Head Space-Trap GC-MS method development and validation for the analysis of volatile organic compounds in water samples

By

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Declaration of authorship

I declare that I am the author of this work, which is original. The work cites the other authors and works which are adequately referred in the text and are listed in bibliography.

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Muhammad Ramzan

Dedication

Dedicated to my beloved father whose memories will always be in my heart.

Acknowledgement

This thesis is written as integral part of Erasmus Mundus masters in Quality in Analytical Laboratories (EMQAL). The experimental work of this thesis has taken place from February 2015 to July 2015 in Water quality lab of Aguas do Algarve (AdA), Tavira, Portugal. A part of the thesis was conducted earlier in October 2014 to November 2014 in the Department of Chemistry and Pharmacy of Faculty of Science and Technology of University of Algarve, Portugal.

My thesis would not have reached its completion without the extended support of my supervisor Maria da.Conceição.Mateus. Her talent and knowledge have greatly inspired me to work through all the obstacles I have faced during the project work.

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Abstract

A head space solid phase microextraction (HS-SPME)-GC-MS and a head space trap (HS-Trap)-GC-MS method were developed for the analysis of volatile organic compounds (VOCs) in water. The HS-SPME-GC-MS method was developed using 75 µm Car/PDMS fused silica SPME fiber and an Agilent 6890/5973 GC-MS system. A set of 7 chlorinated volatile hydrocarbons (Cl-VHCs) was used during the optimization of the HS-SPME-GC-MS method. The HS-Trap GC-MS method was developed using Perkin Elmer Clarus 500/560 GC-MS with Turbomatrix HS-40 Trap autosampler for the analysis of 8 volatile organic compounds (VOCs): benzene, 1,2-dichloroethane, trichloroethene (TriCE), tetrachloroethene (TetCE), chloroform ($CHCl_3$), bromoform ($CHBr_3$), dichlorobromomethane ($CHCl_2Br$) and dibromochloromethane ($CHBr_2Cl$). Performance characteristics the HS-Trap-GC-MS method were established by a method validation study. The obtained method detection limits were found between 0.1 to 0.5 µg/L. The coefficient of determination (R^2) was >0.995 for all the compounds. The sensitivities as determined by the slope of the regression line ranged from 0.069 to 1.22. The % RSDs for repeatability, ranged from 2.8 to 18 % and for intermediate precision ranged from 3.7 to 29.5 %. The % recoveries determined, in treated water intended for human consumption, ranged from 101 to 125 %. The coefficient of variation of the method ranged from 1.8 to 4.8 %while the accuracy of the method determined as z-score was less than 2 for all the compounds. However a method validation study needs to be done to establish the performance characteristics of the HS-SPME-GC-MS method.

Keywords: VOCs, Headspace-Trap, Method development, Method Validation, SPME, GC-MS

Resumo

Neste trabalho foram implementados dois métodos para a análise de compostos orgânicos voláteis (VOCs) em água por cromatografia gasosa associada à espectrometria de massa. Designadamente, por microextração em fase sólida em espaço de cabeça (HS-SPME)-GC-MS e armadilha de espaço de cabeça (HS-Trap)-GC-MS.

O método HS-SPME-GC-MS foi desenvolvido utilizando fibras SPME de silica fundida com revestimento de Car/PDMS 75 µm e um sistema Agilent 6890/5973 GC-MS e foi otimizado o método para a análise de 7 compostos orgânicos voláteis. O método por HS-Trap GC-MS foi desenvolvido utilizando um sistema Perkin Elmer Clarus 500/560 GC-MS com amostrador automático Turbomatrix HS-40 Trap para a análise de 8 VOCs: benzeno, 1,2-dicloroetano, triclroeteno, tetracloroeteno, cloroformio, bromoformio, diclorobromometano e dibromoclorometano. As características de desempenho do método por HS-Trap-GC-MS foram estabelecidas através do estudo de validação do método. Os limites de deteção obtidos situaram-se entre 0,1 e 0,5 µg/L. O coeficiente de determinação (R2) foi superior a 0,995 para todos os compostos. A sensibilidade do método, determinada a partir do declive da reta de regressão linear, apresentou uma amplitude de 0.069 a 1.22. A % RSDs expressa em termos de repetibilidade, variou de 2,8 a 18 % e expressa em termos de precisão intermédia variou de 3,7 a 29,5 %. As percentagens de recuperação foram determinadas para todos os compostos em água tratada para consumo humano, apresentando valores entre 101 e 125 %. O coeficiente de variação do método variou de 1,8 to 4,8 sendo a exatidão do método, determinada a partir do cálculo do "z-score", inferior a 2 para todos os compostos. A validação do método necessita contudo de um estudo experimental mais alargado para confirmar as características de desempenho do método de análise por HS-SPME-GC-MS.

Executive Summary

This thesis is divided into 6 sections: 4 chapters, references and annexures.

Chapter 1 includes problem statement, background information on the subject matter and objectives of the study, gives an overview of the existing methodologies for the analysis of volatile hydrocarbons, explanation of the methodology used in the current study, an insight into validating analytical methodologies, importance and requirements of the method validation studies.

Chapter 2 details materials, standards and solution preparations and all the experimental work conducted to achieve the objectives of the study.

Chapter 3 details the findings of the study on the head space-trap (HS-Trap)-GC-MS method development and validation. The results are discussed and concluded in the same chapter.

Chapter 4 includes work carried out in the department of Chemistry and Pharmacy of Faculty of Sciences and Technology of University of Algarve. The aim of the work was to develop and validate a head space solid phase microextraction (HS-SPME)-GC-MS method for the analysis of chlorinated volatile hydrocarbons (Cl-VHCs) in water and waste water samples. The work was stopped unfinished due to the breakdown of MS turbo-molecular pump. However preliminary results are presented and discussed here.

Annexures includes important raw data and chromatograms obtained during the study.

List of Abbreviations

DC	Direct current
EC	European commission
EI	Electron ionization
ECD	Electron capture detector
ELCD	Electrolytic conductivity detector
eV	Electron volt
HS	Head space
HS-SPME	Head space solid phase microextraction
HS-Trap	Head space trap
ISO	International organization for standardization
kPA	kilo pascals
LOD	Limit of detection
LOQ	Limit of Quantification
MRM	multiple reaction monitoring
mm	millimeter
MSD	Mass spectrometric detector
m/z	mass/charge
PMT	photomultiplier tube
P&T	Purge and Trap
psi	pound square inch
RF	Radio frequency
SPME	Solid phase microextraction
S_{res}	residuals standard deviation
SIM	Selected ion monitoring
THMs	Trihalomethanes
USEPA	United States environment protection agency
VOCs	Volatile organic compounds

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Problem statement

European Union Directive (2004/42/EC) defines volatile organic compounds (VOCs) as organic compounds with boiling points below 250 °C at a standard atmospheric pressure of 101.3 kPa. VOCs found in surface or ground waters are grouped into three categories based on their origin:

1. Chlorinated solvents
2. Fuel components
3. Disinfection byproducts

Most of the VOCs enter water bodies mainly from careless industrial practices of effluent discharge. Chlorinated solvents found in ground waters have sometimes been traced to sources such as home septic tanks, municipal landfills, hazardous waste dumps and industrial facilities. Accidental spills from fueling operations, leaking gasoline pipelines, leaking gasoline storage tanks, storm water runoff, and atmospheric inputs may be the sources of fuel components found in water bodies. Trihalomethanes (THMs): chloroform ($CHCl_3$), bromoform ($CHBr_3$), dichlorobromomethane ($CHCl_2Br$) and dibromochloromethane ($CHBr_2Cl$), are formed in water when chlorine combines with naturally occurring organic material, such as decomposing leaves or animal waste. Since groundwater rarely contains high levels of organic matter, chlorinated private water supplies whose source is groundwater are less susceptible to the formation of THMs. The THMs are most often found in chlorinated surface waters used for public drinking water supplies (USEPA 2009).

VOCs have adverse effects on human health, even at very low concentrations, as they have toxic, carcinogenic or mutagenic properties (Richardson, Plewa et al. 2007; Lopez, Schuhmacher et al. 2008; Fan, Wang et al. 2009; USEPA 2009). That's why analytical monitoring of VOCs in drinking water is a subject of concern for many researchers today. Regulatory values of 9 VOCs in drinking water were established by European Directive of 1998 in European Union; sum of the 4 THMs =100 µg/L, 1,2-dichloroethane=3 µg/L, benzene=1 µg/L, trichloroethene (TriCE) and tetrachloroethene (TetCE)-total= 10µg/L, vinyl chloride= 0.5 µg/L.

Objectives of the study

The major objectives of the study were followings:

HS-Trap-GC-MS

- Development and validation of a HS-Trap-GC-MS method using Perkin Elmer Clarus 500/560 GC-MS system and Turbomatrix HS-Trap 40 autosampler. The method should be based on USEPA method 8260 B for the simultaneous determination of 8 VOCs in water samples and should meet the regulatory requirements of Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. The EU Directive specifies 9 VOCs, 1,2-dichloroethane, trichloroethene (TriCE), tetrachloroethene (TetCE), benzene, chloroform ($CHCl_3$), bromoform ($CHBr_3$), dibromochloromethane ($CHBr_2Cl$), dichlorobromomethane ($CHCl_2Br$) and vinyl chloride. Vinyl chloride was not attempted in this study because is very volatile and may involve different instrumental parameters.

HS-SPME-GC-MS

- Development and validation of a HS-SPME-GC-MS method for the analysis of 7 chlorinated VOCs in ground water samples using 75µm Carboxen/polydimethylsiloxane fused silica SPME fiber and Agilent 6890/5973 GC-MS system.

1 Theoretical Background

Methodologies for the analysis of VOCs

This section reviews the most common analytical methods applied for the determination of VOCs in water samples. Gas chromatography (GC) is a widely applicable technique with flame ionization detector (FID), electron capture detector (ECD) (Popp and Paschke 1997), electrolytic conductivity detector (ELCD) or coupled with mass spectrometry (MS) for the determination of VOCs in water samples. However elimination of water before chromatographic run is the main requirement and needs an extraction step. The VOCs are found in natural and drinking waters in the range of ng/L to µg/L and that necessarily requires a pre-concentration step to achieve a concentration level of analyte that falls in measurement range of the analytical instrument. Sensitive analytical methods are required to meet regulatory requirements, to estimate human exposure to VOCs and to optimize current drinking water treatment practices by chlorination. It is sample preparation step which mainly determines analytical performance, simplicity of optimized method and time of analysis (José Luis Pérez Pavón 2008). The following review focuses on most commonly applied extraction techniques, chromatographic columns and detectors for routine analysis of VOCs in water samples.

1.1 Extraction and Pre-concentration Techniques

Dynamic head space or Purge and trap (P&T), static headspace or simply headspace (HS) and solid-phase microextraction (SPME) have mainly been applied for extraction and pre-concentration of VOCs from water samples (José Luis Pérez Pavón 2008). Each technique has its own advantages and disadvantages. A mini review on the P&T, HS and SPME is presented below. Section 1.1.4 explains the working principle of headspace-trap (HS-Trap) autosampler which was used in this study. A mini review of the main sample extraction and pre-concentration techniques is given below.

1.1.1 Dynamic headspace or Purge and Trap (P&T)

Purge and Trap (P&T) in combination with gas chromatography was 1st described by Swinnerton and Linnebom in 1962 (Swinnerton 1962). An inert gas is bubbled through a 5 mL water sample contained in a specially-designed purging chamber at ambient temperature. The VOCs are efficiently transferred from the aqueous to the vapor phase and swept through a sorbent trap with the purge gas where the VOCs are adsorbed. After purging is complete, the trap is heated and back flushed with the carrier gas to desorb the VOCs onto a gas chromatographic column.

Owing to its exhaustive extraction nature, purge and trap (P&T) is generally believed to provide lower LODs and it is the most usual choice in environmental laboratories for the extraction and pre-concentration of volatile organic compounds in surface, ground, and wastewater samples. USEPA methods 502.2, 524.2, 601, 624, 8260 B and many other standard methods for the analysis of VOCs are based on P&T concentrators.

Apart from complex instrumentation of P&T, the methodology has some important limitations. Purging causes evaporation of water thus water is inevitably transferred to the GC during the trap desorption step. However the current designs of purge and trap systems include dry purge cycle (Restek Corporation 2003) to eliminate water prior to the analytes are desorbed to GC column which further increases the analysis time. Moreover, the system is prone to carry-over effects and it is necessary to clean the system between every sample. The device must be washed, rinsed with distilled water, and dried before the analyses of next sample. Reagent blanks are often required to make sure sample to sample carry over effect has been eliminated which decreases the sample throughput of the system. Samples containing analytes at concentrations greater than 200µg/L can saturate the trap and contaminate the lines and other parts of the system which requires the trap and other parts of the system must be baked and purged resulting in increased downtime to clean the system. To avoid such severe problems a pre-screening step is required which requires extra efforts. Impurities in the purge gas may also be a major interference (Supelco 1998).

Another important limitation is incompatibility of P&T with the mass spectrometer (MS) operations under high vacuum conditions. Efficient removal of VOCs from the trap, during desorption step, requires carrier gas flow rates greater than 5mL/min which demands long wide bore capillary columns (0.53 mm internal diameter) which also results in longer analysis times

because of greater lengths of GC columns. Using jet separators after wide bore columns allows use of an MS but increases the detection limits. Narrow bore capillary columns are best suited for MS operations but capillary column can be operated under maximum carrier gas flow rate of 2mL/min which is also under the limitations of mass spectrometer (Restek Corporation 2003). Splitting the flow after the trap makes it possible to use a narrow bore capillary columns but splitting the flow can greatly decrease the amount of analytes entering the column by 90-95 % which obviously results in increased detection limits like jet separators (Supelco 1998). Cryofocusing or cold trap (secondary trapping) at very low temperatures like -160 °C, offers another possible replacement of jet separators but increases both cost and analysis time (Restek Corporation 2003).

1.1.2 Static Head Space (HS)

Static headspace may be the simplest technique; it is fast and may not require expensive materials or extensive laboratory work when gas tight syringe is used to transfer head space volumes for chromatographic run. However reproducibility issues are main concern with this technique because of possible sample loss. As the sample is transferred from the vial to the injection port, some of it may be lost because of the pressure differences between the vial and atmospheric conditions. A HS autosampler based on balanced pressure system (balanced pressure system explained in section 1.1.4, step 1-3) on the other hand provides excellent repeatability and reproducibility as the number of moving parts are minimized which decreases the chance for compound adsorption and loss *via* leaks. Head space autosamplers based on fixed loop systems are prone to carry over effects and may cause ghost peaks (Restek Corporation 2000).

Headspace (HS) is partial equilibrium technique and furthermore a portion of HS aliquot of volatiles is sampled which is 1 mL, 2mL or whatever the size of sample loop. Head space equilibrium is also greatly affected by the sample matrix (Restek Corporation 2000). Due to these limitations, sometimes the achieved LODs are not enough to comply with some environmental regulations, for example in case of benzene, the LOD cannot be achieved below 0.5µg/L (Barani, Dell'Amico et al. 2006). When more volume of aliquot is injected into GC to achieve lower detection limits, it results in the loss of resolution due to band broadening effect. Cryogenic focusing strategies are being applied to avoid band broadening effect. When Cryogenic focusing is coupled with static head space, it is possible to inject large volumes of HS aliquot into the

column and achieve better detection limits even equal or better than those achieved by P&T. (José Luis Pérez Pavón 2008). One such cold trap is cold trap 9000 (Brechbühler, Switzerland). However, the use of cold traps further increases cost, time of the analysis and productivity of an analytical laboratory.

1.1.3 Fiber Solid Phase Microextraction (SPME)

Fiber solid phase microextraction or simply known as solid phase microextraction (SPME) technique is explained here in more details as a part of the master project was done using SPME as sample preparation technique (chapter 4). Solid phase micro-extraction involves the extraction of analytes from the sample matrix to a liquid or solid coating supported on glass or fused silica fiber. The transport of the analytes begins as the coated fiber is brought in contact with the sample. When the distribution equilibrium is achieved, it is considered that the maximum extraction is achieved because once the equilibrium is achieved there is no net gain in the quantity of the analytes extracted by the fiber. The quantity of the analyte extracted by the fiber is directly proportional to the initial concentration of the analyte in the sample. The fiber is then analyzed to determine the concentration of the analyte in the sample. The extraction can be interrupted prior to equilibrium at some fixed interval and the fiber can be analyzed. The latter determinations are called pre-equilibrium determinations. Equilibrium determinations are easy to perform. To get reproducible data in pre-equilibrium determinations, constant agitation conditions, temperature and careful extraction timings are required (page 15 (Pawliszyn 1997)).

Since SPME is an equilibrium technique, the extraction by the fiber is not exhaustive. The fiber extracts a very small amount of the analyte from the matrix, which becomes a barrier to achieve very detection limits. However, this makes SPME a special and very useful for studying the natural systems where it can facilitate speciation without disturbing the equilibrium of the system. SPME is performed in following three modes depending on the volatility of the analyte and the nature of the matrix (page 16 (Pawliszyn 1997)):

1. Direct Immersion SPME: In the direct immersion mode SPME fiber is directly inserted in the sample and the transport of the analyte is directly from the sample matrix to the fiber. The position of the fiber while immersed in the sample matrix does not make any difference as the

quantity of the analyte extracted is always the same, if all other conditions are kept constant. Direct immersion mode is referred as DI-SPME.

2. Headspace SPME (HS-SPME): In the head space mode, the analytes are transported to the fiber through the air above the sample matrix. It allows protection of the fiber from the interferences due to sample matrix. It also allows modifications in the sample matrix, like pH changes, without damaging the fiber. The position of SPME fiber, in the headspace, may significantly affect the extraction efficiency of the HS-SPME operation.

3. Membrane protected SPME: The SPME fiber can be closed in semi permeable membrane, which can allow the transport of the analyte molecules to and from, avoiding the large molecular weight interferences from the sample matrix. The large molecular weight compounds can adsorb to the fiber surface making it partially or totally unavailable for interactions with the analyte molecules.

Nature of the coating material and thickness of the coating, temperature, agitation condition, pH, salt concentrations are the important parameters for SPME. Selection of the coating material depends on the nature of the analyte. Polydimethylsiloxane (PDMS) is widely used coating material for non-polar hydrophobic organic compounds like PAHs. Thickness of the coating affects the equilibrium time or speed of the analysis. The total amount extracted by the fiber depends on the volume of coating of the extraction material. Agitation conditions affect the mass transfer rate and equilibrium time between fiber coating and the analytes in the sample (page 89 (Pawliszyn 1997)). Temperature is very important parameter and affects the coating/sample distribution coefficient of the analyte and extraction efficiency. Salt concentration and pH affect SPME in similar fashion to solvent extraction (page 24 (Pawliszyn 1997)).

SPME was invented by Pawliszyn and Belardi (R.P Berlardi 1989) and became commercially available in 1993. SPME is fast, easy and compatible with narrow bore GC capillary columns. Even though SPME is relatively new technique but it is evolving rapidly. The development and availability of commercial autosampler devices such as TriPlus (Thermo Fisher scientific; Milan, Italy), Combi-PAL (CTC Analytics; Zwingen, Switzerland), MPS 2 (Gerstel Inc.; Mulheim and der Ruhr, Germany) made it possible to further increase the sample throughput (Spietelun, Marcinkowski et al. 2013).

SPME is well suited for a wide range of concentrations. Like head space, SPME is also equilibrium or partial extraction technique but may offer better detection limits than head space depending on the type and thickness of the fiber coating. Many studies have been carried out to find out the most suitable polymeric coating for the target compounds. A carboxen/polydimethylsiloxane (CAR/PDMS) fiber has the best extraction efficiency and sensitivity (Cho, Kong et al. 2003; Garcia-Esteban, Ansorena et al. 2004; O'Reilly, Wang et al. 2005) but a 100 µm PDMS fiber offers a wider range of linearity (Nakamura and Daishima 2005; Spietelun, Marcinkowski et al. 2013). A PDMS/DVB is a better choice in terms of repeatability along with better detection limits and broader linear range (San Juan, Carrillo et al. 2007). A DVB/CAR/PDMS provides narrower chromatographic peaks and better chromatographic resolutions (Lara Gonzalo)(José Luis Pérez Pavón 2008). In spite of being widely used, these fibers still have some drawbacks such as lower thermal and chemical stability, high cost, less reusability (less than 100 times).

SPME as compared with P&T meets the thresh hold limits but cannot compete in analytical performance especially LODs and precision. SPME produces higher broadening of peaks hence less chromatographic resolution as compared with P&T (Lara-Gonzalo, Sanchez-Uria et al. 2008).

1.1.4 Head Space-Trap (HS-Trap)

In the HS-T methodology, sample vapors from a head space vial are transferred to the trap by using balanced pressure principle. The analytes adsorbed to the trap are desorbed by rapid heating of the trap to high temperatures while passing the career gas flow through the trap to the column. Figure 1 shows simplified schematics of a HS-T operation during trap load. The analysis sequence of HS-T system is described below step by step:

1. *Equilibration*

A HS vial with the sample is heated in the incubation oven to a fixed temperature for a set constant time called “thermostattation time” in order to reach equilibration between the sample and its vapors in the headspace. The heating temperature is defined by the sample characteristics.

