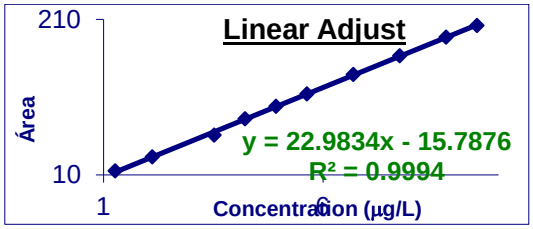
VT= -4.847E+00

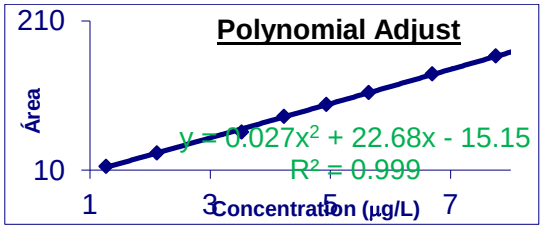
$F_{(1,7)95\%}$= 6.61

If VT<F, a linear calibration function leads to good fit of experimental points.

o-Xylene

Concentration (µg/L)	Área	Linear Adjust Area	Polynomial Adjust Area
1.27	15	12.46680	13.65795
2.11	33	32.03102	32.91493
3.52	61	64.63807	65.09826
4.23	82	80.94159	81.23133
4.93	98	97.24511	97.39200
5.64	114	113.54863	113.58029
6.69	139	138.00391	137.91447
7.75	163	162.45920	162.31077
8.81	187	186.91448	186.76919
9.51	202	203.21800	203.10931





Linear Adjust	$(y-y_i)^2$	***Polynomial Adjust***	$(y-y_i)^2$
	6.417E+00		1.801E+00
	9.389E-01		7.236E-03
	1.324E+01		1.680E+01
	1.120E+00		5.909E-01
	5.699E-01		3.697E-01
	2.037E-01		1.762E-01
	9.922E-01		1.178E+00
	2.925E-01		4.750E-01
	7.314E-03		5.327E-02
	1.484E+00		1.231E+00
Soma =	2.526E+01	Soma =	2.268E+01
N-2 =	8	N-3 =	7
$S_{y/x}$ =	1.777E+00	$S_{y/x(2^a)}$ =	1.800E+00

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

DS^2= 2.583E+00

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

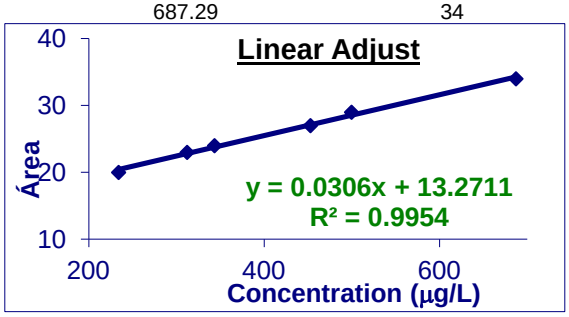
VT= 7.973E-01

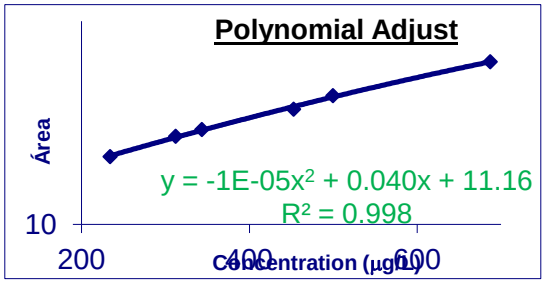
$F_{(1,7)95\%}$= 5.59

If VT<F, a linear calibration function leads to good fit of experimental points

Cyclohexanone

Concentration (µg/L)	Área	Linear Adjust Area	Polynomial Adjust Area
234.30	20	20.43532	20.17862
312.41	23	22.82338	22.93817
343.65	24	23.77861	24.00783
452.99	27	27.12189	27.59792
499.85	29	28.55473	29.06333
687.29	34	34.28607	34.48580





Linear Adjust		***Polynomial Adjust***	
	$(y-y_i)^2$		$(y-y_i)^2$
	1.895E-01		3.190E-02
	3.119E-02		3.823E-03
	4.901E-02		6.125E-05
	1.486E-02		3.575E-01
	1.983E-01		4.011E-03
	8.184E-02		2.360E-01
Soma =	5.647E-01	Soma =	6.333E-01
N-2 =	4	N-3 =	3
$S_{y/x}$ =	3.757E-01	$S_{y/x(2^a)}$ =	4.595E-01

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

DS^2= -6.864E-02

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

VT= -3.251E-01

$F_{(1,7)95\%}$= 10.13

If VT<F, a linear calibration function leads to good fit of experimental points

Annex 3 Rikilt Test.