2. *Pressurization*

After equilibration, the vial is pressurized to a suitable pressure. Vial pressurization is done by a needle which pierces the septum and allows the carrier gas at a pre-set pressure to enter the vial to set the internal pressure to a particular value termed as "vial pressure".

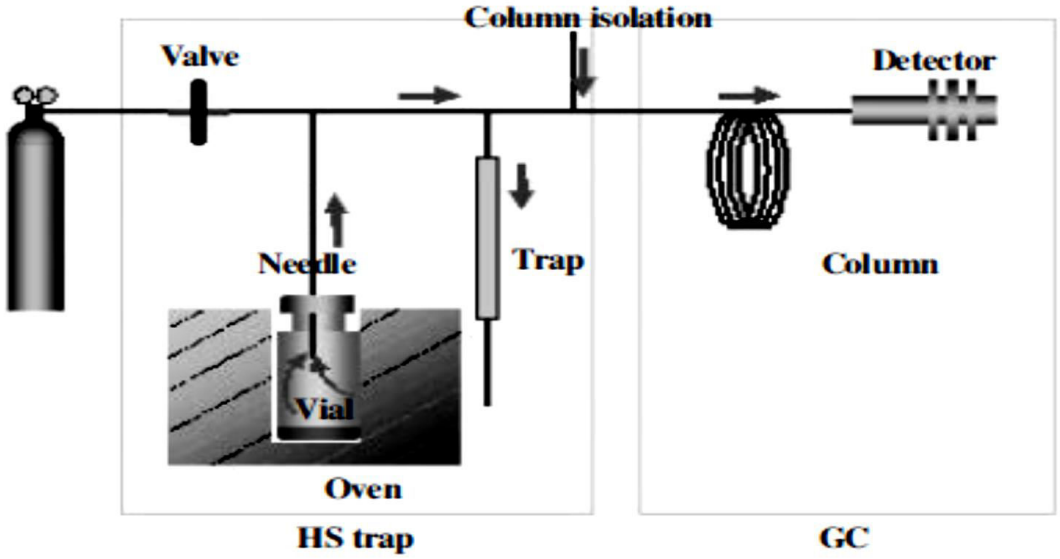

Figure 1 Turbomatrix HS Trap during trap load (Barani, Dell'Amico et al. 2006). Vapors from the HS vial, mixed with career gas, flow towards the column but column isolation flow prevents the branching of the flow from the vial to the column. The gaseous mixture from the vial ends up in passing only through the trap where VOCs get adsorbed.

3. *Trap load*

After vial pressurization, a solenoid valve interrupts the carrier gas flow towards the GC column. The pressurized vial then acts as a reservoir of the carrier gas (mixed with sample vapors) towards the column. A column isolation flow, also provided by the carrier gas reservoir, prevents branching of the decay flow to the GC column resulting in a flow of the mixed head space gas from the vial to the trap only and lasts until the pressure in the vial has decayed to its lowest value (decay time). This trap load step can be repeated up to four times for each vial. The number of times vial pressurization and trap load is performed per vial is termed as "cycle number" (Perkin Elmer Inc. 2008). The trap load step in HS-Trap methodology is illustrated in Figure 1. Keeping the transfer line and the analytical column pneumatically isolated constantly supplied with an isolation carrier gas flow during sample preparation by a pressurized vial is known as balanced pressure HS system.

4. *Trap dry-purge*

After vapor extraction is complete, the cold loaded trap is purged with dry carrier gas to remove moisture from the trap. The moisture to the trap is not avoidable even if the adsorbent material is mostly hydrophobic and the vial equilibration temperature is kept low. Moisture can damage the capillary column and worsen the detection limits by increasing the baseline. The amount of time needed for the dry purge is termed as "dry purge time" which depends on the type of sample and the HS-Trap "cycles number". Higher number of Trap re-loadings and higher HS oven temperature gives rise to more moisture to the trap.

5. *Trap desorb and trap hold*

After dry purge is complete, the trap temperature is rapidly increased to a desired high value (desorb temperature) to release the trapped analytes. It is kept at that value for a specified time (Trap hold time) to clean it, avoiding any possible carry-over. A recommended trap hold time is five minutes. As soon as the trap is heated, the column isolation is stopped and the GC run begins. The trap is desorbed in the backflush mode with an optional split provided in the trap desorb flow. The HS-Trap pneumatics provides flow from the other end of the trap as well for backflushing the trap during the trap desorb. The trap desorb flow is generally set to 50 mL/min. If a split, in the desorb flow, is required for GC-MS analysis, the outlet split allows a fixed split flow of 15 mL/min. This is equivalent to split/splitless injection but the change in the split ratio is not accessible to the user in this case.

Combining head space with SPME (HS-SPME) offers greater sensitivity over simple head space sampling by concentrating the analytes in the extraction phase for GC analysis but analytical precision is better in case of simple headspace-GC analysis (Flórez Menéndez JC 2004). Combining balanced pressure head space extraction with a trap (PerkinElmer, Wellesley, MA) offers the potential to achieve good detection limits like purge and trap systems and a very good repeatability and reproducibility. HS-Trap system is relatively simple compared with purge and trap systems. Avoidance of purge gas eliminates interferences caused by impurities in the purging gas. Unlike P&T systems, trap in HS-Trap system is less prone to accumulation of water because there is no purging which causes relatively more evaporation of water compared with head space. Analytical methods based on HS-Trap-GC-MS offer the opportunity to simulate the standard methods based on purge and trap and GC methodology (Griffith 2004). Along with several

advantages, the use of trap in HS-Trap still requires pre-screening in order to avoid contamination of trap from samples with high concentrations of analytes. However, the presence of spilt vent in the HS-T system minimizes efforts to dilute samples found to be highly contaminated during pre-screening, if the split vent option is used in the HS-Trap system.

1.2 Gas chromatography-Mass Spectrometry (GC-MS) for VOCs Analysis

GC-MS is a hyphenated technique which couples the good separation quality of gas chromatography with excellent qualitative and quantitative capabilities of mass spectrometry. The coupled GC-MS technique was pioneered in the 1950's by Fred W. McLafferty and Roland S. Gohlke (Gohlke and McLafferty 1993) and has since found a wide range of applications. Compared to other hyphenated chromatography and spectroscopic techniques (e.g. CE-MS, LC-MS and LC-NMR), GC-MS offers low costs and unsurpassed chromatographic reproducibility and resolution. Principles of gas chromatography and mass spectrometry are explained below separately.

1.2.1 Gas Chromatography

Gas chromatography (GC) is an important analytical method widely used in laboratories around the world. It's a frequently used method to separate and analyze volatile compounds ranging from 2 to above 1000 Da (McNair H.M. and Miller J.M 2011).

In gas chromatography, a column is the central part of the chromatographic system. The stationary phase inside the column is a solid or a liquid coated on a solid support or directly on the column wall. When a sample, introduced into the column, is swept through the column with a carrier gas, analytes in the sample are separated based on differences in their vapor pressures and differences in their interactions with the stationary phase in the column (page 80 (C.F Poole 2003)). Basic components and schematics of a gas chromatograph are shown in Figure 2 and explained below.

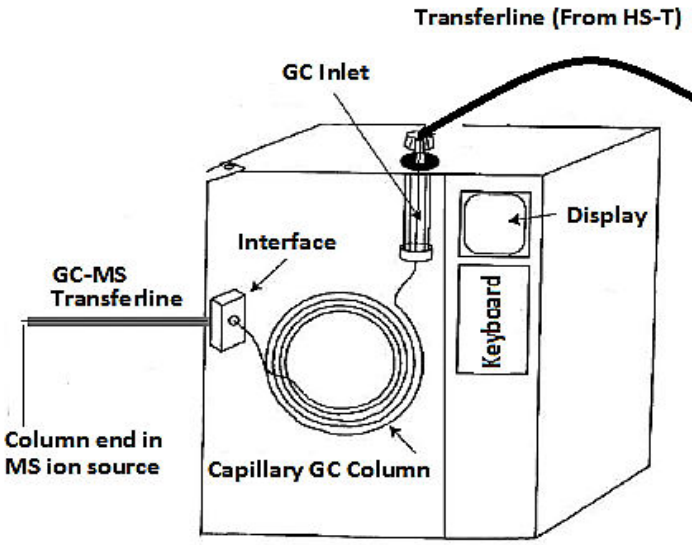

Figure 2 Schematic of a simplified gas chromatograph (page 8 (McMaster 2008)

1.2.1.1 Carrier gas

In GC an inert gas such as nitrogen, helium or hydrogen is used as carrier gas and the main function of which is to carry the sample through the column. The choice of carrier gas is based on efficiency, availability, cost and safety as well as compatibility with the detector. The carrier gas reservoir is connected to the system at the sample injection chamber where the carrier gas is mixed with the sample vapor. The carrier gas flow is controlled by a flow controller or pressure regulator placed between the carrier gas reservoir and the sample injection chamber, (page 83-86 (C.F Poole 2003)).

1.2.1.2 Sample inlet

In typical GC, a sample is introduced into an injection chamber also termed as sample inlet which provides means by which the sample is vaporized and mixed with carrier gas prior to the start of the separation in the column. Various inlet types are available specific to the type of column and chromatographic application. The inlet should facilitate sample introduction into the column without reduction of the separation potential of the column; without thermal degradation, adsorption or rearrangement of sample components; without discrimination of sample components by boiling point, polarity or molecular weight; and with quantitative recovery for both trace and major sample components. It is also preferable that changes in the column operating conditions should not affect the sampling process. There is no universal inlet design

which can manage the full range of gas chromatographic applications and column types (page 180 (C.F Poole 2003)).

In the HS-Trap methodology, a HS-Trap autosampler can be connected to the GC in 3 ways. In the "on-column" configuration, capillary column is directly connected to the head of HS-Trap autosampler by passing through the GC inlet and transferline. In the "direct operation" configuration, HS-Trap transferline is passed through the GC inlet and connected to the GC column inside the oven. The third configuration, involves the HS-Trap transferline connection to the GC inlet. The column is connected to the GC inlet in usual configuration. For HS-Trap operations, the third mode is not generally recommended by the instrument manufacturer because of the complexity of the chromatography pressures and flows involved (page 60,61(Perkin Elmer Inc. 2008)).

1.2.1.3 Column

There are two main types of columns usually used in GC, packed columns and open tubular (capillary) columns. Packed columns contain a finely divided solid support material coated with the liquid stationary phase (page 33 (C.F Poole 2003). They range in size from 1 to 5 m in length and 2-4 mm in diameter. A typical capillary column used for GC is a coiled capillary tube of fused silica with an internal coating of stationary phase (page 10 (McMaster 2008)). Capillary columns used in GC have a length ranging from 10 to 100 meters and can further be divided into two groups based on their inner diameter (i.d): wide bore capillary columns (i.d$\geq$0.53 mm) and narrow bore capillary columns (i.d $\leq$0.5) (page 393(Robert L. Grob 2004)). In general, fused-silica capillary columns applied for VOCs analysis have a dimethylpolysiloxane stationary phase (non-polar). It can be combined with different phenyl or cyanopropylphenyl groups, achieving different degrees of polarity suitable for different sample matrices for the analysis of VOCs. (José Luis Pérez Pavón 2008). Bonded and cross-linked stationary phases provide thermal stability and increase column life by avoiding loss of stationary phase due to evaporation while working at higher temperatures which can be as high as 400 ˚C. Loss of stationary phase due to evaporation is called Column bleeding which can offer problems in chromatographic separations and detection of the analytes. Ideally, a stationary phase in GC column should have a boiling point of more than 100 ˚C higher than the maximum working temperature (Douglas A. Skoog 2007).

Narrow bore capillary columns provide better separations than wide bore capillary columns and are compatible with MS operations but due to their smaller diameters they have low sample capacity. Sample capacity of an analytical column increases with increased diameter. Wide bore capillary columns due to larger diameters provide more sample capacity. Environmental samples with high analyte concentrations are better analyzed with wide bore columns. Table 1 lists common columns and stationary phases used for VOCs analysis.

Table 1 Columns used for VOCs analysis (H.J Th. Bloemen 2012)

Type of column	Stationary phase composition	Polarity	Operating temperature °C
Packed column			
SP-2100	100% Dimethylpolysiloxane	non polar	0 to 180
OV-17	50% Diphenyl, 50% Dimethylpolysiloxane	mid polarity	-10 to 150
SP-1000	Polyethylene glycol ester	Very polar	40 to 220
Capillary columns			
Elite volatile	5% phenyl, 95% dimethyl polysiloxane	non polar	-20 to 260
OV-1	100% dimethylpolysiloxane	non polar	40 to 130
ZB-5MS	5% phenyl, 95% dimethyl polysiloxane	non polar	-60 to 350
SE-54	5% phenyl, 95% dimethyl polysiloxane	non polar	20 to 120
SE-52	5%-Phenyl)-methylpolysiloxane	low polarity	50 to 220
Ucon HB 5100	Polyethylene/polypropyleneglycol ethers	Polar	25 to 175
VOCOL	(20%) Diphenyl - (80%) Dimethylpolysilioxane	Intermediate	10 to 120
DB-624	6%Cyanopropyl-phenyl,94%dimethylpolysiloxane	low to mid polar	5 to 115

1.2.2 GC Detectors for VOCs analysis

The electron capture detector (ECD) and electrolytic conductivity detector (ELCD) are highly specific for halogenated compounds. Generally, these detectors provide good limits of detection for the analysis of halogenated compounds in water samples but there is always a need of second chromatographic column when analyzing environmental samples to make sure that there was no co-elution and the peak response is just because of the analyte. Furthermore simultaneous analysis of non-halogenated VOCs is not possible along with the halogenated VOCs using ECD or ELCD alone. A mass spectrometric detector (MSD) S is a universal detector and is capable to correctly quantify the analyte response even though there are coelutions with the target analyte. A

rapid qualitative identification of analytes is possible by comparison of their mass spectra with those in a library of spectra of known compounds (José Luis Pérez Pavón 2008).

1.2.3 Mass spectrometry

Mass spectrometry is a micro-analytical technique that can provide more structural information about a unit analyte than any other known analytical technique. Structural determination and compound characterization is based on the measured mass-to-charge (m/z) ratio of an analyte molecule. The sample molecules are first ionized by an ion source, the ions are then repelled towards the mass analyzer. Repulsion is done by a repeller plate at the back of the ion source provided with variable voltage of the same sign as the ionized fragments. Right after the pinhole, a series of focusing electrical lenses with variable voltage but same polarity like ionization fragment ions, squeezes the ion bean into an intense ion stream and it enters the mass analyzer (page.43-44 (McMaster 2008)). The process is illustrated in Figure 3. The mass analyzer separate the ions based on their m/z ratio. The selected masses from the mass analyzer strike the detector surface and produce signals. Main components of a mass spectrometer are briefly explained in the following text.

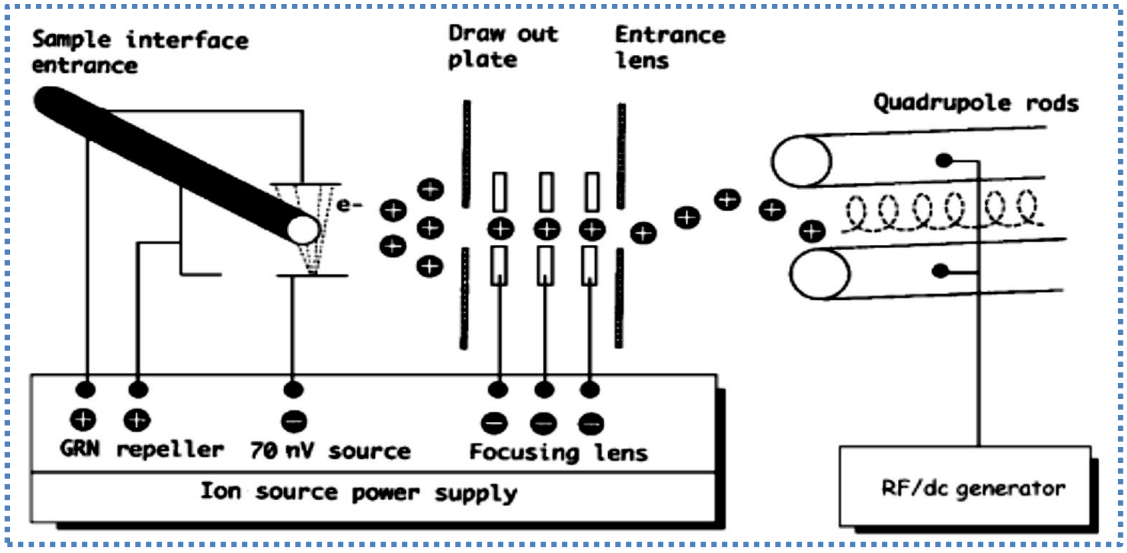

Figure 3 Schematics of mass spectrometric operation with EI as the ion source and quadruple mass analyzer (page 44 (McMaster 2008)

1.2.3.1 Ion source

The ion source produces ions in gas phase. There are many different ionization methods currently available depending on physical and chemical properties of the analyte and the internal energy transfer required during the ionization process. In the electron ionization (EI) source, sample from the gas chromatograph interface is exposed to a stream of 70-eV electrons from a filament. The EI is a highly energetic ionization technique, which typically offers extensive fragmentation. The advantage is that fragmentation patterns are reproducible. Characteristic fragments with specific masses can be used for either quantitative or qualitative analysis. Qualitative analysis offers structural determination, while quantitative analysis provides information about the concentration (J. Throck Watson 2007).

1.2.3.2 Mass analyzer

Several types of mass analyzers are in use. All mass analyzers use static or dynamic electric and magnetic fields that can be alone or in combination with each other. Most of the basic differences between the various common types of mass analyzer lie in the manner in which such fields are used to achieve separation. A quadruple mass analyzer is based on a combination of direct current (DC) and alternating current (AC) of radio frequency (RF) in four symmetrically arranged parallel rods. Diagonally aligned rods are electrically connected to make a pair. At any given time the two pairs have a potential of same magnitude, but with opposite signs. When accelerated ion stream enters a quadruple, the ions are forced into a corkscrew, three dimensional sine wave. The trajectories of the ions are determined by their m/z under the combined given DC/RF field. The combined DC/RF field is swept together for higher or lower field strength, upsetting the trajectories of all other ions, making them collapse with the walls of the rods and disappear, except the ions with one selected m/z as illustrated in Figure 4.

Heavy ions have low charge density and will therefore be less influenced by the attractions and repulsions while the lighter ions are more influenced by the changes in the AC electrodes due to high charge density. In other words, by sweeping DC/RF fields up or down, larger or smaller masses can be selectively directed towards the detector (page. 44 (Marvin McMaster)).

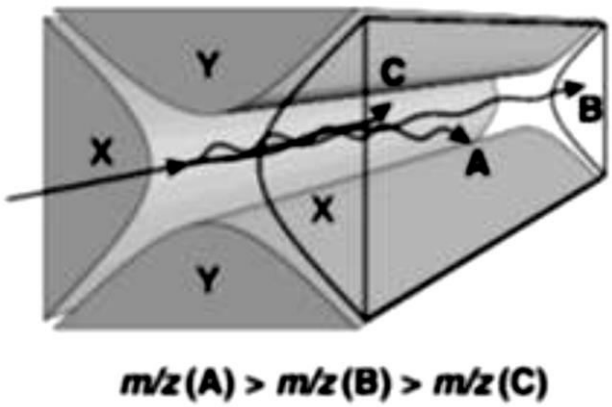

Figure 4 Principle and Scheme of a quadruple mass analyzer (Rolf Ekman 2009). Molecule A and C will not make through the quadruple since they are not within the allowed m/z range. They will collapse with the walls of the analyzer and disappear.

1.2.3.3 Detector

Detectors in the MS convert the energy of incoming particles into electrical signals. The energy is registered by electronic devices and transferred to a computer that translates these electrical signals into mass spectrometric information about the analyte. The signal received by the detector is generally low, so the energy of the incoming particles is usually amplified by the structural configuration of the detector. When ions from the mass analyzer strike the detector, the energy of the impact causes a secondary emission of electrons or photons depending on the nature of the detector (page 49-50 (Rolf Ekman 2009)). Several types of detectors are available for mass spectrometers. The choice of a particular detector is made by considering the design of the instrument in connection with analytical applications (Hoffman 2007)

1.2.3.4 Coupling GC with MS

The type of interface between GC and MS depends on the flow rate of carrier gas in the GC column. Direct coupling of MS is preferred with a GC where the flow rate in GC column is <2 ml/min. Higher flow rates are not compatible with MS operations. At a flow rate >2 ml/min even with the most efficient 2 stage vacuum systems, it is very difficult to achieve required vacuum necessary for MS operations. The life of EI source filament is also greatly affected at higher flow rates. (Chromacademy).

1.3 Validating Analytical Methodologies

Analytical measurements play crucial role in the modern world. Decisions making about the control of the manufacturing process of a product, assessing whether a product complies with regulatory limits or taking decisions about legal affairs, international trade, supporting health care will not be possible without accurate and reliable analytical measurements. Checking the quality of drinking water or the environment to comply with regulatory requirements, international environmental standards or for public health are some other crucial applications of analytical measurements. That's why an analytical lab must ensure that its analytical measurements are reliable and reproducible by other independent laboratories anywhere in the world and the user can use these results with great confidence for important decision making. Quality of analytical results can be guaranteed if they are obtained under the quality system developed by international analytical institutions. This system is based on four key concepts:

1. The laboratories which produce analytical results must operate under quality assurance principles of ISO/IEC 17025:2005 and should be accredited by this standard.
2. The laboratory must participate in proficiency testing schemes, in accordance with the "International harmonized protocol for proficiency testing of analytical laboratories"(M. Thompson 2006).
3. The laboratory must use internal quality control procedures which comply with the "Harmonized guidelines for internal quality control in analytical chemistry" (M. Thompson 1995).
4. The laboratory must use validated methods of analysis.

Using validated analytical methodology is one of the 4 key concepts to produce reliable and reproducible results. ISO 17025:2005 defines validation as confirmation by examination and the provision of objective evidence that particular requirements for a specific intended use are fulfilled (ISO/IEC 17025 2005). In fact, method validation is the means by which analysts have to demonstrate that the method is fit-for-purpose and the customers can trust the reported results.

1.3.1 When to validate a method

Method validation is necessary whenever it is needed to verify that performance characteristics of an analytical method are adequate for a particular analytical problem. ISO 17025:2005 requires

method validation in the following situations to confirm that the method is fit for an intended use. ((ISO/IEC 17025 2005) clause 5.4.5.2))

- New method developed for a particular problem
- Established method revised to incorporate improvements or extended to a new problem;
- Established method used in a different laboratory, or with different analysts or different Instrumentation
- To demonstrate the equivalence between two methods, e.g. a new method and a standard.
- When quality control indicates an established method is changing with time;

Method validation is hard to separate from method development when a new method is developed for an analytical problem. Many of the method validation parameters are usually evaluated, at least approximately, as part of method development and it is usually not possible to determine exactly where method development finishes and validation begins. (EURACHEM 1998). It is recommended as the method development proceeds, regular review should be carried out that customer needs are still being fulfilled. In case of chromatographic method development and validation, this can be well taken care of when method development and validation is broken down into four following steps (Bliesner 2006).

1. Method evaluation and further method development
2. Final method development and trial method validation
3. Formal method validation
4. Data review and method validation report

1.3.2 Components of Method Validation

The basic method validation parameters are commonly derived by using statistical procedures and usually refer to the reliability of the method. The trueness, accuracy, linearity, precision, selectivity, sensitivity, range, ruggedness, limit of detection and limit of quantification are the commonest and essential parameters that are required to be assessed in order to check whether a method satisfies previously defined analytical requirements and performance criteria and are required by ISO 17025:2005. These parameters are also called analytical method performance characteristics. Other complementary parameters are cost, ease of use, availability of material,

instruments and trained staff and sample throughput etc. (Ricard Boqué 2002). The latter parameters are not the main focus of this study.

The extent of method validation depends on the type of the method going to be applied or nature of the changes made in reapplying a method to different laboratories. The type of changes may include instrumentation, operators and the circumstances in which the method is going to be used. The type of method and extent of validation required accordingly is as follows:

- **Standard methods:** when applied with their full specifications do not require full method validation but the lab will have to have data to show that it can achieve the level of performance which the standard specification claims for the method.
- **Documented in house methods based on standard methods**: Extent of validation depends on the extent of the departure from the standard specifications.
- **In-house methods which are the laboratory's own methods:** need a high level of validation.

For method validation study to establish the performance characteristics of the method, mainly ISO 17025:2005 requirements and ISO 8466 and ISO 5725 approaches were attempted with the available data. However the method performance characteristics and their importance are supported in the following text by other method validation guides as well.

1.3.2.1 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection (LOD) is the lowest amount of an analyte to be examined in a test material that can be detected and regarded as different from the blank value with a given probability but not necessarily quantified.

The Quantitation limit (LOQ) is the lowest amount of an analyte to be examined in a test material that can be quantitatively determined under the experimental conditions described in the method with a defined variability given by coefficient of variation.

In practice, LOQ is generally more relevant than LOD. It is quite common to estimate LOD and LOQ as part of method development. There are several approaches to calculate LODs. The LOQ, by convention, is 3 times of LOD. The EU directive defines limit of detection as 3 times the

relative within batch standard deviation of a natural sample containing a low concentration of the parameter(Council Directive 98/83/EC 1998).

Two very common ways are based on regression statistics obtained during linearity study:

$$LOD = \frac{3.3 * S_{res}}{b} \qquad \text{Equation 1}$$

Where standard, S_{res}= standard deviation of residuals as found in the linear regression of the data for linearity study

$$LOD = \frac{3 \times S_a}{b} \qquad \text{Equation 2}$$

Where S_a is the standard deviation at the intercept point of the calibration function. The S_a is calculated by following expression:

$$S_a = S_{res}\sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n.\sum_{i=1}^{n}(x_i - \bar{x})^2}} \qquad \text{Equation 3}$$

Where n= number of standard concentration levels used for linearity study, $\bar{x}$ is the mean of all the reference values for standard concentrations, x_i= accepted value of i^{th} standard.

The LOQ is more important. Once LOQ is calculated a standard concentration near or equal to the LOQ is must to test to ascertain whether the accuracy and precision achieved is satisfactory.

1.3.2.2 Working range

The working range of a method is defined as "the interval being experimentally established and statistically proved by the calibration of the method, between the lowest and highest quantity and mass concentration" (ISO 8466).

Working range may or may not be linear but for a method to be effective, the curve should be repeatable from day to day. Within a linear working range, signal response will have a linear relationship to analyte concentration (EURACHEM 1998).

While establishing a preliminary working range for linear functions, following requirements must be met:

1. The working range shall cover, as far as possible, the application range of analysis

2. The most frequently encountered sample concentration should lie in the center of the working range.
3. A linear correlation between the measured values concentration.
4. The lower limit of working range should be equal to or greater than the limit of quantification.
5. Information or measured values are independent of the concentration. Statistically speaking, the difference between the variance of the lowest and highest concentrations in the working range. This is called test for homogeneity of variances. Ten independent replicates of the lowest and highest standards in the working rang are recommended and comparison is made as follows.

$$\frac{S_{10}^2}{S_1^2} = PG \quad \text{Equation 4}$$

Where S_1^2 is the variance of 1st standard and S_{10}^2 is the variance of last standard. The PG value is compared with the tabulated value of the distribution F (Fisher) for n-1 degrees of freedom and a 1% significance level. Where n is the number of standard replicas measured. If PG <F then the differences in the variances of the lowest and highest standards in the working range are not significant and the range of the work is well adjusted. If PG> F then the differences in variances are significant. The range of the work should be readjusted to that where the difference in the variances for the first and last standard are not significant i.e PG<F(ISO 8466).

1.3.2.3 Linearity

The linearity of a method is its ability, within a given range, to provide an informative value or results proportional to the amount of analyte to be determined in the test material (ISO 8466).

For linearity study, ISO 8466-1 requires at least 5 standards in the calibration data but recommends regularly distributed over the studied range of values. Linearity may be ensured by following three points:

1. The statistical calculations

A data may have good statistics even with outliers and points of influence. An outlier is a data point which is significantly different from the rest of the data set. A point of influence is a

calibration point which has a disproportionate effect on the position of the regression line (LGC Limited 2003). The graphical representation of the calibration data with the calculated regression line will make outliers and point of influences evident and any nonlinearity may be detected.

A visual inspection of plot of the residuals against concentration is strongly recommended to highlight problems with the calibration data that may not be immediately obvious from a simple scatter plot of the data. (EURACHEM 1998).

2. The statistical calculations:

Residual standard deviations from linear regression model and with a non-linear regression model of polynomial 2 for the same set of data set are compared as in followings.

$$DS^2 = (N-2)S_{res}^2 - (N-3)S_{res}'^2 \qquad \text{Equation 5}$$

Where S_{res}^2 is variance of residuals for linear regression, $S_{res}'^2$ is variance of residuals for 2nd degree polynomial model and N is the number of reference materials/standards used. The DS^2 and the variance of the nonlinear calibration function are compared by the F-test to determine whether there are significant differences or not.

$$PG = \frac{DS^2}{S_{res}'^2} \qquad \text{Equation 6}$$

The value PG is compared with the limit value *F1-α* given by the Fischer-Snedecor table for a confidence level "1-α" and a degree of freedom 1 and (N-3). If PG ≤ F1-α: the nonlinear calibration function does not result in an improved adjustment; for example, the calibration function is linear. If PG > F1-α: the work scope must be made as narrow as possible to obtain a linear calibration function: otherwise, the information values from the analyzed samples must be evaluated using a nonlinear calibration function.

3. **The correlation coefficient:**

The correlation coefficient "R" and related parameter coefficient of determination (R^2) are a measure of the strength of the degree of correlation between the y and x values. And depending on the extent of correlation "r" can take any value between +1 and –1. Values closer to 1, show stronger correlation. The correlation coefficient is commonly used in analytical measurement and it is easily and frequently misinterpreted, because:

• Correlation and linearity are only loosely related. It should be noted that coefficient r is a measure of correlation not a measure of linearity. A low r value does not necessarily mean that there is no correlation. There could be a relationship between the y and x values, but not a linear one. (LGC Limited 2003)

• Apparently data may show good correlation. A plot of the data is necessary to make sure that the data is satisfactory for the purposes of calibration.

• Predictions made from the calibration curve would have small uncertainties when r is very close to unity (1).

1.3.2.4 Sensitivity

Sensitivity is defined as the slope of the calibration function of the complete analytical method inclusive of all procedural steps within the working range in question (ISO 8466).

In other words, it is the ratio between the variation of the information value of the analysis method and the variation of the analyte quantity and is given by the following equation:

$$S = \frac{\Delta L}{\Delta C} \qquad \text{Equation 7}$$

ΔL - Increase in the value read

ΔC – Change in concentration

For the calibration curve, the sensitivity (slope of line) should be constant over the entire range. Sensitivity is the ability of the method to discriminate between differences in analyte contents. More sensitive methods are better able to distinguish small changes in analyte concentration (NATA Australia 2006).

The ratio of sensitivity and residual standard deviation is termed as standard deviation of the method (S_{xo}) which is a characteristic of analytical method and a measure for the quality of the analytical procedure. Standard deviation of a method is expressed as:

$$S_{xo} = \frac{S_{res}}{b} \qquad \text{Equation 8}$$

As can be seen from the equation; the quality of analytical method increases with increasing sensitivity and decreasing residual standard deviation. Different analytical methods may have

different working ranges. The coefficient of variation of a method is a normalized expression and can be used to compare the analytical efficiency of new developed methods with that already in use or with the standard analytical methods even with different working ranges. The coefficient of variation of a method is expressed as percentage by the following expression (ISO 8466):

$$V_{xo} = \frac{S_{xo}}{\bar{x}} \times 100 \quad \text{Equation 9}$$

1.3.2.5 Selectivity/Specificity

If a method responds to only one analyte, it is called specific. Generally analytically methods respond to multiple analytes. The ability of a method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test is termed as selectivity (NATA Australia 2006). During method development, it should be established first that the signals attributed to the analyte are being produced only due to analyte. The presence of something chemically or physically similar or arising as a coincidence is not contributing to the produced signals. The selectivity of analytical method can be assessed by determining % recoveries of the analytes in the real sample matrices (EURACHEM 1998).

1.3.2.6 Precision

Precision is the closeness of agreement between independent test results obtained under stipulated conditions (ISO 5725).

Precision is a measure of random errors and is normally determined for specific circumstances which in practice can be very varied. The three precision measures are:

1. Repeatability
2. Intermediate precision
3. Reproducibility

Repeatability and reproducibility represent 2 extreme cases of precision which can be obtained while analyzing the same sample, repeatability being the smallest and reproducibility the largest one. Intermediate precision is an intermediate case. (EURACHEM 1998).

Reproducibility study involves the analysis of same sample by the same method, in different laboratories, by different operators and different equipments. However for a single laboratory validation study this parameter is not assessed.

Purpose of the precision experiments is to find the limits within which results may vary in practice in. This information is very important for establishing rejection criteria by which the operator makes a conclusion to accept or reject results obtained in different circumstances in practice.

Precision is usually stated in terms of standard deviation or relative standard deviation (RSD) (EURACHEM 1998). It is recommended that for precision estimation, at least 7, preferably ≥10 determinations should be made on the same sample or standard under repeatability and intermediate precision conditions (IUPAC 1999).

1.3.2.6.1 Repeatability

Repeatability refers to tests performed on identical test items during a short interval of time in same laboratory by same operator using the same equipment. In other words repeatability is precision under conditions that are as constant as possible (NATA). Repeatability being the smallest expected precision (EURACHEM 1998).

Repeatability standard deviation S_{ri} is given by the equation:

$$S_{ri} = \sqrt{\frac{1}{n-1} . \sum_{i=1}^{n} (x_i - \bar{x})^2} \qquad \text{Equation 10}$$

Where n=number of replicates made under repeatability conditions for same sample or standard.

x_i = individual determination made in repeatability experiment

$\bar{x}$ = mean of all replicates for same sample/standard in repeatability experiment

When repeatability is determined on 2 different concentration levels in samples/standards, a comparison can be made between the two recoveries, even if determinations were done separately but for determinations on each sample/standard, identical conditions were kept constant. Repeatability of alternative method should be compared with that of the standard method.

The value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95% is termed as repeatability limit (ISO 5725 1997). Repeatability limit is expressed as "r" and is evaluated by following equation:

$$r = 1.96.\sqrt{2}.S_{ri} = 2.8\sqrt{S_{ri}^2} \qquad \text{Equation 11}$$

Where

S_{ri}- Standard deviation of repeatability

S_{ri}^2 - Variance of repeatability

1.3.2.6.2 Intermediate Precision

Repeatability is a useful indicator of method performance, but in general a lab has multiple operators and the analysis of same samples may be required to repeat on different day by different analysts. So repeatability underestimates the spread of results that can be expected under normal operating conditions over the longer term (NATA Australia 2006).

Intermediate precision is precision measured between different analysts, over extended timescales, within a single laboratory. While stating intermediate precision, exact conditions should be given (EURACHEM 1998).

For assessing intermediate precision 2 strategies may be used:

1. A similar equation to standard deviation for intermediate precision can be used when determinations are made on the same standard level:

$$S_i = \sqrt{\frac{1}{n-1}.\sum_{k=1}^{n}(y_k - \bar{y})^2} \qquad \text{Equation 12}$$

Where

n = number of determinations made in different intermediate precision conditions

y_k= individual determination made in intermediate precision experiment

$\bar{y}$ = mean of all determinations for same sample standard in intermediate precision experiment

2. When individual determinations in intermediate precision experiment are made in duplicates on a range of standards or samples concentration, following calculations should be done:

$$\bar{X}_J = \frac{(y_{j1}+y_{j2})}{2} \quad (13a) \qquad d_j = y_{j1} - y_{j2} \quad (13b) \qquad r_j\,(\%) = \frac{|d_j|}{\bar{X}_J} \times 100 \quad (13c)$$

$$S_{pi}\,(\%) = \frac{\left[\sum_{j=1}^{p} r_j\,(\%)\right]/p}{1.128} \qquad \text{Equation 13}$$

S_{pi}= RSD in % for intermediate precision.

p - Total number of samples analyzed in duplicate

y_{j1} - First result obtained for the sample j

y_{j2} - Second result obtained for the sample j

1.3.2.6.3 Relative standard deviation

Precision standard deviation is a measure of dispersion of test results under specific precision conditions (ISO 5725) and is dependent on analytical method and analyte concentration. When the working range is large, precision should be studied at several concentration levels across the working range and precision values should be compared with fixed criteria. For small working range, relative standard deviation is largely constant and may be more useful, because concentration has been factored out (EURACHEM 1998).

The Relative standard deviation (RSD) is expressed in % and is given by:

$$RSD = \frac{S}{\bar{x}} \times 100 \qquad \text{Equation 14}$$

$\bar{x}$= Average of the values determined on same concentration level.

For residual method validations, it is recommended that precision should be assessed at minimum 2 concentration levels, the lowest and the highest ones to cover the entire working range (IUPAC 1999).

1.3.2.7 Accuracy

Accuracy is defined as the closeness of agreement between test results and accepted reference value (ISO 5725). In other words, accuracy of an analytical method is a measure of the extent to which the test results generated by the method agree with the true value. The term accuracy when applied to test results involves both precision and trueness. Precision expresses random errors while trueness expresses systematic errors in the measurement. Trueness is defined as closeness

of agreement between the average value obtained from a large series of test results and an accepted reference value. Bias is quantitative expression of trueness (ISO 5725)

Several approaches are in practice for the assessment of accuracy. Following are the most common ones.

1.3.2.7.1 Analyzing Certified Reference materials

The best way to estimate accuracy is to analyze a matrix certified reference material (CRM) containing a stated concentration (or amount) of the analyte with the analytical method under study. Analytical Certified reference materials are generally accepted as providing traceable values; the reference value is then the certified value of the CRM.

$$z = \frac{X_{lab} - X_{ref}}{S_{interlab}} \qquad \text{Equation 15}$$

If $z \leq 2$, the results are considered accurate

1.3.2.7.2 Recovery

Recovery tests on matrix blanks or on fortified samples demonstrate how successful the method has been at extracting the analyte from the matrix. If the analytical method fails to determine all of the analyte present, it may reflect an inherent problem in the method resulting in the bias associated with sample preparation, extraction of the analyte from a sample or other analytical steps prior to determination (NATA).

Test portion of matrix is spiked or fortified at various concentration levels, then spiked/fortified test portions are extracted and analyte concentration is determined. Recovery is calculated in following way.

$$\%\ Recovery = \frac{(C1 - C2)}{C3} \times 100 \qquad \text{Equation 16}$$

Where

C1 = concentration determined in fortified sample

C2 = concentration determined in unfortified sample

C3 = concentration of fortification

The evaluation of the accuracy with recovery testing may be performed by calculating the average relative error of recoveries:

$$E_r = \frac{|\overline{X_{rec}} - R_{rec}|}{R_{rec}} . 100 \qquad \text{Equation 17}$$

Where

$\overline{X_{rec}}$ = Average concentrations recovered for each level of fortification;

R_{rec}= Concentration that theoretically should recover for each fortification level.

The value of the relative error is compared with a predefined value.

1.3.2.7.3 Measurement Uncertainty (MU):

Measurement uncertainty is defined as an estimate attached to a test result, which characterizes the range of values within which a true value is asserted to lie (ISO 3534 2006). Measurement uncertainty is a single parameter usually a standard deviation or confidence interval which characterizes the dispersion of the analysis results around the true value. A measurement uncertainty estimate takes account of all recognized effects operating on the result; the uncertainties associated with each effect are combined according to well-established procedures into a single standard deviation called standard uncertainty u(x), or in an expanded form (U)

$$U = k.u \qquad \text{Equation 18}$$

Generally k = 2, for a 95% confidence interval.

The MU is an increasingly common expression of accuracy and provides a single figure expression of accuracy (EURACHEM 1998). The MU estimate is necessary to ensure results are fit for purpose, results are traceable to international or national standards, to effectively compare results between laboratories and/or specifications, legal tolerances or regulatory limits, make informed decisions, to improve test methods (NATA Australia 2006).

Three main approaches have been applied for the estimation of MU with their own advantages and disadvantages among which top down approach based on interlaboratory data is the simplest one. This approach was presented by Analytical Methods Committee" (AMC) of the "Royal Society of Chemistry" This approach is extremely simple. According to the top down approach reproducibility standard deviation (S_R) from interlaboratory comparison can simply be used as a

working estimate of measurement uncertainty, provided that laboratory's bias and intermediate precision is not larger than the reproducibility standard deviation. However this technique overestimates the MU. The expanded uncertainty is selected in order to define a range which is expected to contain the true value of the result with high probability (EURACHEM/CITAC 2000):

$$U = k.S_R \qquad \text{Equation 19}$$

Bottom up approach presented by ISO guide for the expression of uncertainty in measurements (GUM) is based on the quantification and combination of all the individual sources of uncertainty, associated with the random and systematic errors that can be responsible for the measurement error. The uncertainty estimate with this approach is most realistic but the methodology for most chemical measurements is extremely difficult and in some cases, even impossible for the production of objective measurement uncertainty values (EURACHEM/CITAC 2000).

Top down approach based on in house validation data is an intermediate approach and is based on a combination of top down and bottom up approaches. It estimates MU from the performance of the analytical method in inhouse method validation and/or routine analysis quality control a broader frame (i.e., several days, different operators or equipments, etc.) in intralaboratory environment (EURACHEM/CITAC 2000).