This test accesses the linear range by giving a specific range within which the calibration data should be made. It mainly determines if instrument calibration should be done using response factor instead of the calibration curve.

Each calibration point mus lie within 90 and 110 to meet the requirement.

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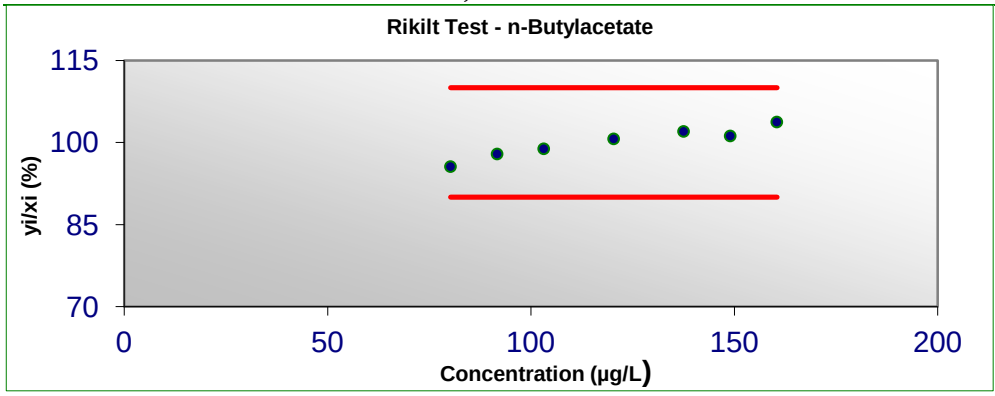
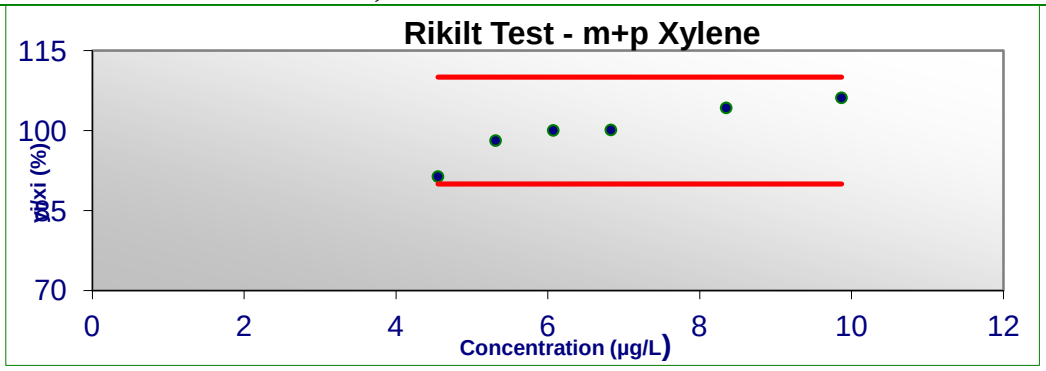
n-Butylacetate

Concentration (µg/L) = xi	Área = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
80,23	88	1,09686	96	110	90
91,69	103	1,12334	98	110	90
103,15	117	1,13425	99	110	90
120,34	139	1,15502	101	110	90
137,54	161	1,17060	102	110	90
149,00	173	1,16109	101	110	90
160,46	191	1,19034	104	110	90
	Média	**1,1473577**			



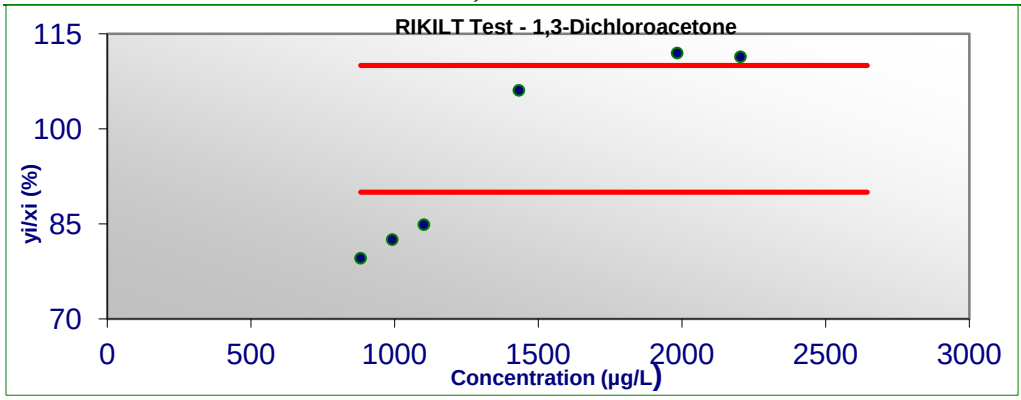
m+p Xylene

Concentration (µg/L) = xi	Área = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
4,56	87	19,09771	91	110	90
5,31	109	20,50887	98	110	90
6,07	127	20,90870	100	110	90
6,83	143	20,92699	100	110	90
8,35	182	21,79174	104	110	90
9,87	219	22,18779	106	110	90
	Média	**20,9036347**			