2 Experimental Part (HS-Trap-GC-MS)

2.1 Chemicals and Reagents

The individual standards of benzene, 1, 2-dichloroehthane, trichloroethene (TriCE), tetrachloroethene (TetCE) each at a concentration 2000 µg/mL, a mix standard of the 4 THMs; chloroform ($CHCl_3$), bromoform ($CHBr_3$), dibromochloromethane ($CHBr_2Cl$) and dichlorobromomethane ($CHCl_2Br$) each at 2000 µg/mL each and a mixture of flurobenzene and chlorobenzene-d5 each at 2500 µg/mL were obtained from two different sources; RESTEK (USA) and Reagecon (Ireland). Pesticide quality methanol (MeOH) was obtained from VWR UK. Suprapure hydrochloric acid (30%) was supplied by Merck (Darmstadt, Germany). Reagent grade ascorbic acid was supplied by Sigma Aldrich Portugal. Bottled water Agua de Nascente Portugal was found free of the VOCs under study and was used for preparing calibration standards. In the further texts it will be referred as dilution water.

2.2 Materials and Instrumentation

The analytical instrument used was Perkin Elmer Clarus 500 GC directly coupled with Clarus 560 MS operated in electron ionization mode. PerkinElmer Elite Volatiles column 30 m × 0.25-mm i.d and 1.4-µm film thickness was used for gas chromatographic separations. The GC-MS was connected with Turbomatrix Headspace 40 Trap autosampler in "on column" configuration (on-column configuration explained in section 1.2.1.2).

Clear glass 20 ml HS vials with 18 mm magnetic screw cap were obtained from ILC Portugal. Gas tight micro syringes of volumes 50, 100 and 500 µL were purchased Hamilton USA. Electronic pipette 10 mL was purchased from Eppendorf Germany. A freezer at -16 (±2) °C was used for storing standards. A Refrigerator at 4 (±2) °C was use for sample storage.

2.3 Analytical Procedures

2.3.1 Preparation of stock and working standard solutions

Following stock solutions and standards were prepared and used in this study.

2.3.1.1 Mixed standard Stock solution:

A mixed secondary standard with each of the 4 THMs at 40 µg/mL each, benzene at 5 µg/mL, 1,2 dichloroethane, TriCE and TetCE at 10 µg/mL each was prepared in MeOH in following way. Appropriate volumes of each of the primary standard solution (Table 2) were added to 9.8 mL of MeOH in a 10 mL volumetric flask. The volume was made upto the mark with methanol. This mixed standard stock solution was transferred to several 300 µL vials filled completely and capped without headspace. These vials were stored at -16 °C in a freezer for maximum 3 weeks or otherwise once opened for use; whichever happened earlier.

Table 2 Preparation of mixed standard stock solution

Compound	Primary standard concentration µg/mL	Volume of primary standard solution added in 10 mL in MeOH (µL) solution	Conc. in stock solution (µg/mL)
THMs	2000	200	40
Benzene		25	5
1,2 dichloroethane		50	10
Trichloroethene		50	10
Tetrachloroethene		50	10

2.3.1.2 Working standard solution:

A working standard solution was prepared fresh each day by transferring 250 µL of the mixed standard stock solution to 1mL methanol in a 1.5 mL vial. The concentration of each standard achieved in the working standard solution is given below in Table 3. The calibration standards were prepared by appropriate dilutions of the working standard solution.

Table 3 Working standard solution concentration

Standard	Working standard solution concentration (µg/mL)
Benzene	1
1,2 DCE, Tri & Tetrachloroethene	2
THMs	8

2.3.1.3 Internal standard solution:

5 µg/mL internal standard solution was prepared by adding 20 µL of the mixed primary internal standard solution (2500 ug/mL) to approximately 9.8 mL of MeOH in 10 mL flask. The volume was marked up to 10 mL with MeOH. This stock internal standard mixture was transferred to 1.5 mL vials filled completely, stored and kept in a freezer at -16 °C for maximum 3 weeks otherwise

once opened for use. A 10 µL of this working standard in 10 mL sample or calibration standard would give a concentration of 5 µg/L.

2.3.1.4 Calibration Standards:

The working standard solution was diluted directly into HS vials to prepare 6 concentration levels calibration standards. The volume of the working standard solution and dilution water in each vial is given below in Table 4.

Table 4 Preparation of calibration standards

Calibration standard number	Calibration standard conc. level achieved (µg/L)			Working standard solution added (µL)	Water volume (mL)
	Benzene	1,2 DCE, Tri and Tetrachloroethene	THMs		
1	0.5	1	4	5	9.995
2	1	2	8	10	9.99
3	2	4	16	20	9.98
4	3	6	24	30	9.97
5	4	8	32	40	9.96
6	5	10	40	50	9.95

2.3.2 Sampling and sample collections

Three kinds of water samples were collected: surface water, treated water intended for human consumption and waste water from water treatment facilities of Aguas do Algarve. Each sample was collected in a set of four, in 40 mL glass vials already containing 0.25 mg of Ascorbic acid for sample preservation. The vials were completely filled and capped immediately without headspace. All sample vials stored at 4 °C during transportation and in the laboratory at 4 °C till analysis.

2.3.3 Method development and Optimization HS-Trap-GC-MS conditions:

Method development and optimization of the HS-Trap, GC separations and MS conditions was performed on 10 mL test portions of 100 µg/L of mixed standard solution of each of the standards in VOCs free, in the HS vials. The vials were spiked with 10 µL of IS mixed standard solution, immediately screw capped and transferred to HS-Trap autosampler for further analysis.

2.3.4 pH effect on extraction efficiency of HS-Trap opeartion

A mixed standard solution of all the VOCs with benzene= 2 µg/L, THMs= 16 µg/L and 1,2-dichloroethane, TriCE and TetCE= 4 µg/L was prepared by direct dilution of the working standard solution in HS vial and analyzed in 2 sets; each in duplicates. One set of duplicates was added with 25 µL 1:1 HCl solution after the internal standard addition and the other was analyzed without any acid addition. The average GC-MS peak areas were compared to observe any effect of acid addition on the extraction efficiency of the HS-Trap operation.

2.3.5 Optimized HS-Trap GC-MS method

Optimized conditions were used for the HS-Trap-GC-MS analysis for validation study. GC Equilibrium time 2min, GC oven was programmed at 40 °C startup time, hold up at 40 °C for 2.00 min, then ramped at 10.0°C/min to 100°C, with no hold up time, then ramped at 40.0°C/min to 230°C with a final hold up for 5.0 min. Helium was used as carrier gas in constant pressure mode at 25 psi.

Optimal headspace parameters were: vial temperature 80°C; needle temperature 90°C; transfer line temperature 120°C; trap material: air toxics; trap load temperature 40°C; trap desorption temperature 280°C; thermostattation time 20 min; cycles number 4; vial pressurization time 1 min; vial pressure decay time 2 min; trap desorption time 0.5 min; trap hold 6min; dry purge time 5 min; vial pressure 35 psi; trap desorption pressure 10 psi; Trap outlet split: on.

GC-MS transfer line temperature was 200°C. The MS was operated with 200 °C ion source temperature, 100 µA trap emission, 70 eV electron energy and 600 mV photomultiplier voltage. Selected ion monitoring mode with 0.001 sec inter channel delay, 0.01 sec or 0.015 sec dwell times was used for quantification. All the HS-Trap-GC-MS conditions are summarized below in the Table 5 and MS acquisition parameters are given in Table 6. The mass spectrometer tune parameters are shown in Figure 5.

Table 5 HS-Trap-GC-MS Conditions for validation study

Sample introduction	Turbomatrix HS 40 Trap
Needle temperature	90 °C
Vial thermostatting time	20 min
Vial pressurization time	1 min
Vial pressure	35 psi
Decay time	2 min
Vial Oven temperature	80 °C
HS-Trap-GC transferline	Fused silica 1.5 m x 320 µm
Transferline temperature	120 °C
Trap low temperature	40 °C
Trap High temperature	280 °C
Dry purge time (Helium)	5 min
Trap dry purge temperature	40 °C
Trap hold time	6 min
Desorb time	0.5 min
Desorb pressure	10 psi
Clarus 500 GC	
Carrier gas	Helium
Column pressure	25 psi
GC Oven program	Startup 40 °C, 2 min hold up at 40 °C, 10 °C/min rise till 100 °C no hold up at 100 °C, 40 °C/min till 240 °C, final hold up for 5 min.
Column	Elite volatile: 30 m × 0.25-mm i.d and 1.4-µm film thickness
MS Clarus 560	
GC-MS transferline temperature	200 °C
Electron energy	70 eV
Trap emission	100 µA
Photomultiplier voltage	600 mV
Acquisition mode	Selected ion monitoring
Dwell time	0.01 sec or 0.015 sec. (Table 6)
Inter channel delay	0.001 sec
Span (Da)	0.5

Table 6 MS Acquisition parameters for qualification and quantification

Compound	Quantifier ion	Qualifier ion	Dwell time (sec)	Retention time (min)	Scan window (min)
Chloroform	85	83	0.01	2.53	2.31-2.80
1,2-dichloroethane	66	64		3.00	2.78.325
Benzene	78	77		3.36	3.10-3.55
Trichloroethene	95	97		3.95	3.40-4.10
Flurobenzene	82	112		3.57	3.45-3.83
Dichlorobromomethane	85	83		3.96	3.75-4.25
Dibromochloromethane	129	127		5.68	5.38-6.00
Chlrobenzene-d5	117	82		6.92	6.61-7.20
Bromoform	173	171		7.46	7.33-8.00
Tetrachloroethene	166	164	0.015	6.18	5.89-6.50

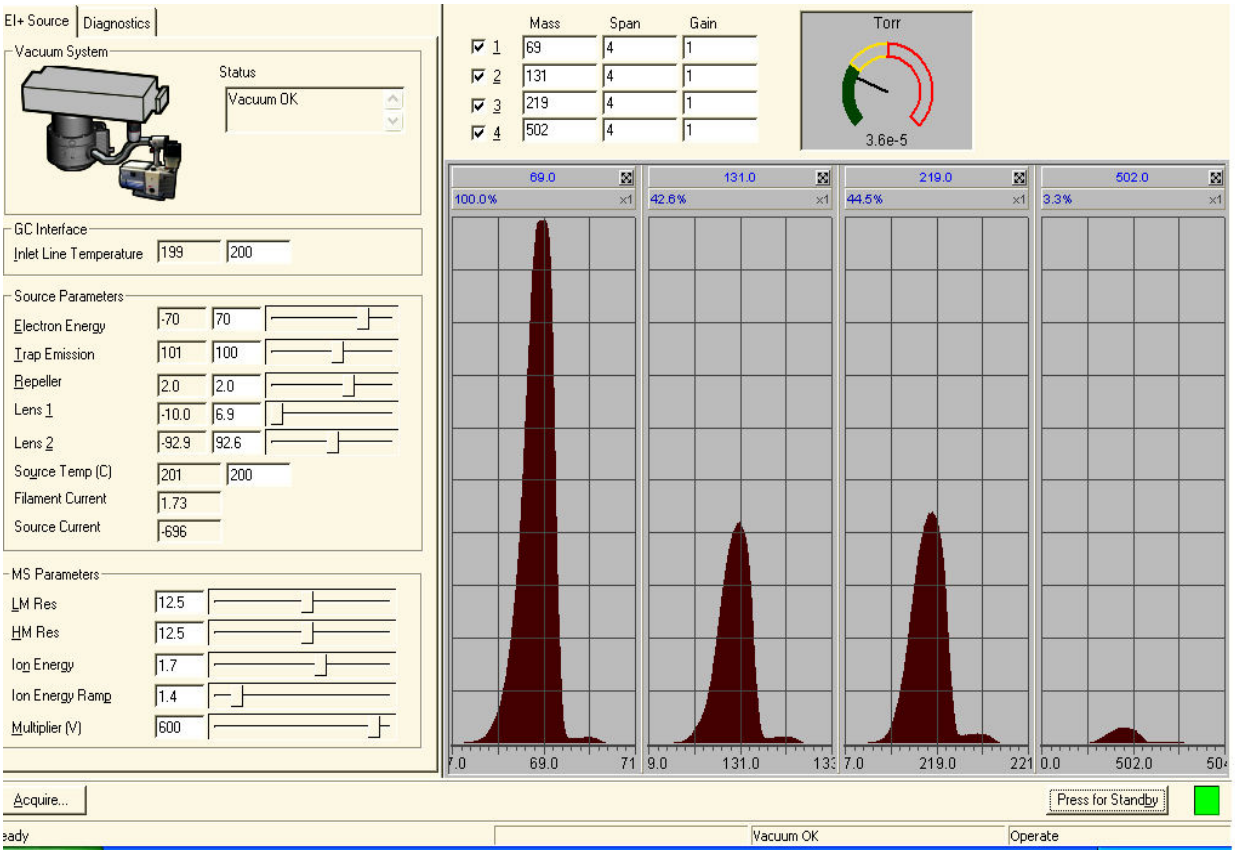

Figure 5 Perkin Elmer Clarus 560 MS tune parameters

2.4 Method Validation

2.4.1 Calibration

Six concentration level standards for each of the VOC were analyzed through all the steps of the method. The measured GC-MS peak areas were normalized with the internal standard responses. The data sets consisting of the 6 standard concentrations and corresponding measured values (normalized GC-MS responses) were subjected to regression analysis. Coefficients of determination (R2), intercept "a" and slope of the regression line "b" and residual standard deviation (Sres) were found. Confidence intervals (CI) of coefficients "b" and "a" of the calibration functions were calculated by using following expression:

$$CI = \pm(t_{(a,df)}.S) \qquad \text{Equation 20}$$

Where "t" is the 2 tailed student t distribution value at n-2 degrees of freedom and "S" is the standard deviation. Standard deviation of "b" was calculated using following equations:

$$S_b = \frac{S_{res}}{\sqrt{\sum_{i=1}^{N}(x_i - \bar{x})^2}} \qquad \text{Equation 21}$$

Where S_b is the standard deviation of the "b". Standard deviation of "a" was calculated using equation 3 given in section 1.3.2.1.

The confidence interval of the predicted y values in the least square fit regression was calculated by following equation.

$$\Delta y = t_{(a,df)}.S_{res}\sqrt{1 + \frac{1}{n} + \frac{n(\bar{x} - x_i)^2}{n.\sum x_i^2 - (\sum x_i)^2}} \qquad \text{Equation 22}$$

Calibration standards were prepared by diluting working standard solution with the dilution water directly in the HS vials. For calibration standard preparation, specified volume of dilution water was transferred to HS vial with a calibrated electronic pipette. Then appropriate volume of working standard solution was dispensed close to the bottom of HS vial using a 50 µL syringe with Teflon tip. The specified volumes of the mixed standard working standard solution and dilution water are given in Table 4. Immediately after the addition of working standard solution of mix standards, 10 µL of working standard solution of internal standard was dispensed in a

similar fashion followed by 25 µL of 1:1 HCl solution. The HCl solution was dispensed with the wall of the HS vial, well above the water surface. The vial was screw capped immediately and inverted upside down thrice to mix the solution. The capped vials were transferred to the HS-Trap autosampler for the HS-Trap-GC-MS analysis. Quantification was performed using internal standard method. Flurobenzene was used as internal standard for chloroform, 1,2-dichloroethane, benzene, trichloroethene and dichlorobromomethane. Chlorobenzene-d5 was used as internal standard for dibromochloromethane, tetrachloroethene and bromoform.

2.4.2 Linearity

Calibration data was plotted graphically and regression lines were obtained to observe evidence of unlinearity. Correlation coefficients and plots of residuals were obtained. Statistical test of linearity was performed on the calibration data by calculating and using linear, non-linear calibration functions with their residual standard deviations using ISO 8466 approach as given in section 1.3.2.3. The test PG values were compared with the tabulated PG value to check if the linear calibration function is valid in the selected working range.

2.4.3 LODs and LOQs of the method

LODs and LOQs were calculated using the approaches discussed in section 1.3.2.1.

2.4.4 Precision

Repeatability of the developed method for each analyte was determined by analyzing a mixed standard in dilution water with benzene= 2 µg/L, THMs= 16 µg/L and 1,2-dichloroethane, TriCE and TetCE= 4 µg/L. The standard was prepared by direct dilution of the working standard solution into HS vials. Ten replicates of the standard were analyzed by the HS-Trap-GC-MS method in the same run. The Intermediate precision was determined by analyzing the mixed standard on different days. It only involved one source of variation which was time. The analysis was performed by the same operator. The final results were treated by Dixon's Q-test for gross error (outliers). After removing the outliers from each set of data, % RSDs of repeatability and repeatability limits (r) were calculated. The %RSDs for intermediate precision were calculated using equation 12 as given in the section 1.3.2.6.2.

2.4.5 Working range

Working range was pre-selected based on laboratory's analytical requirements. Six independent measurements of the 1st and the last standards in the working range were attempted on different days. Variances in the both data sets: the replicates of the lowest standard and the highest standard, were subjected to F-test to determine if the variances are statistically significant or not. The test for homogeneity of the variances is explained in section 1.3.2.2.

2.4.6 Sensitivity

Sensitivity was determined as the slope of the calibration line for each parameter by the linear regression model. The % RSD was calculated for the slopes of the calibration curves produced under the intermediate precision conditions for each of the compound. The coefficient of variation of the method (as described in 1.3.2.4) was also calculated for each parameter.

2.4.7 Recovery

The recovery for each analyte was estimated at one concentration level for each compound Table 7 gives the concentration of each compound in the recovery samples. Surface water, treated water and waste water samples were spiked in duplicates directly into head space vials. Each spiked sample was immediately added with 10 µL of internal standard solution followed by 25 µL of 1:1 HCl solution, screw capped and transferred to HS-Trap autosampler. Table 7 gives spiked concentrations in the recovery samples for all the compounds.

Table 7 Preparation of recovery samples

Concentration level after spiking (µg/L)			Volume of working standard solution added (µL)	Volume of sample (mL)
Benzene	1,2 DCE, Tri and Tetrachloroethene	THMs		
2	4	16	20	10

The % Recoveries were calculated using equation 16 described in section 1.3.2.7.2.

2.4.8 Accuracy

The accuracy was estimated by analyzing CRM in ground water matrix. Proficiency testing (PT) samples from LGS Aquacheck were obtained and used as CRM after the PT cycle was complete. The CRM was analyzed as real samples as described in 2.5. Accuracy was calculated as z-score by equation 15 described in section 1.3.2.7.1.

2.4.9 Quantification of Real Water samples

The real water samples were analyzed in duplicates. A 10 mL of water sample was transferred to head space vial and added with 10 µL internal standard solution followed by 25 µL HCl solution (1:1) and screw capped immediately. The screw capped vials were transferred to the HS-Trap autosampler for further analysis following the HS-Trap-GC-MS method.

Quantification was performed by using minimum 4 point calibration curves in the linear working range. Calibration standards were freshly prepared and run in the same run with the real samples.

3 Results and Discussion (HS-Trap-GC-MS)

3.1 Method Development and optimization

3.1.1 Chromatographic Separations

Chromatographic separations for all the 8 VOCs were achieved in less than 8 minutes; however the total GC run time was 17 minutes which included a 5 minute final hold up at 240 °C to clean the column. The total GC cycle was 25 minutes which included the run time and cool down period to get back at 40 °C for the next run. A run time of 15 to 35 minute is reported in the literature for long narrow capillary columns necessary to achieve the separation of these 8 VOCs. Different GC oven programs were attempted to achieve chromatographic separations for these compounds. A 40 °C startup oven temperature with initial hold up of 2 minutes, then rise at 10 °C/min till 100 °C, no hold up at 100 °C, 2nd gradient at 40 C/min till 230 °C with a final hold up of 5 min to clean the column was found optimum GC oven program. All the analytes and internal standard peaks were well separated except TriCE and $CHCl_2Br$ which co-elute at around 4 minutes as can be seen in the overlaid SIM chromatogram shown in Figure 6. A 6 minutes initial hold uptime at 40 °C startup was attempted to achieve better separations between TriCE and CHCl2Br but no significant improvements were observed. On the other hand it resulted in considerable peak broadening of all the peaks.

Better separations are possible between TriCE and Cl_2BrCH when 35 °C startup temperature is used with longer initial hold up time but the peak shapes are almost twice the widths of those obtained at 40 °C startup and 2 minutes hold up time.

A 35 °C start up with initial longer hold up time was not opted because of following reasons along with broadened peaks:

- Cool down time increases about 3 minutes as compared with analysis is started at 40 °C.
- During summer, at the cooling conditions provided in the instrument room, sometimes, the instrument was not able achieve or maintain a 35 °C start up temperature.

A 40 °C startup with initial hold up 2 min was found a good compromise. Several column pressures were also attempted but 25 psi was found optimum for the chromatographic separations.

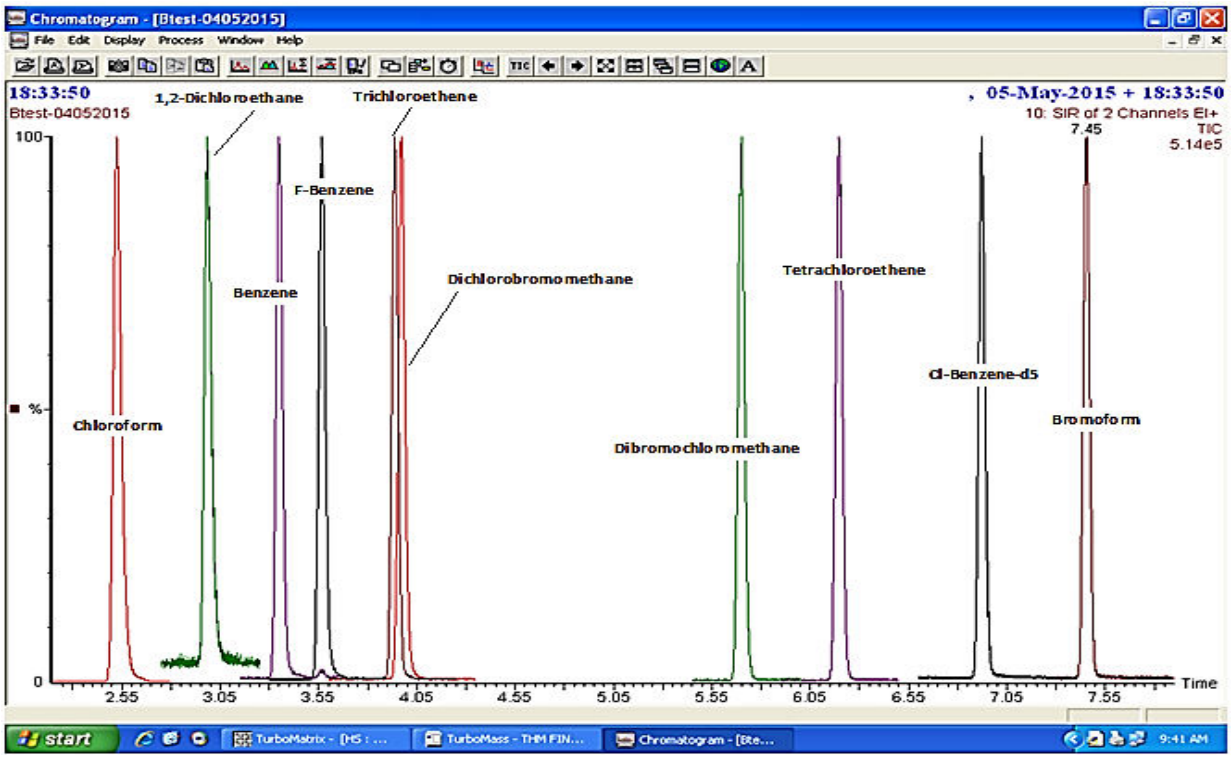

Figure 6 Overlaid SIM Chromatogram for the target compounds and internal standard peaks showing separation and coelutions in the optimized method. (THMs= 40, Benzene= 5, 1,2-dichloroethane, TriCE and TetCE =10 µg/L)

3.1.2 Head Space-Trap (HS-Trap)

HS-Trap parameters for VOCs analysis can simply be chosen for maximum extraction efficiency by careful considerations consulting HS-Trap user' guide prior to method development, saving significant time in method development stage. If the time permits, they can further be optimized to reduce the analysis time in the overall analysis cycle.

A vial thermostatation time of 20 minutes was chosen with 80 °C vial oven temperature. A thermostatting time of 10 minute was attempted but it resulted in the lower extraction efficiency of the earlier vials. The thermostatting function overlaps with the analysis of previous vial with no extra time for thermostatting except for the 1st vial. During trap desorption step in the analysis of one vial, the system picks up the next vial for thermostatting. Moreover lower time intervals

cannot save significant time and will not result in any significant increase in the sample throughput.

A pressure=35 psi was used for vial pressurization. A pressure, 10 psi higher than the column pressure is recommended to allow the system to transfer the maximum of the headspace vapor volume to the trap in each cycle. Higher pressures have an associated risk of subjecting the system to gas leaks. F.Barani *et al* have reported that there was no significant increase in the extraction efficiency at pressures higher than 30 psi and lower pressures are associated with lower head space volume transfers and hence lower signal intensity (Barani, Dell'Amico et al. 2006).

Vial pressurization period of 1 minute was selected. A 1-3 minutes vial pressurization period is recommended when using capillary columns to ensure homogeneity of the gas phase in the vial and good precision. The manufacturer recommends 2 minutes vial pressurization period for good reproducibility and 3 minutes for optimum reproducibility. F. Barani et al and Meng Yuan (Barani, Dell'Amico et al. 2006; Meng Yuan 2009) also have used 1 minute as vial pressurization period for the target analytes along with other VOCs. Due to limited availability of the instrument for this project, a precision comparison at 1 minute and 2 and 3 minutes was not attempted. However, a lower pressurization time leads to decreased analysis time and considerable increase in the overall sample throughput. A cycle number of 4, the maximum provided in the instrument software by default, was selected in the method development stage. The maximum extraction efficiency might be achieved in fewer cycles. A comparison needs to be done as if the maximum efficiency is achieved in 3 cycles eliminating the 4^{th} one will save 3 minutes in analyzing each sample leading to significant increase in overall sample throughput.

A 90 °C needle temperature was chosen. It is recommended to have needle temperature 10 °C higher than the vial oven temperature. The needle temperature is also safer for the septum to cause any septum burns.

Three trap desorb temperatures: 280 °C, 300 °C and 320 °C, were attempted. A 280 °C desorb temperature was found optimum. A 280 °C trap desorb temperature is high enough for complete desorption and trap clean. No carryover effects were observed at the temperature. A 10 psi trap desorb pressure was chosen. F. Barani *et al* have reported that higher desorb pressures resulted in increased sensitivity but in their study, a 0.3 min desorb time was used. Significant sensitivity

was obtained with 0.5 min trap desorb time and 10 psi trap desorb pressure. A trap desorb time of 0.5 minutes is considered more than enough for trap desorb of VOCs. Lower desorb time was not attempted because no significant advantage of time saving in the analysis was seen in the reduction of this parameter.

Transferline temperature was chosen 120 °C. Transfer line temperatures below needle temperature are not recommended as it may cause condensation in the transfer line. Too high temperatures may cause oxidation of the analyte in the heated transfer line as it also contains traces of oxygen. Injector temperature higher than transferline is not recommended so injector temperature was also set 120 °C. Outlet split during trap desorb was kept open. A significant increase in the sensitivity of the developed method can be obtained with little extra efforts if the outlet split option is not used. Based on literature survey, it was considered advantageous to avail the outlet option in the initial experimental work. During the course of the work it was realized that the type of the samples lab is receiving and under the instrumental conditions should be kept closed but the rest of the experimentation was still carried out with the trap outlet split open for the homogeneity of the data, needed to be obtained in the limited available time and the instrument.

3.1.3 MS Quantification

A dwell time of 0.01 sec was used for the quantifier and qualifier ions for all the compounds except tetrachloroethene for which 0.015 dwell time was selected. Sensitivity comparison at 0.01 and 0.05 sec dwell times during MS method optimization showed no significant differences on increased dwell times for all the compounds except for tetrachloroethene. Figure 7 demonstrates obtained sensitivities for benzene and tetrachloroethene at 0.01 and 0.05 sec dwell times. A 0.015 sec dwell time for tetrachloroethene was selected for better sensitivity while obtaining more than 5 data points per peak. Dwell times higher than 0.015 sec would not give sufficient data points per peak. Minimum 5 data points per peak are required for analytical precision to minimize the effect of chromatographic variations. The difference in the sensitivity for tetrachloroethene at 0.01 and 0.015 sec may or may not be significant. It was not verified with further experimentation.

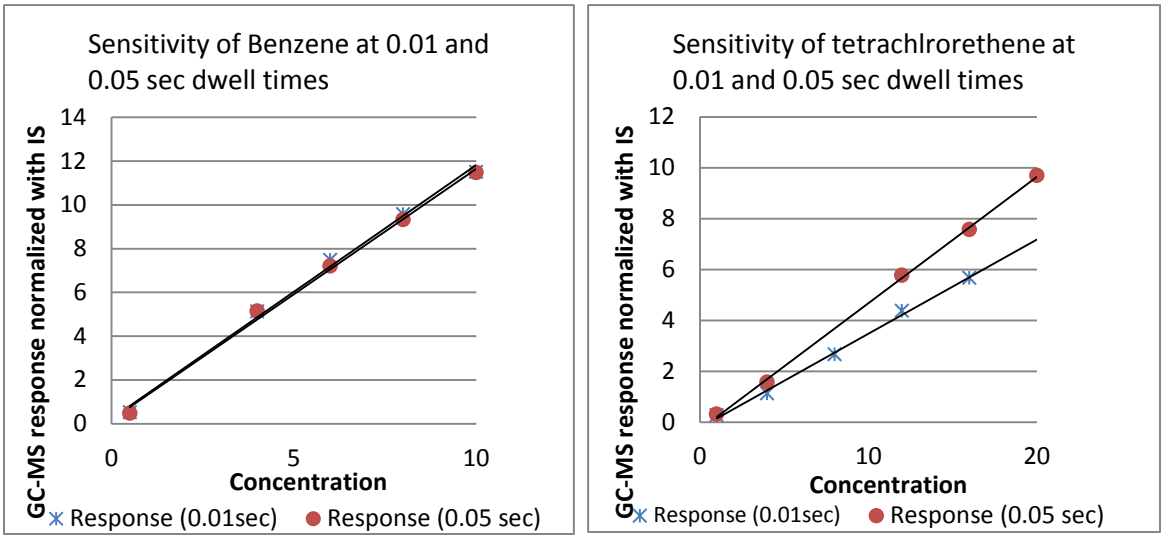

Figure 7. Sensitivity at different dwell times. The sensitivities can be seen as the slopes of line given in the linear regression equations for calibration curves at 0.01 and 0.05 sec dwell times for benzene (left side: slope of the line= 1.16 with dwell time 0.05 sec and 1.15 with0.01 sec dwell time) and tetrachloroethene (right side: slope of the line= 0.5 with 0.05 sec dwell time and 0.37 with 0.01 sec dwell time).

A multiplier voltage of 600 mV was unavoidable to maintain the necessary gain from the aged photomultiplier tube (PMT). GC-MS instruments are designed to have an optimum gain. With the aged multipliers it is necessary to apply increased voltage to maintain the gain. A 600 mV multiplier voltage for PMT is generally considered very high as the upper voltage limit of the PMT is 700mV. Higher multiplier voltages increase the noise, degrade the S/N and detection limits and decrease the signal stability. It may affect precision of the analysis to significant extent.

One quantifier and one qualifier ion was selected for each compound. The characteristic qualifier and quantifier ions used for each compound are given in Table 6. Compound identification was done with retention time and the quantifier, qualifier ratios.

3.1.4 Calibration standards and sample preparation

Calibration standards preparation strategy used by F. Barani *et al* (Barani, Dell'Amico et al. 2006) was modified. Initially a working standard solution in water was prepared as done by F. Barani *et al* (Barani, Dell'Amico et al. 2006). Calibration standards were prepared in triplicates in head space vials by the direct dilution of this aqueous working standard solution from the same vial. The results suggested that the stability of this aqueous standard solution is not enough to

prepare one set of calibration standards in triplicates in normal time required for standard preparation. Each time when a volume was withdrawn from the working standard solution vial, the increased headspace resulted in the decreased amount of VOCs in the aqueous phase. The succeeding standards result in lower signals than expected. A sharp decline in case of tetrachloroethene for 5^{th} and 6^{th} standard level concentrations might be explained with its very low water solubility and higher tendency to move to head space as compared with the other compounds. The attempted calibration curves are shown in Figure 8. The working standard solution was prepared in methanol instead of water to solve the problem.

The preparation of calibration standards in different flasks by the dilution of working standard solution as given in the USEPA methods 8260 B is more time taking and reagent consuming. Direct dilution of working standard into head space vials saves significant time of the operator used for the preparation of calibration standards and reagents as well.

Acid addition to lower pH of the calibration standard solutions is essential in USEPA method for VOCs analysis in water samples, as the samples for VOCs analysis are preserved at or below pH 2 to avoid biological transformations of certain VOCs.

No significant differences were observed in the HS-Trap extraction efficiency under the experimental conditions when the calibration standards were prepared in the dilution water with or without acid addition to obtain pH <2. Figure 9 shows the GC-MS responses for the 4 VOCs in the calibration standards prepared with and without pH adjustment. The data suggested that acid addition might not be necessary in the calibration standards. However more data is recommended for any conclusive remarks.

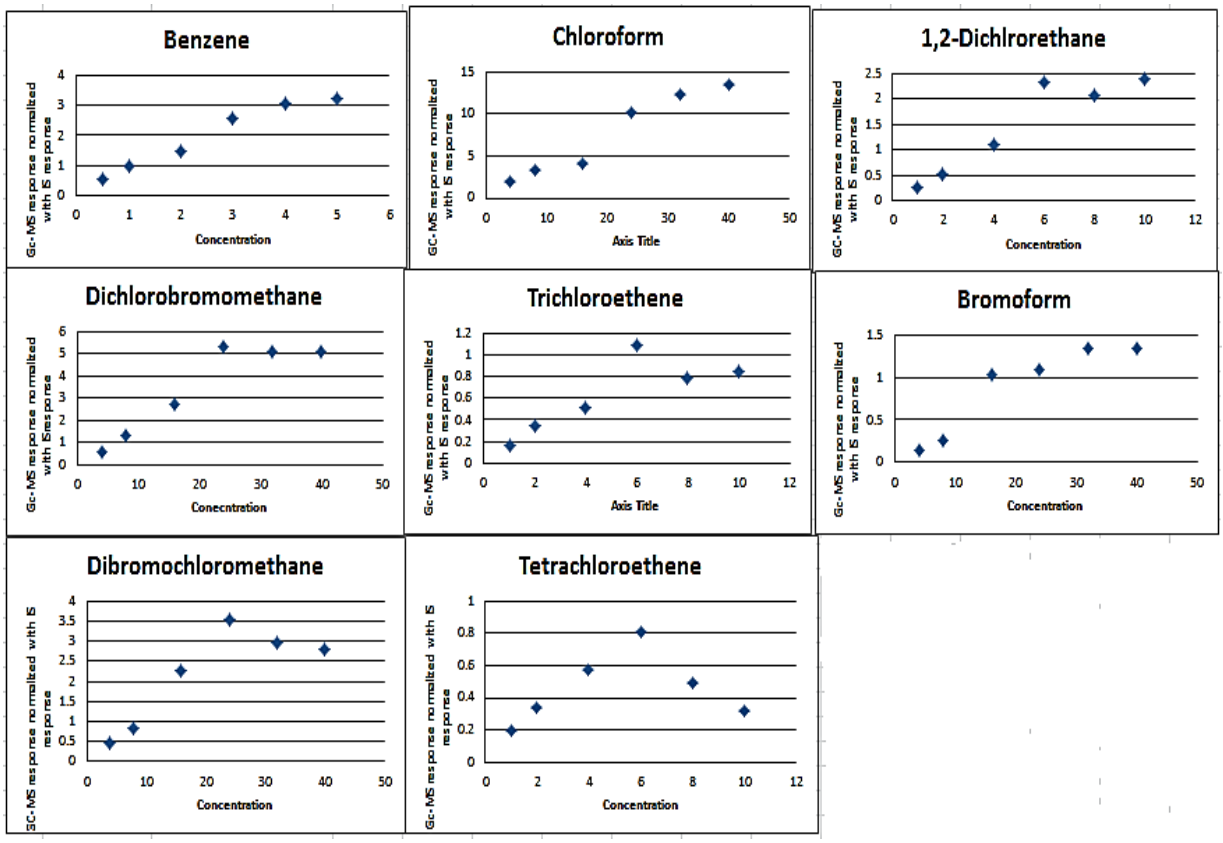

Figure 8 Calibration curves obtained when working standard solution was prepared in water. The parallel points for all the compounds were obtained by analyzing same set of vials. The x-axis is the concentration and y-axis shows the GC-MS responses normalized with internal standard response.

F. Barani *et al* reported conversion of tetrachloroethene to trichlroethene in water solutions at pH 9 and demonstrated that such phenomenon may appear at neutral pH as well in the HS vials during thermostatation at 70 °C or above (Barani, Dell'Amico et al. 2006). The dilution water has pH range between 4.8 and 5.7 and no such effect was observed when standards were prepared in the dilution water. However when sodium carbonate was introduced at 500 mg/L carbonate concentration into duplicates of a standard solution prepared in the dilution water, during the the HS-Trap-GC-MS analysis, tetrachloroethene peak intensity decreased only in one of the duplicates to significant extent but no significant increase in the peak area of trichloroethene was observed for the same vial. Figure 10 shows the comparison as a bar chart along with for the parallel duplicates without any carbonate addition but pH<2. More data is required to make any conclusions.

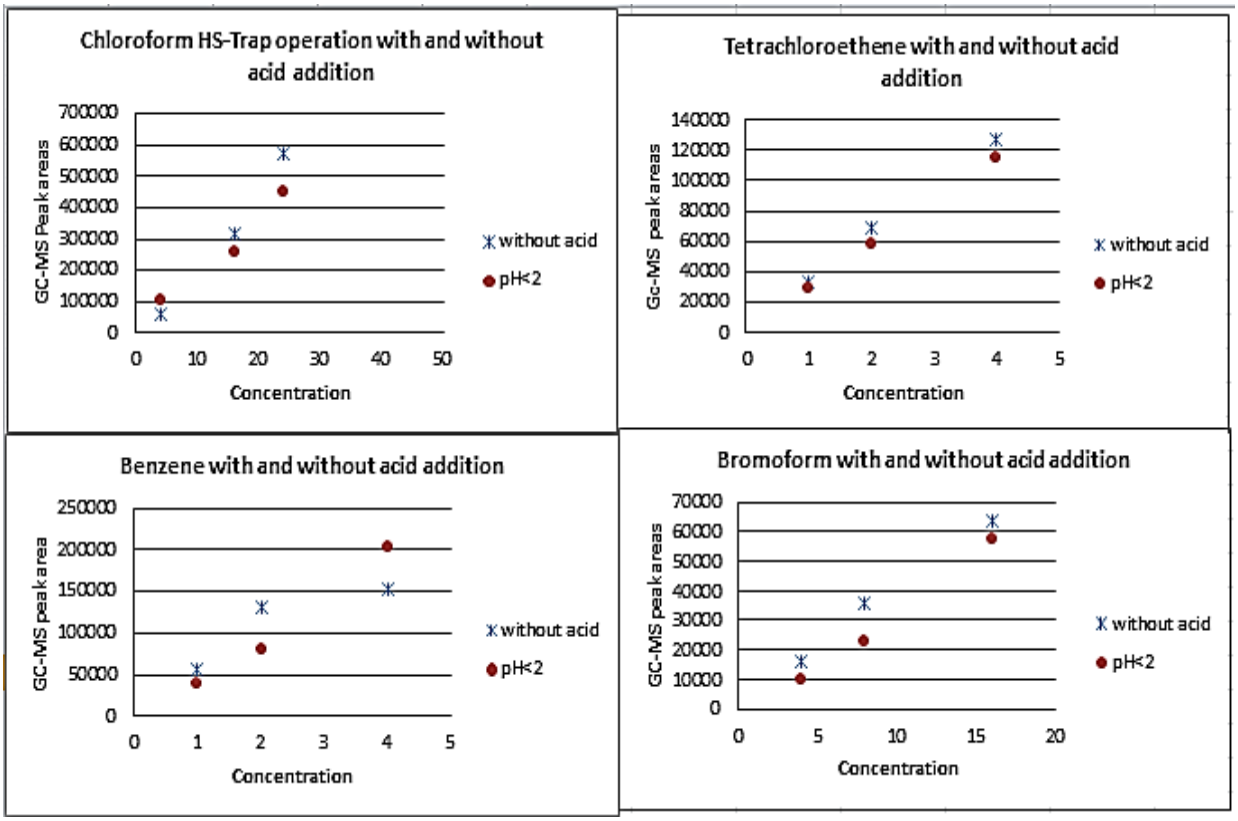

Figure 9. HS-Trap extraction efficiency with standards at pH<2 and without any acid addition: The red dots show GC-MS peak areas for standards with pH<2 as a result of acid addition while the blue marks show GC-MS responses for calibration standards without any acid addition.

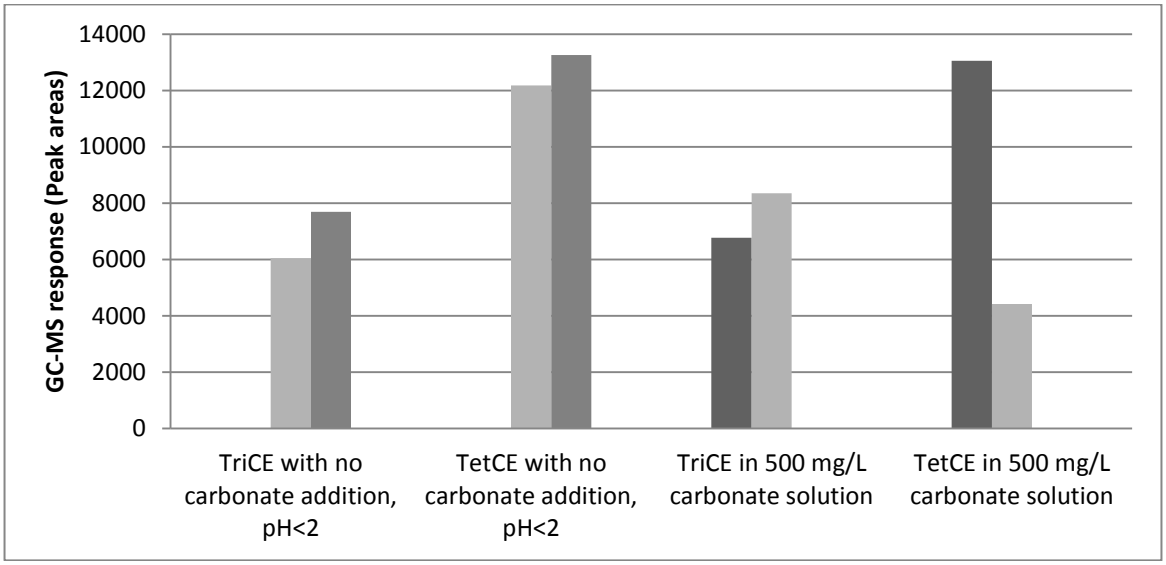

Figure 10 Effect of carbonate addition on the HS-Trap-GC-MS analysis of TetCE. The bar chart shows the results of HS-Trap-GC-MS analysis of 2 sets of vials in duplicates: one with acid addition to obtain pH< 2, while the other set of duplicates with added carbonates to increase the pH.