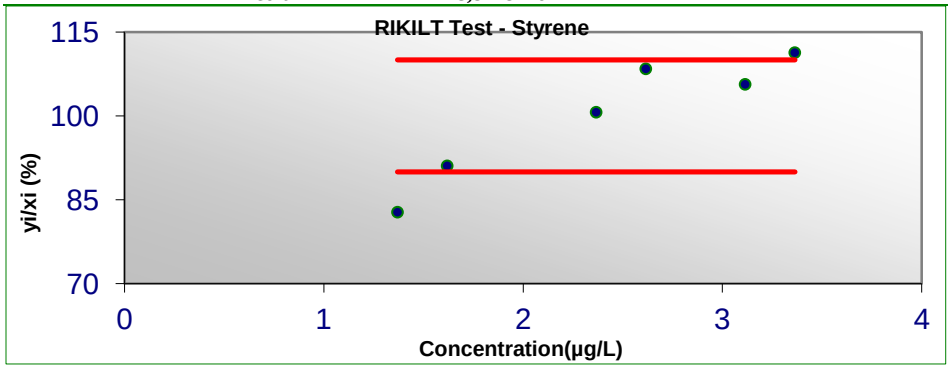
1,3- Dichloroacetone.

Concentration (µg/L) = xi	Área = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
881,5	6	0,00681	80	110	90
991,7	7	0,00706	82	110	90
1101,9	8	0,00726	85	110	90
1432,4	13	0,00908	106	110	90
1983,4	19	0,00958	112	110	90
2203,7	21	0,00953	111	110	90
2644,5	28	0,01059	124	110	90
	Média	**0,0085569**			



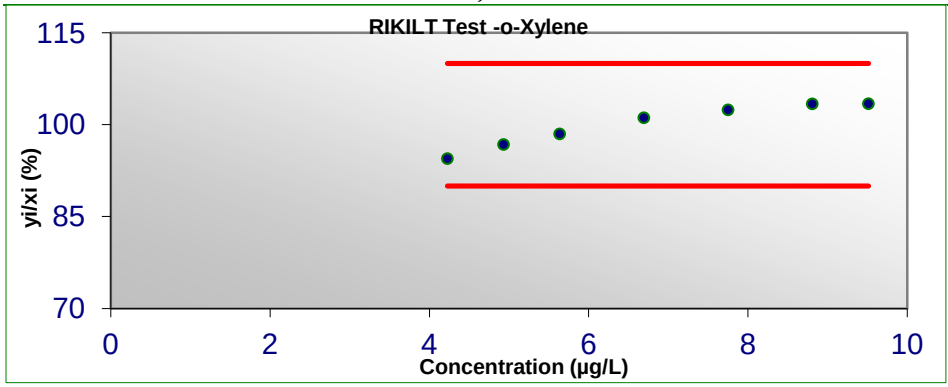
Styrene

Concentration (µg/L) = xi	Área = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
1,37	10	7,29686	83	110	90
1,62	13	8,02654	91	110	90
2,37	21	8,87144	101	110	90
2,62	25	9,55541	108	110	90
3,11	29	9,31079	106	110	90
3,36	33	9,81022	111	110	90
	Média	**8,8118746**			



o-Xylene

Concentration (µg/L) = xi	Área = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
4,23	82	19,39525	94	110	90
4,93	98	19,86830	97	110	90
5,64	114	20,22309	98	110	90
6,69	139	20,76462	101	110	90
7,75	163	21,02944	102	110	90
8,81	187	21,23070	103	110	90
9,51	202	21,23490	103	110	90
	Média	**20,5351852**			



Cyclohexanone

Concentration (µg/L) = xi	Área = yi	Ratio yi / xi	% yi / xi	Upper Limit	Lower Limit
234,30	20	0,08536	129	110	90
312,41	23	0,07362	112	110	90
343,65	24	0,06984	106	110	90
452,99	27	0,05960	90	110	90
499,85	29	0,05802	88	110	90
687,29	34	0,04947	75	110	90
	Média	**0,0659854**