3.2 Method validation Results

3.2.1 Calibration

The coefficients "a" and "b" of the calibration function along with their uncertainties at 95 % confidence interval (CI) are listed in Table 8. A 6 point calibration curve for benzene along with its confidence band at 95 % confidence interval (CI) is shown in Figure 11. Calibration data sheets, calibration curves along with their confidence bands for all the compounds can be found in Annex A.

Table 8 Coefficients of the calibration functions

Compound	Regression Equation for linear functions	Coefficients of the calibration functions @ 95% CI, n=6	
		$b \pm t.S_b$	$a \pm t.S_a$
Benzene	$y=(b \pm t.S_b)x+ (a \pm t.S_{a)}$	1.22±0.04	0.02±0.12
TriCE		0.26±0.01	-0.04±0.06
TetCE		0.53±0.02	-0.1±0.1
1,2-DCE		0.14±0.01	0.01±0.04
$CHCl_3$		0.31±0.01	-0.02±0.15
$CHCl_2Br$		0.17±0.01	-0.1±0.2
$CHBr_2Cl$		0.22±0.01	-0.1±0.3
$CHBr_3$		0.069±0.004	-0.02±0.09

*y=predicted information value, x= Concentration, b=slope of the calibration curve, S_b =standard deviation of slope, a= intercept, S_a =standard deviation of intercept, t= student t distribution, n=6

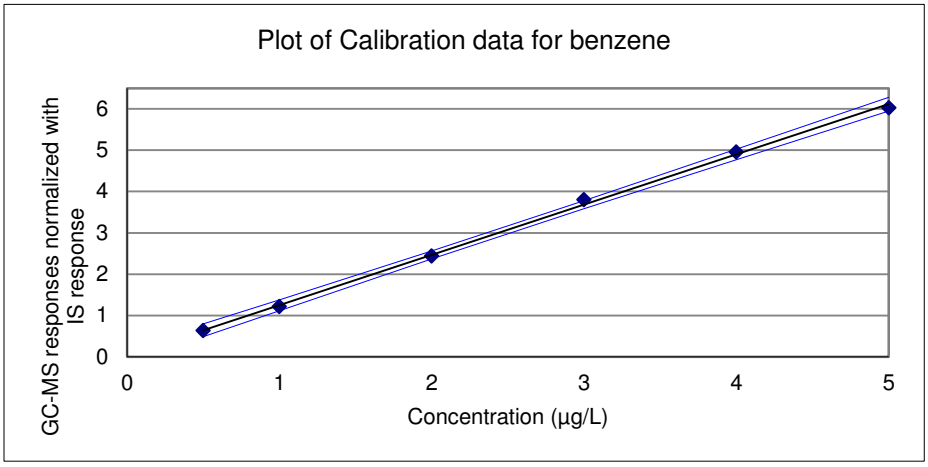

Figure 11 The regression line for benzene with its confidence band. The central line is the least square fit of the regression. The upper (y+CI) and lower (y-CI) lines represent upper and lower confidence intervals (CIs) respectively at 95% confidence level.(y=(1.22±0.04)x+0.02±0.12))

3.2.2 Linearity

The calibration data showed linearity when plotted with the calculated regression lines. When the residuals were plotted random pattern was observed in the plot of residuals. Only dichlorobromomethane failed to pass the statistical test for linearity. The coefficient of determination (R^2) was found greater than 0.995 in all the cases. The R^2 values found for all the compounds are listed in Table 16. Plots of calibration data with the calculated regression line, for all the compounds, demonstrated linearity. Such a plot for benzene is shown in Figure 12 along with a plot of residuals. Graphical representation of the calibration data for all other compounds can be found in the Annexure A.

Table 9 lists the calculated PG values for all the compounds along with the tabulated PG values. The calculated PG for dichlorobromomethane was found 27.77 significantly higher than the tabulated PG value.

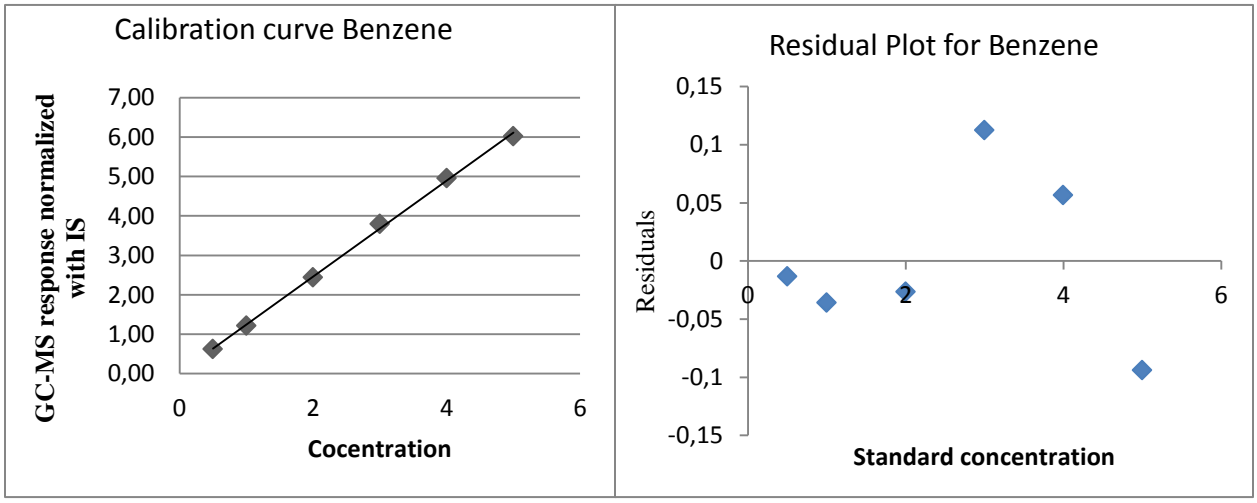

Figure 12 Plot of calibration data along with regression line left side) and plot of residuals (right side) for benzene. Linear calibration function for benzene: y= (1.22±0.04)x+(0.02±0.12) with R^2=0.9988

Table 9 Results of statistical test for linearity

Compound	PG Values (n=6) @ 95% confidence level	
	Calculated	Tabulated
Benzene	2.9	21.2
Trichloroethene	0.4	
Tetrachloroethene	0.1	
1,2-DCE	2.4	
Dichlorobromomethane	27.8	
Dibromochloromethane	0.01	
Chloroform	0.0	
Bromoform	0.6	

3.2.3 LODs and LOQs

The method detection limits calculated as defined the EU drinking water regulation were well below than those required by the EU regulation and ranged between 0.1 and 0.5 µg/L. The EU directive requires that a measurement method should have detection limits 10 % of the regulatory limit for all the listed compounds except for benzene for which it should 25% of the regulatory limit. For individual THMs LOD should be 10 % of 25 % of the regulatory limit and for of trichloroethene and tetrachloroethene it should be 10 % of 50 % the regulatory limit. The regulatory limits for each parameter are given in the objectives of the study.

The method detection limits, found using standard deviation at intercept as given in equation 2 (1.3.2.1), ranged from 0.1 to 1.6 and were higher than the former estimated detection limits but still met the requirements of the EU regulation. The LODs obtained using standard deviation of residuals as given in equation 1 (1.3.2.1) ranged from 0.2 to 2.8 µg/L. The LODs for bromoform, dibromochloromethane and 1,2-dichloroethane calculated by the 3rd approach do not meet the requirements of the EU regulation. For the objectives of the study, the EU regulation approach is required. The other two approaches are affected by the variances in higher standards resulting in high residual standard deviations as residual standard deviation is part of both equations. This bias more strongly appears in equation 1 as compared with equation 2.

The LOQs were calculated as 3 times of the detection limits estimated using the EU drinking water definition for LODs. The obtained LOQs ranged from 0.3 to 1.5 µg/L. These LOQs, for all the compounds, were lower than the lowest standard concentrations in the calibration range. The LODs and LOQs found for all the compounds are listed in Table 10.

Table 10 The HS-Trap-GC-MS method detection and quantification limits estimated in bottled drinking water at standard concentrations: THMs= 4 µg/L , benzene=0.5 µg/L and 1,2-dichloroethane, TriCE and TetCE at 1 µg/L standard solution

Compound	Method LODs Required by The EU directive (µg/L)	The HS-Trap-GC-MS method LODs (µg/L)			LOQs
		The EU directive approach (n=6)	Equation 1	Equation 2	3 times the LODs calculated as defined by the EU directive
CHCl3	2.5	0.2	1	0.6	0.6
CHBr3	2.5	0.5	2.8	1.6	1.5
CHBr2Cl	2.5	0.2	2.6	1.5	0.6
CHCl2Br	2.5	0.2	2.5	1.4	0.6
1,2-Dichlrorethane	0.3	0.2	0.6	0.3	0.6
TriCE	0.5	0.3	0.5	0.3	0.9
TetCE	0.5	0.4	0.5	0.3	1.2
Benzene	0.25	0.1	0.2	0.1	0.3

The LODs estimated by the all 3 approaches are generally higher than those reported in the relevant literature (Barani, Dell'Amico et al. 2006; Meng Yuan 2009). Much lower LODs are possible to obtain using the method but precision remains the main hurdle. Figure 13 shows S/N obtained in SIM mode for trichlroethene, tetrachloroethene, benzene and bromoform when the lowest standard concentrations in the calibration range were analyzed using the HS-Trap-GC-MS method. For bromoform and 1,2-dichloroethane S/N ratios between 100-200 were obtained. The 1,2-dichlooroethane is not shown in the chromatogram. For all other compounds S/N ratios greater than 700 were obtained at the lowest standard concentration level in the calibration range.

Lower precision of linearity as indicated by higher residual standard deviations resulted in the greater LODs obtained. Residual standard deviation is at the core of the Equation 1 and Equation 2 for the estimation of LODs. For all the VOCs no peaks were observed when dilution water blanks were analyzed. An overlaid SIM (selected ion monitoring) chromatogram is given in the annexure B.

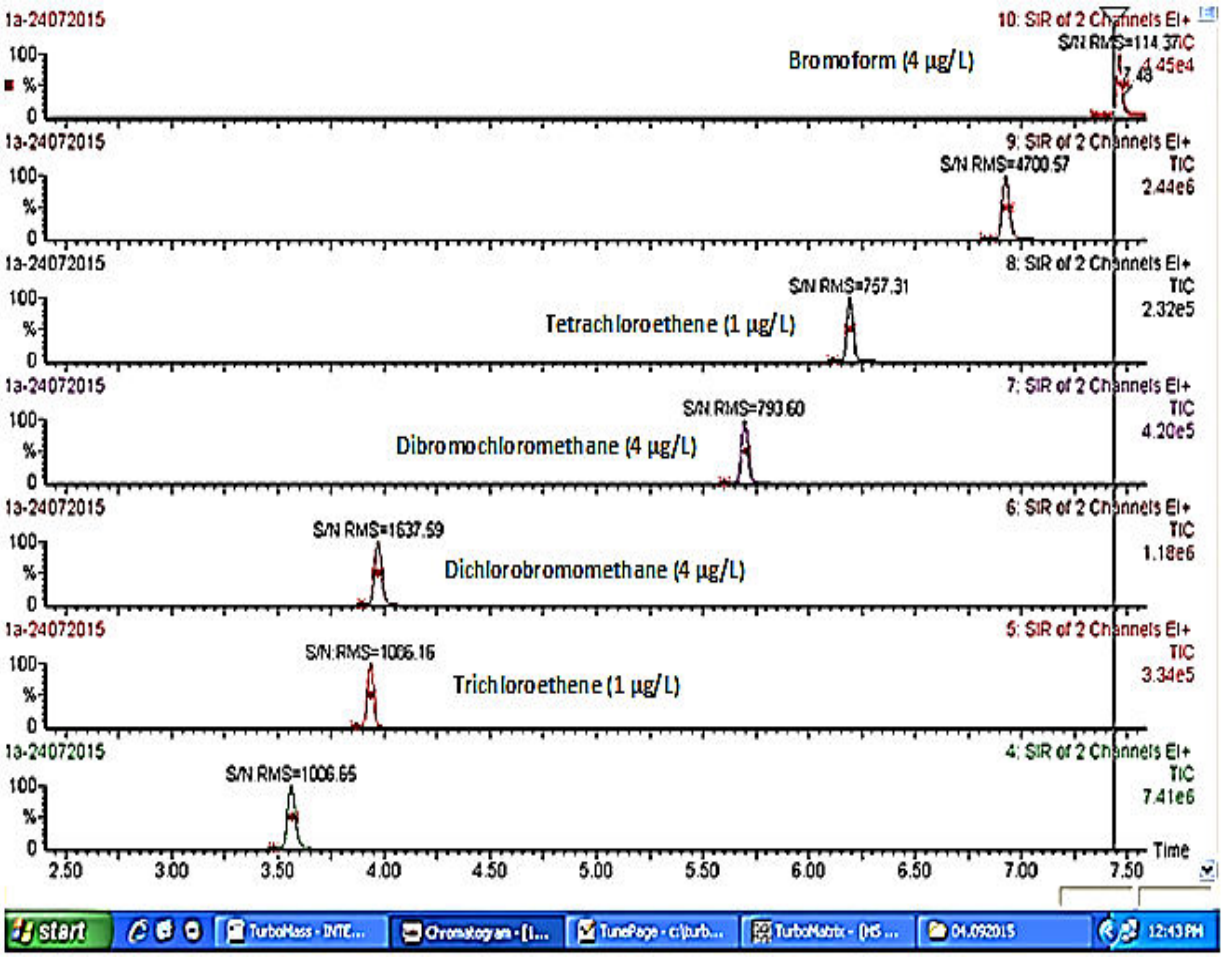

Figure 13 SIM chromatograms with S/N ratios of the VOCs at the lowest standard concentrations in the working range. The S/N ratios shown were calculated using the instrument software which uses maximum height of the chromatographic peak for compound as signal and the root mean square of the baseline in the selected window as noise.(SIM: Selected ion monitoring, SIR= Selected ion recording)

3.2.4 Sensitivity

Sensitivity values calculated as average of the slope of the calibration line found on different days ranged from 1.13 to 0.0692 with the highest value for benzene while the lowest value for bromoform. Tetrachloroethene was found with the 2nd highest sensitivity value as 0.65. The average sensitivities for all the compounds are listed in Table 11 along with %RSDs. The % RSDs found for all the compounds were higher than 10%, except for bromoform and dichlorobromomethane with % RSDs equal to 8 and 10 respectively. The highest % RSD was found for 1,2-dichloroethane with a value of 26%.

Table 11 Sensitivity of the method for the VOCs

Compound	Average sensitivity (n=5)	%RSD
Benzene	1.13	13
Trichloroethene	0.24	20
Tetrachloroethene	0.65	17
1,2-Dichloroethene	0.15 (n=4)	26
Chloroform	0.30	17
Dichlorobromomethane	0.17	10
Dibromochloromethane	0.19	13
Bromoform	0.069 (n=4)	8

The coefficient of variation of the method ranges from 1.8 to 4.8 while being the lowest value for chloroform and the highest for bromoform. The coefficient of variation of the method for all the compounds is listed in Table 16.

3.2.5 Working range

The linear working could not be statistically established for benzene, 1,2-dichloroethane, trichloroethene and bromoform. The PG values obtained for the test for homogeneity of variances were very high for these compounds. The PG values ranged from 1.4 to 130 for all the VOCs: the lowest for tetrachloroethene and the highest for 1,2-Dichloroethane. Table 12 lists the test PG values along with the tabulated PG values at 99 % confidence level and "n" number of measured values on the lowest and the highest concentration levels in the selected working range.

Table 12 Test PG values for homogeneity of variances

Compound	PG Values @ 99% Confidence level		n
	Obtained	Tabulated	
Benzene	54.56	10.97	6
TriCE	100	10.97	6
TetCE	1.40	15.98	5
1,2-Dichloroethene	130	15.98	5
$CHCl_3$ (n=3)	16.15	99.00	3
$CHCl_2Br$	13.52	15.98	5
$CHBr_2Cl$	7.28	15.98	5
$CHBr_3$	122	15.98	5

3.2.6 Precision

The % RSDs for repeatability ranged 2.8 to 18 % while the lowest one being for benzene and the highest one for chloroform. The repeatability limits ranged from 0.2 (benzene) to 11.4 (chloroform). The intermediate precision for all the compounds was well below 20 % RSD except for chloroform with a much higher value of 29.5% (Table 13).

Table 13 Repeatability and intermediate precision

Standard	Conc. (µg/L)	Repeatability (n=10)		Intermediate precision (n=5)
		%RSD	r	% RSD
$CHCl_3$	16	18.0	11.4	29.5 (n=4)
$CHBr_3$		NA	NA	9.5 (n=3)
$CHBr_2Cl$		14.5	6.7	10.8 (n=4)
$CHCl_2Br$		15.9	9.1	13.9 (n=5)
1,2-Dichlrorethane	4	15.1	1.8	8.6 (n=4)
TriCE		5.4	0.7	5.1 (n=5)
TetCE		5.2	0.6	3.7 (n=5)
Benzene	2	2.8	0.2	6.3 (n=6)

Table 13 lists the repeatability limits, % RSDs for repeatability and intermediate precision for each compound along with the concentration level used for each compound. Numbers of determinations (n) under repeatability and intermediate precision conditions are also provided.

The EU regulation requires a measuring method to produce results with a minimum precision of 25 % of the parametric value. The obtained precision meets the requirements of the EU regulation.

3.2.7 Accuracy

Accuracy estimated for all the compounds was found good in the working range under consideration. The z-score values found were lower than 2 for all the compounds. The lowest value of z-score was found 0.16 for 1,2-dichloroethane and the highest value was 1.26 for tetrachloroethene. Table 16 lists the z-score values obtained for all the compounds.

3.2.8 Recovery

Recoveries for all the compounds were found between 80% - 120% except for chloroform in treated water (122% in treated water) dibromochloromethane (40 % in surface water and 125% in

treated water) and dichlorobromomethane (70% in surface water). Care must be taken while interpreting these results as these recoveries may not represent matrix effects. These recovery values were estimated on a single day on duplicate samples. Minimum 5 determinations are recommended by the USEPA method under intermediate precision conditions. Table 14 lists the determined recoveries in the dilution water, surface water, treated water and waste water samples. Since just 2 measurements were made for each recovery, standards deviations of recoveries are not given in the table.

Table 14 % Recoveries at ppb Benzene, 4 ppb 1,2-DCE, TriCE and TetraCE, 16 ppb THMs

Compound	Spiked level	% Recovery (n=2 in repeatability conditions)			
		Method development	Surface water	Treated water	Waste water
$CHCl_3$	16	-	114	122	90
$CHBr_3$		-	-	-	-
$CHBr_2Cl$		80	40	125	107
$CHCl_2Br$		99	70	110	92
1,2-Dichlrorethane	4	-	-	-	-
TriCE		103	99	117	85
TetCE		100	105	117	97
Benzene	2	100	103	102	95

3.2.9 Real Water Analysis

None of the compounds was detected in surface water samples. Only chloroform was found in waste water samples at a concentration of 18 (µg/L). Treated water samples were found with THMs traces. It was not possible to obtain a calibration curve and quantify bromoform concentrations because of the bromoform peak shape problem which appeared in the last experiment and in which real water analysis was carried out. The problem was suspected to be associated with MSD because of the low sensitivity of bromoform and deteriorated S/N ratios. Because of the PMT conditions a greater decrease in S/N ratios for all the compounds was observed in that experiment. When the PMT voltage was raised to 650 mV compared with 600 mV no such distortion was observed and when the outlet split option was not availed, no such distortion was seen. Because of the deadline in the time frame no further experiment was possible. Table 15 contains averages of duplicate results for real water samples analysis.

The results of the real water samples were, as were expected. Generally surface water samples do not contain VOCs because of their low solubility in water and their escaping tendency. However THMs are produced during the treatment process of the surface water when chlorination is one of the steps in the water treatment process due to the chlorination of the dissolved organic material present in surface waters.

Table 15 Results for real water samples analysis

Compound	Surface water (µg/L)	Treated water (µg/L)	Waste water (µg/L)
$CHCl_3$	ND	4	18
$CHBr_3$	-	-	-
$CHBr_2Cl$	ND	20	ND
$CHCl_2Br$	ND	12	BDL
1,2-Dichlrorethane	ND	ND	ND
TriCE	ND	ND	ND
TetCE	ND	ND	ND
Benzene	ND	ND	ND

*ND=not detected, BDL=below detection limit

3.3 Interferences

Following interferences were found effecting efficiency of the analytical method:

3.3.1 Leak Problems

Several screw capped vials were tested for vial seal integrity: after adding few drops of methanol and screwing it tightly, the vial was immersed in hot water with temperature around 80 °C. The vial leak was found a frequent problem. Minor leaks were detected more frequently even after the vials were screwed carefully and as tight as was possible. The chance of the leak even increases when the vial is further pressurized at 80 °C during thermostatation. It is not possible in normal work flow to check each screw capped vial for vial seal integrity before analysis. This might lead to significantly higher variance at the highest standard concentrations level compared with the lowest ones in the calibration range and resulting in the failure of the test for homogeneity of variances for those compounds which are farther from their respective internal standards. The compounds with high volatility like chloroform and 1,2-dichloroethane or those farther from internal standard like dichlororbromomethane and dibromochloromethane suffer

with internal standard bias due to the leak problems. This also often causes internal standard response to deviate even more than 30 % within the same run. Major vial leaks are easy to detect because much lower internal standard responses obtained compared normal IS responses.

3.3.2 Aged photomultiplier tube in the MSD

The S/N ratios degraded significantly during the course of the work. The most effected compounds from this problem were those with low sensitivities: bromoform and 1,2-dichloroethane.

3.3.3 Summary of Method Performance characteristics

Table 16 Summary of the HS-Trap-GC-MS method performance characteristics

Compound	LD (µg/L) estimated	LQ (µg/L) Obtained	Coefficient of determination R^2	%RSD Repeatability	Repeatability limit (r) (n=10)	CV intermediate precision n=5	Sensitivity as slope (n=5)	% Recovery Treated water	Coefficient of variation of the method	Accuracy Z-score
$CHCl_3$	0.2	0.6	0.995	18.0	11.4	29.5 (n=4)	0.22 (n=3)	122	1.8	-0.77
1,2-dichloroethane	0.2	0.6	0.9973	15.1	1.8	8.6 (n=4)	0.15(n=4)	-	4.2	0.16
Benzene	0.1	0.3	0.9988	2.8	0.2	6.3 (n=6)	1.14	101	2.8	-
TriCE	0.3	0.9	0.9994	5.4	0.7	5.1	0.25	117	3.1	-0.23
$CHCl_2Br$	0.2	0.6	0.9983	15.9	9.1	13.9	0.17	111	4.3	-0.51
$CHBr_2Cl$	0.2	0.6	0.9961	14.5	6.7	10.8 (n=4)	0.19 (n=4)	125	4.5	-1.08
TetCE	0.4	1.2	0.9992	5.2	0.6	3.7	0.65	117	3.5	1.26
$CHBr_3$	0.5	1.5	0.9966	-	-	9.5 (n=3)	0.069 (n=4)	-	4.8	-0.87

Note:

1. "n" in each parameter is given in the top row. However wherever it differs within the column, it is specified in that particular case.
2. Recoveries, repeatability and intermediate precisions were determined at THMs= 16 µg/L, benzene = 2 µg/L and 1,2-dichloroethane, trichloroethene and tetrachloroethene= 4 µg/L.
3. LODs and LOQs were determined at standard concentration THMs= 4 µg/L, benzene = 0.5 µg/L and 1,2-dichloroethane, trichloroethene and tetrachloroethene = 1 µg/L. LODs and LOQs given in the table are those obtained by the EU water directive approach..

3.4 Conclusions

A head space-Trap GC/MS method was developed with 40 minute analysis time per sample for the analysis of 8 high priority volatiles for low-level concentrations in water using Perkin Elmer Turbomatrix Head space-Trap 40, and Clarus 560 MSD in selected ion monitoring mode. The developed method has the LODs well below than those required by the EU directive, good accuracy, potential to demonstrate linearity by all means and precision to meet the requirements of the EU drinking water regulation. However, following problems were identified during method validation study:

1. Linear working range was not statistically proven for benzene, trichlroethene, 1,2-dichloroehane and bromoform
2. Dichlorobromomethane did not pass the statistical test for linearity
3. The % RSDs for sensitivity are significantly higher than 10 % for 6 out of 8 compounds
4. The recoveries of dibromochloromethane (40 %), dichlorobromomethane (70 %) in surface water samples are significantly lower than the required criteria.

The above identified problems were found to be associated with the head space vial seals and an aged PMT. These two factors predominantly resulted in lower precision of linearity and also lead to higher LODs than required when calculated using linearity approach, significantly higher variances in higher standards lead to failure of the test for homogeneity of the variances. The lower recoveries of dibromochloromethane and dichlorobromomethane need further data for any conclusive remarks. The developed method has the potential to meet all the regulatory requirements for simultaneously determination of the 8 VOCs if the identified issues are resolved.

3.5 Recommendations for further work

1. Crimp seal vials recommended by the HS-Trap autosampler's manufacturer should be used. Hand crimp should be adjusted and vial seal should be checked for seal integrity. Once the crimp is adjusted for proper seal, only then it should be used in the routine analysis
2. Head space-Trap leak test must be performed before the analysis is started
3. Trap outlet split function should be unchecked for the calibration range
4. Lower 'final column hold up" times and fewer "cycle numbers" should be attempted as it can save significant time of analysis leading overall cost reduction and increasing laboratory productivity. A final column hold up time 2 minutes and a cycle number 3 is suggested. However the impacts of these should be validated.
5. An MS ion source temperature 230 °C instead of 200 C° will lead to significant increase in ionization efficiency of the ion source for the VOCs. In the head space solid phase microextraction (HS-SPME)-GC-MS method development and optimization it a 230 C° ion source temperature was found resulting in increased ionization efficiency of the MS ion source (Figure 17). A 200 °C ion source temperature in this study was opted to maximize work flow and avoid frequent tuning of the instrument as the instrument was being used for other applications as well. However the S/N ratios should be compared under the instrumental conditions.
6. Recoveries and precision should be determined at the lower concentration nd higher concentration levels as well. Due to limited availability of the instrument for this study, recoveries and precision was determined at only one concentration level for each compound.

4 Head Space-Solid phase microextraction (HS-SPME)-GC-MS

4.1 Experimental

4.1.1 Reagents and Materials

The individual standards trichloroethene: 5000 µg/mL, tetrachloroethene: 200 µg/mL, 1,1,1-trichloroethene:200 µg/mL, dichloromethane:200 µg/mL, carbon tetrachloride:200 µg/mL, 1, 1-dichloroehthene:1000 µg/mL and chloroform:200 µg/mL were purchased from sigma Aldrich. Pesticide quality methanol (MeOH) was obtained from VWR UK. Suprapure hydrochloric acid (30%) was supplied by Merck (Darmstadt, Germany).

4.1.2 Apparatus and Instruments

The analytical instrument used was Agilent 6890 GC with split/splitless inlet, directly coupled with Agilent 5973 MS operated in electron ionization mode. Zebron column 30 m × 0.25-mm i.d and 0.5-µm film thickness was used for gas chromatographic separations. Clear glass 20 ml HS vials with 18 mm magnetic crimp caps were obtained from VWR. A Supelco SPME holder and 75 µm Carboxen/polydimethylsiloxane (Car/PDMS) fiber assembly were purchased from Sigma Aldrich. Gas tight micro syringes of volumes 10, 250 and 100 µL were purchased Hamilton USA. A refrigerator at 4 °C (±2) °C was used for storing standards. A clamp stand was used to hold the SPME holder while the fiber was exposed to the head space of the sample vial.

4.1.3 Solutions

A 100 µg/L mixed standard solution was prepared fresh each day in deionized water, by the dilution of primary standard solutions. A 50 µL of each of the primary standard solutions except trichloroethene for which 2 µL and 10 µL for 1,1-dichloroethene were transferred to 100 mL flask with about 98 mL of deionized water. The volume was made up to the mark. A 5 µg/L solution aqueous solution was prepared by diluting the 100 µg/L solution.

4.1.4 HS-SPME sample preparation

A 10 mL of mixed standard aqueous solution at specified concentration of all the VOCs was transferred to a 20 mL SPME vial. The vials were crimp capped immediately. The vial septum was pierced with the holder needle with the SPME fiber inside. The fiber was then exposed into the head space of the SPME vial above the sample for a specified time. The height of the clamp on the stand holding the SPME holder was adjusted and kept same to expose the SPME fiber in the middle of the head space in each SPME vial. After the HS-SPME step was complete, the loaded fiber was immediately transferred to the inlet of the GC ready for chromatographic run. The SPME fiber was desorbed at a fixed depth in the GC inlet, estimated to be the center of the inlet and most heated area.

4.1.5 MS Data Acquisition

Initially the MS was operated in scan mode with m/z scan 30-250. Once the standards and solvent peaks were identified along with their retention times, MS was operated in SIM mode with 100 ms dwell times and 1.75 minutes solvent delay. MS quadruple temperature was 150 °C in both acquisition modes. Table 17 lists the characteristic ions for each compound recorded in SIM mode.

Table 17 MS data acquisition on 5973 MSD: SIM parameters

Compound	Ions
1,1-dichloroethene	61, 83, 96
Dichloromethane	49, 84, 86
Chloroform	47, 83, 85
Trichloroethene	95, 97, 130
1,1,1-trichloroethene	61, 97, 99
Tetrachloroethene	129, 164, 166
Carbon tetrachloride	117, 119, 121

4.2 Results (HS-SPME-GC-MS method development and optimization)

4.2.1 GC separations

Initially GC separations were optimized by injecting the vapor phase of a mixture of all the Cl-VHCs with a gas tight syringe. Various GC oven programs were attempted along with 220, 250 and 270 °C injector temperatures and 0.5, 0.8 and 1 and 1.2 mL/min column flows to obtain best chromatographic separations. One parameter was changed at a time. After an initial estimate of the optimum conditions, the loaded SPME fibers were desorbed in the GC-injector at 250 and 270 °C with 0.1, 0.3, 0.5 and 2 minutes fibers desorption times. Higher desorb temperatures were not attempted to prolong the SPME fiber life. A 0.3 minute desorption time for the fiber was found optimum at 270 °C. The optimum GC conditions found are listed in Table 18.

Table 18 Optimized GC conditions in the HS-SPME-GC-MS method

Injector type	Split/splitless
Injector conditions	270 °C, Splitless mode, SPME fiber desorption time=0.3 min
Carrier gas	Helium
Career gas flow	0.8 mL/min
GC Oven program	Equilibration time= 0.5 min Startup temperature= 40 °C 1st hold up = 2 min hold up at 40 °C 1st ramp= 20 °C/min rise till 130 °C 2nd hold up= 2 min hold up at 130 °C 2nd ramp= 50 °C/min till 220 °C Final hold = 2 min 220 °C

All the compounds were well separated in less than 5 minutes with good peak shapes under these conditions. A total run time of 12.3 minute was achieved. A chromatogram in SIM mode obtained under these conditions is given in Figure 14.

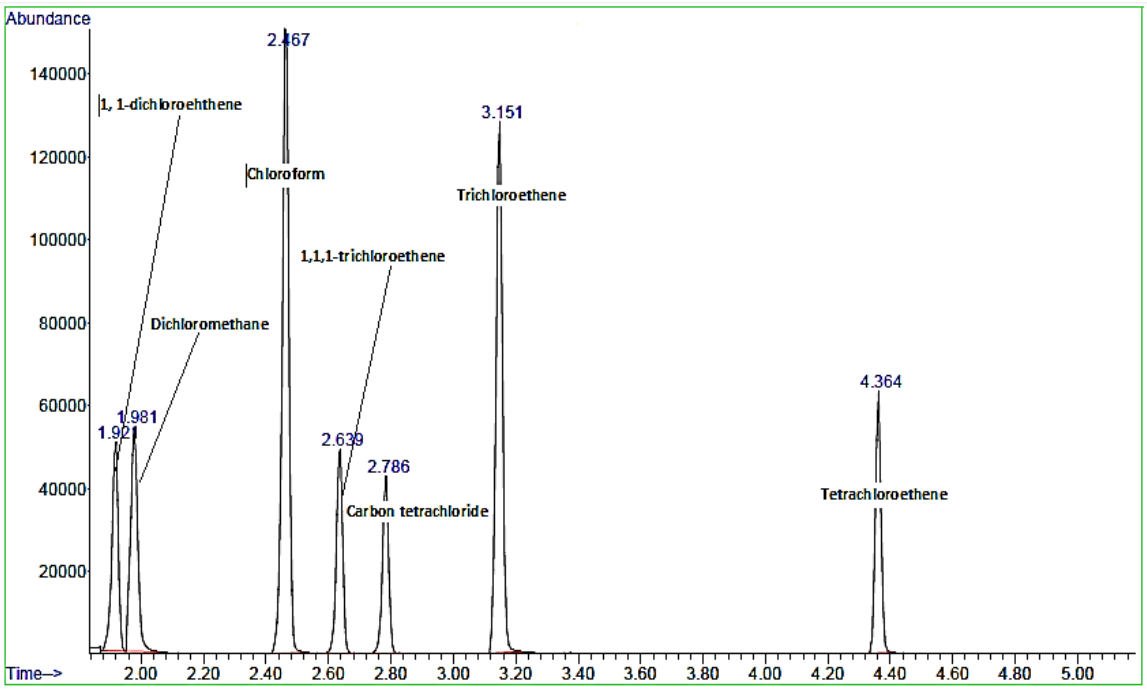

Figure 14 GC separations achieved during SPME-GC-MS method development Sample was introduced in the GC-System by desorption from the SPME fiber in the injector at 270 °C. The peaks are labeled with the retention times The x-axis= GC run time and y-axis=GC-MS responses obtained in SIM mode.

4.2.2 SPME Optimization

4.2.2.1 Optimization of fiber exposure time in the sample head space

A 5 minute SPME fiber exposure to the vapor phase of the sample in the head space of SPME vial was found optimum. No significant increase in the GC-MS responses was observed for increased exposure times. Figure 15 shows GC-MS responses plotted against fiber exposure time. It can also be seen in the figure that a small deviation in the exposure time will not result in significant deviations of the GC-MS response.

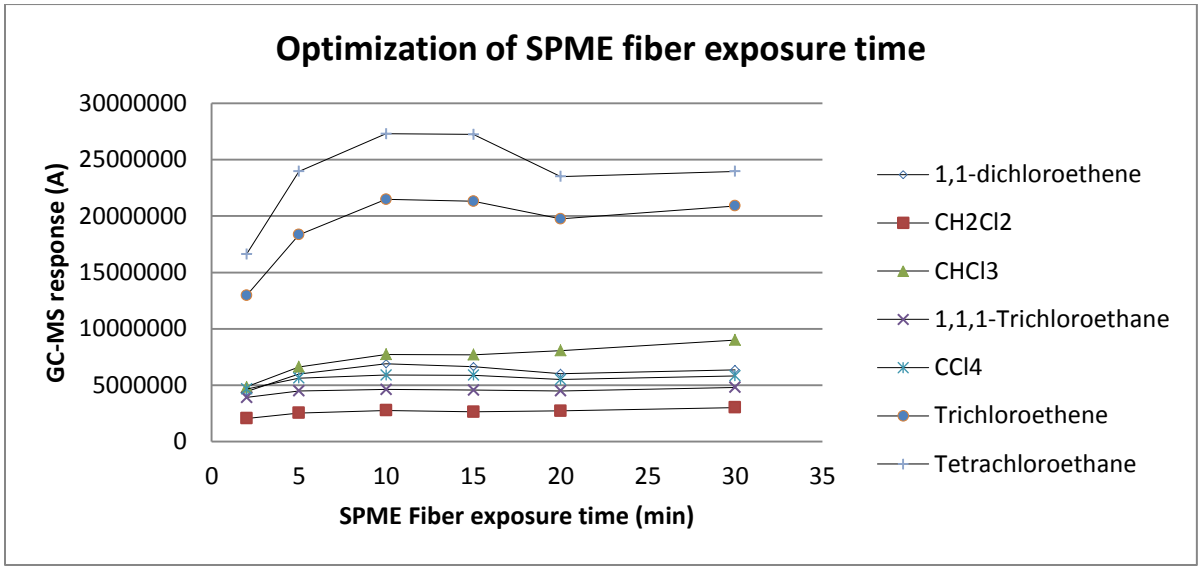

Figure 15 Optimization of fiber exposure time using Head space SPME for the extraction of Cl-VHCs. A 5 min exposure time is optimum for all the Cl-VHCs as further exposure results in no significant increase in the extraction of Cl-VHCs as observed by GC-MS peak areas.

4.2.3 pH and salt out effect on HS-SPME operation

Three parallel vials were prepared one with 3 grams NaCl, 2nd with 2 drops of HCl (1:1) to lower pH of the sample to <2 and in the 3rd vial both conditions were combined prior to vial sealing. GC-MS responses from all 3 vials were compared. Best extraction efficiency was achieved when salt and acid additions were combined as can be seen in the Figure 16: a comparison of GC-MS peak areas for three compounds under the three different conditions. The GC-MS peak areas shown in the figure are averages of duplicate results

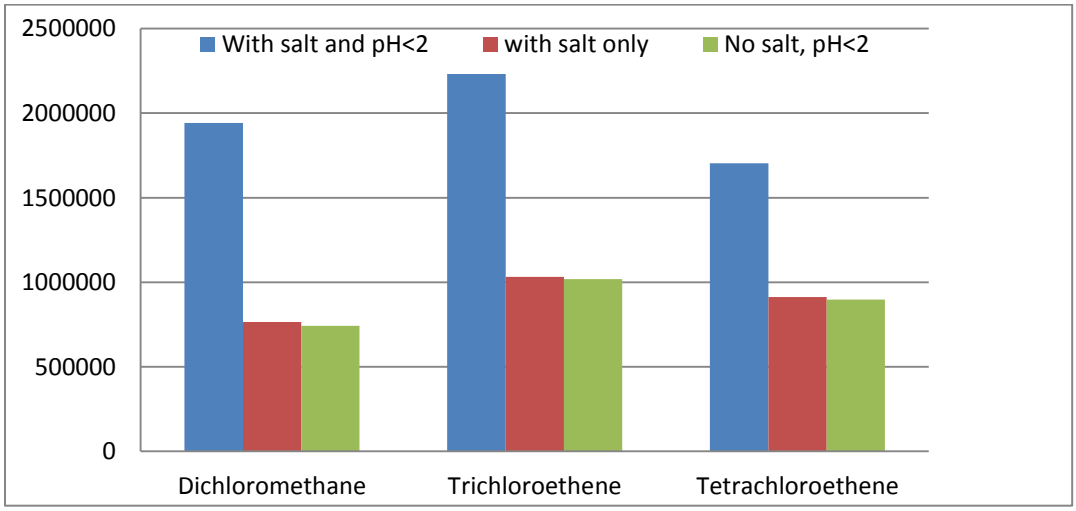

Figure 16 pH and salt out effect on the extraction efficiency of the HS-SPME operation

4.2.4 Optimization of ion source temperature

Ionization efficiency of the ion source for the given compounds was compared on 150, 200 and 230 °C source temperatures along with 150 and 200 °C transfer line temperatures. It was found that maximum ionization efficiency for the target compounds is achieved at 230 °C among the given temperatures. No significant differences were seen when the transfer line temperature was increased from 150 to 200 °C as can be seen in the bar chart shown in the Figure 17. A mixed standard solution at 5 µg/L concentration of each of the Cl-VHCs was used for optimization of the ion source.

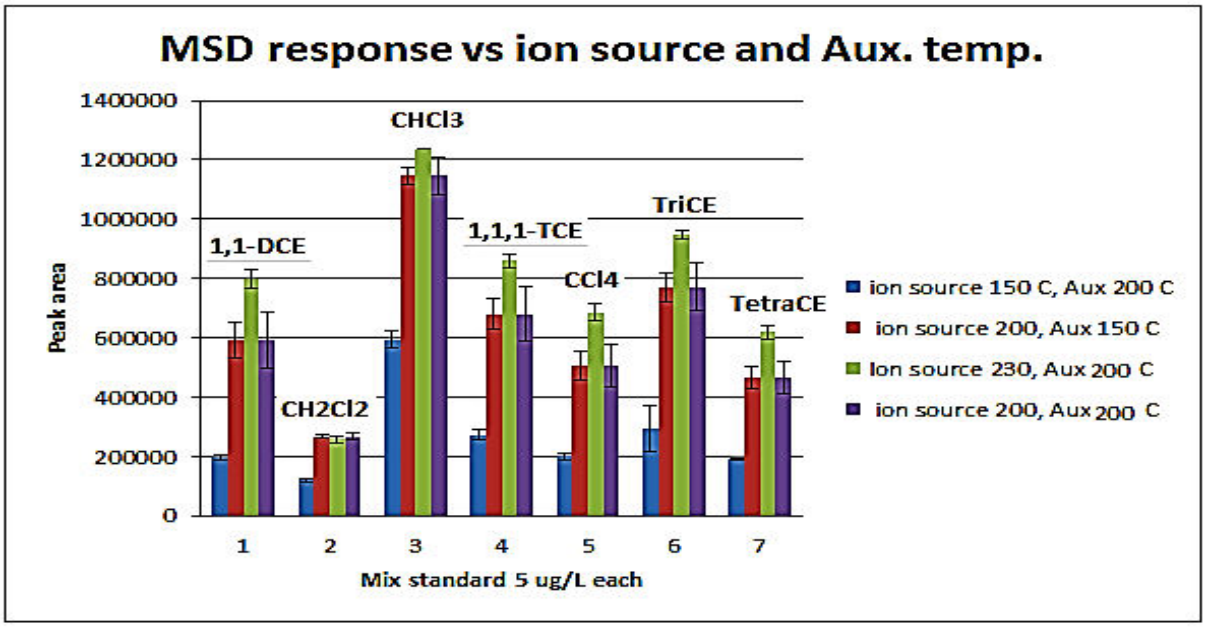

Figure 17 Optimization of ion source and MS transfer line temperatures. Each group of bars shows the ionization efficiency of ion source under 4 given conditions for the specified compound. The standards were run in duplicates under each condition. The error bars show the range of the duplicate results. It can be seen that maximum ionization efficiency is achieved at 230 °C compared with lower temperatures. No significant difference is seen when transfer line temperature is increased from 150 to 200 °C.

4.3 Interferences

VOCs were found in the lab environment. Nitrogen purging of SPME vials was compulsory just prior to sample preparation.

4.4 Conclusion

A HS-SPME-GC-MS method was developed using 75µm Car/PDMS SPME fiber, Agilent 6890/5973 GC-MS system for the analysis of 7 chlorinated volatile hydrocarbons. With 75 µm car/PDMS fused silica fiber can be obtained with sample pH <2 and 3 grams NaCl per 10 mL sample in 20 mL HS vial. With the SPME fiber a minute fiber exposure time to sample vapors in the sample HS is optimum for the HS-SPME of the given chlorinated volatile hydrocarbon compounds. An MS ion source temperature 230 °C should be used as lower temperatures result in significantly low sensitivity.

4.5 Further Work

- Ionization efficiency at 230 °C and 250 °C may be compared. Higher temperatures may not result in any further increase in ionization efficiency.
- A validation study needs to be conducted after the choice of an internal standard to establish performance characteristics of the developed method.

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6 Annexure

Annexure A

Data Table for LODs as defined by the EU directive

Compound	Concentration (µg/L)	Within batch GC-MS responses					
		R1	R2	R3	R4	R5	R6
Benzene	0.5	0.535	0.584	0.643	0.484	0.535	0.534
TriCE	1	0.22	0.133	0.122	0.196	0.139	0.213
TetCE	1	0.178	0.253	0.276	0.148	0.132	0.19
1,2-dichloroethane	1	0.245	0.322	0.308	0.241	0.3	0.257
Chloroform	4	1.698	2.288	2.112	1.425	1.97	1.516
Bromoform	4	0.122	0.226	0.111	0.085	0.158	-
Dichlorobromomethane	4	0.75	0.588	0.53	0.642	0.516	0.661
Dibromochloromethane	4	0.551	0.472	0.538	0.409	0.339	0.48

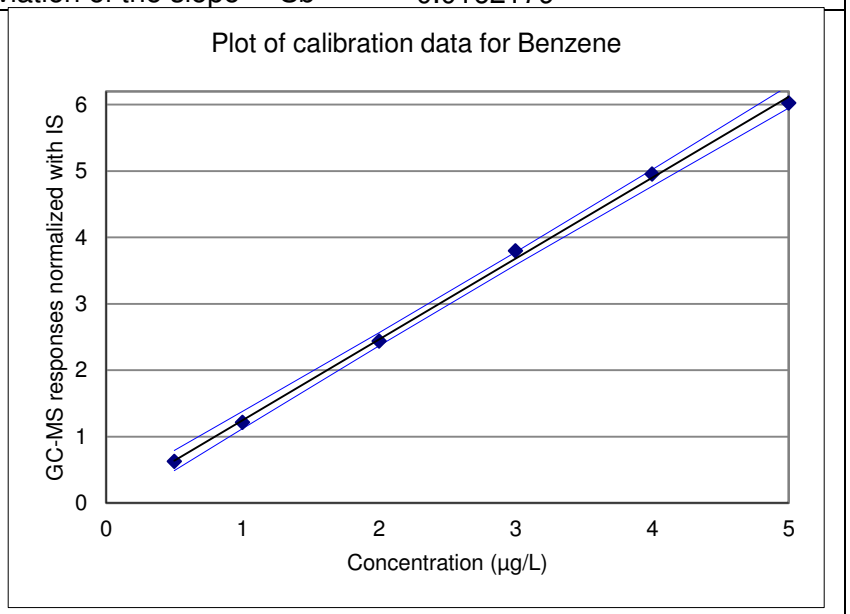
Plots of Calibration data

Benzene

Experimental data	
x	y
0.5	0.63
1.0	1.21
2.0	2.44
3.0	3.79
4.0	4.95
5.0	6.02

Derived values			
Slope, m	m	1.216	SLOPE(y,x)
Intercept, b	b	0.032	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{yx}	SYX	0.082	STEYX(y,x)
Average x	XAVG	2.583	AVERAGE(x)
SSX	SSX	15.208	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.045563	
Standard deviation of the slope	Sb	0.0162179	

Regression line confidence interval			
x	CI	y+CI	y-CI
0.5	0.15	0.79	0.49
1	0.13	1.38	1.12
2	0.10	2.56	2.37
3	0.10	3.78	3.59
4	0.12	5.02	4.77
5	0.17	6.28	5.95

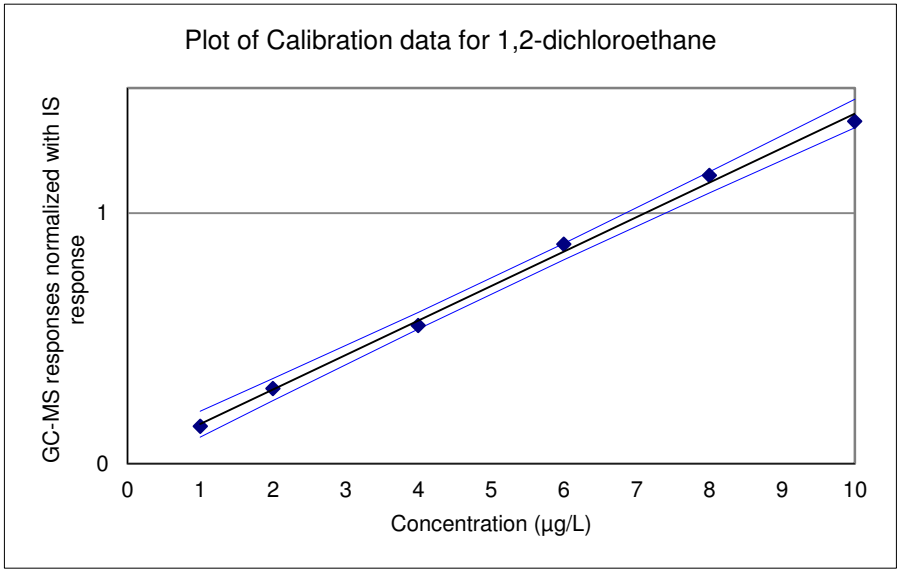


1,2-dichloroethane

Experimental data	
x	y
1.0	0.15
2.0	0.30
4.0	0.55
6.0	0.88
8.0	1.15
10.0	1.37

Derived values			
Slope, m	b	0.138	SLOPE(y,x)
Intercept, a	a	0.020	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{yx}	Sres	0.028	STEYX(y,x)
Average x	XAVG	5.167	AVERAGE(x)
SSX	SSX	60.833	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.0158377	
Standard deviation of the slope	Sb	0.0028187	

Regression line confidence interval			
x	CI	y+CI	y-CI
1.0	0.05	0.21	0.11
2.0	0.04	0.34	0.25
4.0	0.03	0.60	0.54
6.0	0.03	0.88	0.81
8.0	0.04	1.16	1.08
10.0	0.06	1.45	1.34

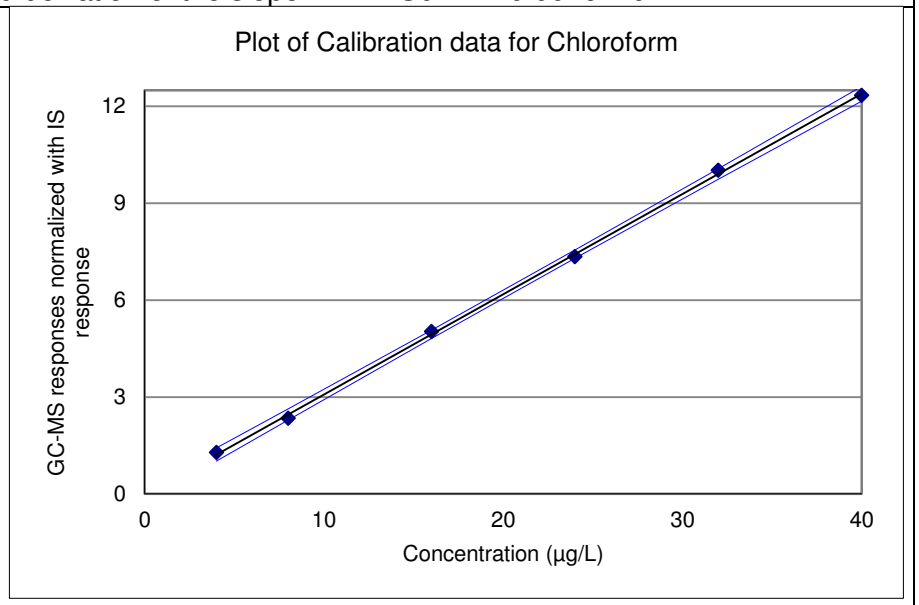


Chloroform

Experimental data	
x	y
4.0	1.28
8.0	2.34
16.0	5.02
24.0	7.34
32.0	10.01
40.0	12.34

Derived values			
Slope, m	b	0.310	SLOPE(y,x)
Intercept, a	a	-0.028	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{yx}	Sres	0.109	STEYX(y,x)
Average x	XAVG	20.667	AVERAGE(x)
SSX	SSX	973.333	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.0601189	
Standard deviation of the slope	Sb	0.0026749	

Regression line confidence interval			
x	CI	y+CI	y-CI
4.0	0.20	1.42	1.01
8.0	0.17	2.63	2.28
16.0	0.13	5.07	4.81
24.0	0.13	7.55	7.29
32.0	0.17	10.07	9.74
40.0	0.22	12.61	12.16

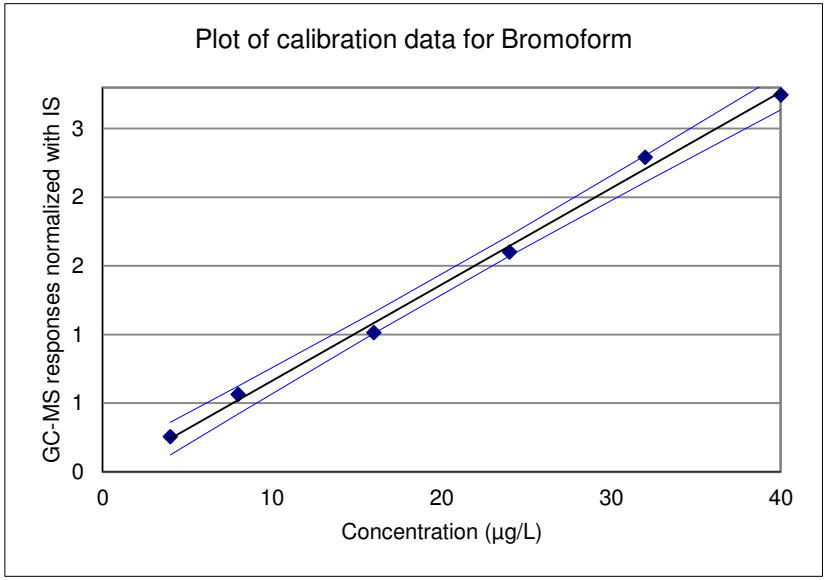


Bromoform

Experimental data	
x	y
4.0	0.26
8.0	0.56
16.0	1.01
24.0	1.60
32.0	2.29
40.0	2.74

Derived values			
Slope, m	m	0.070	SLOPE(y,x)
Intercept, b	b	-0.038	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{yx}	SYX	0.064	STEYX(y,x)
Average x	XAVG	20.667	AVERAGE(x)
SSX	SSX	973.333	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.0364863	
Standard deviation of the slope	Sb	0.0016234	

Regression line confidence interval			
x	CI	y+CI	y-CI
4	0.12	0.36	0.12
8	0.10	0.62	0.42
16	0.08	1.16	1.01
24	0.07	1.72	1.57
32	0.10	2.30	2.11
40	0.13	2.90	2.64

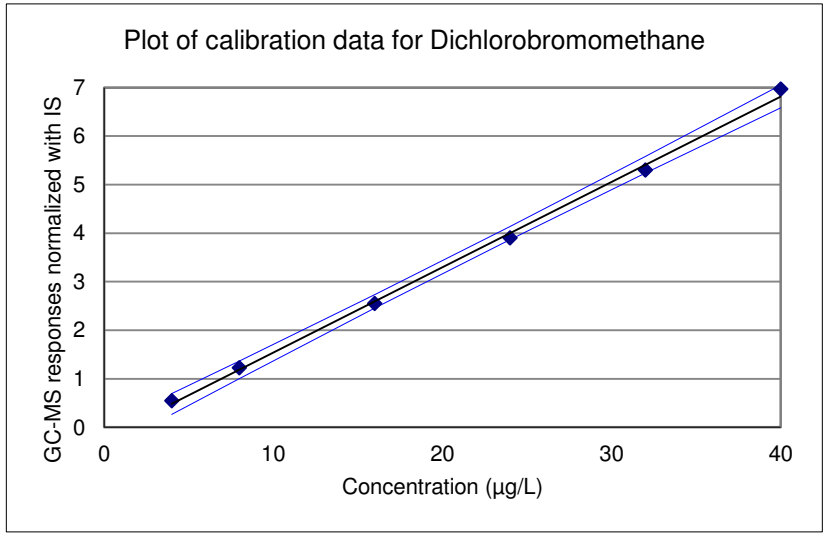


Dichlorobromomethane

Experimental data	
x	y
4.0	0.55
8.0	1.23
16.0	2.55
24.0	3.90
32.0	5.30
40.0	6.97

Derived values			
Slope, m	m	0.176	SLOPE(y,x)
Intercept, b	b	-0.221	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{yx}	SYX	0.114	STEYX(y,x)
Average x	XAVG	20.667	AVERAGE(x)
SSX	SSX	973.333	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.0802042	
Standard deviation of the slope	Sb	0.0035685	

Regression line confidence interval			
x	CI	y+CI	y-CI
4	0.21	0.70	0.27
8	0.18	1.37	1.00
16	0.14	2.73	2.46
24	0.13	4.13	3.87
32	0.17	5.58	5.23
40	0.23	7.05	6.58

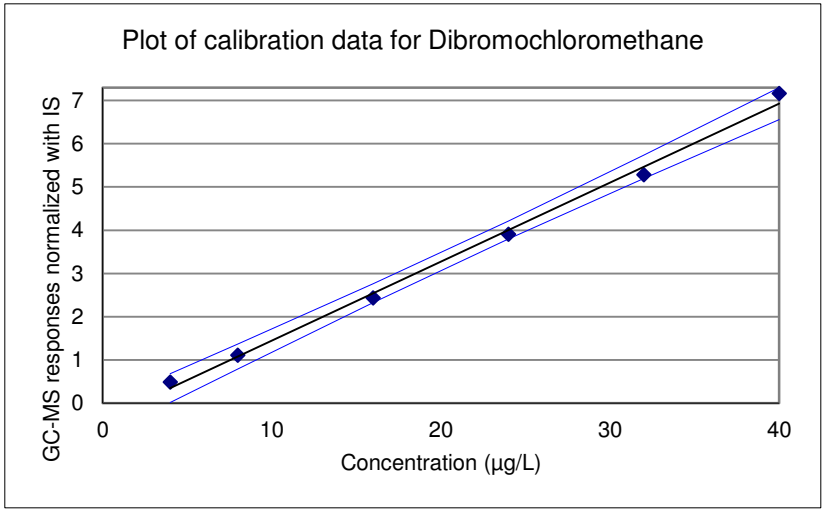


Dibromochloromethane

Experimental data	
x	y
4.0	0.49
8.0	1.11
16.0	2.43
24.0	3.90
32.0	5.28
40.0	7.16

Derived values			
Slope, m	m	0.183	SLOPE(y,x)
Intercept, b	b	-0.378	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{yx}	SYX	0.179	STEYX(y,x)
Average x	XAVG	20.667	AVERAGE(x)
SSX	SSX	973.333	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.1052927	
Standard deviation of the slope	Sb	0.0046848	

Regression line confidence interval			
x	CI	y+CI	y-CI
4	0.33	0.69	0.02
8	0.29	1.37	0.80
16	0.22	2.76	2.33
24	0.21	4.21	3.79
32	0.27	5.74	5.19
40	0.37	7.29	6.56

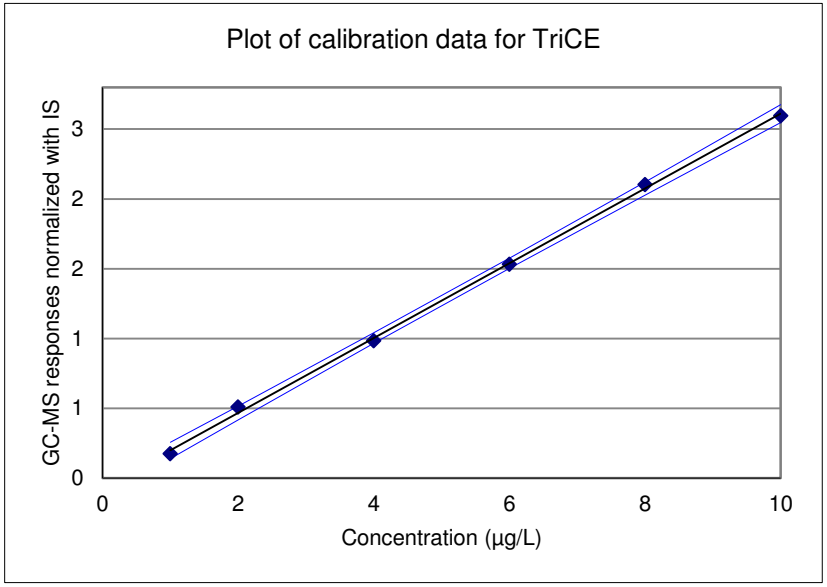


Trichloroethene

Experimental data	
x	y
1.0	0.17
2.0	0.51
4.0	0.98
6.0	1.53
8.0	2.10
10.0	2.60

Derived values			
Slope, m	m	0.268	SLOPE(y,x)
Intercept, b	b	-0.068	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{vx}	SYX	0.031	STEYX(y,x)
Average x	XAVG	5.167	AVERAGE(x)
SSX	SSX	60.833	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.0225493	
Standard deviation of the slope	Sb	0.0040132	

Regression line confidence interval			
x	CI	y+CI	y-CI
1	0.06	0.26	0.14
2	0.05	0.52	0.42
4	0.04	1.04	0.97
6	0.04	1.58	1.50
8	0.05	2.12	2.03
10	0.06	2.67	2.55

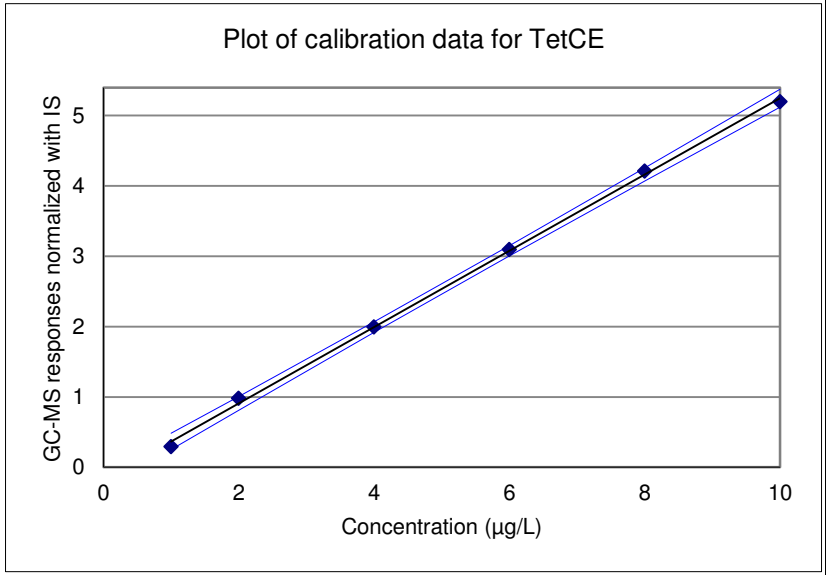


Tetrachloroethene

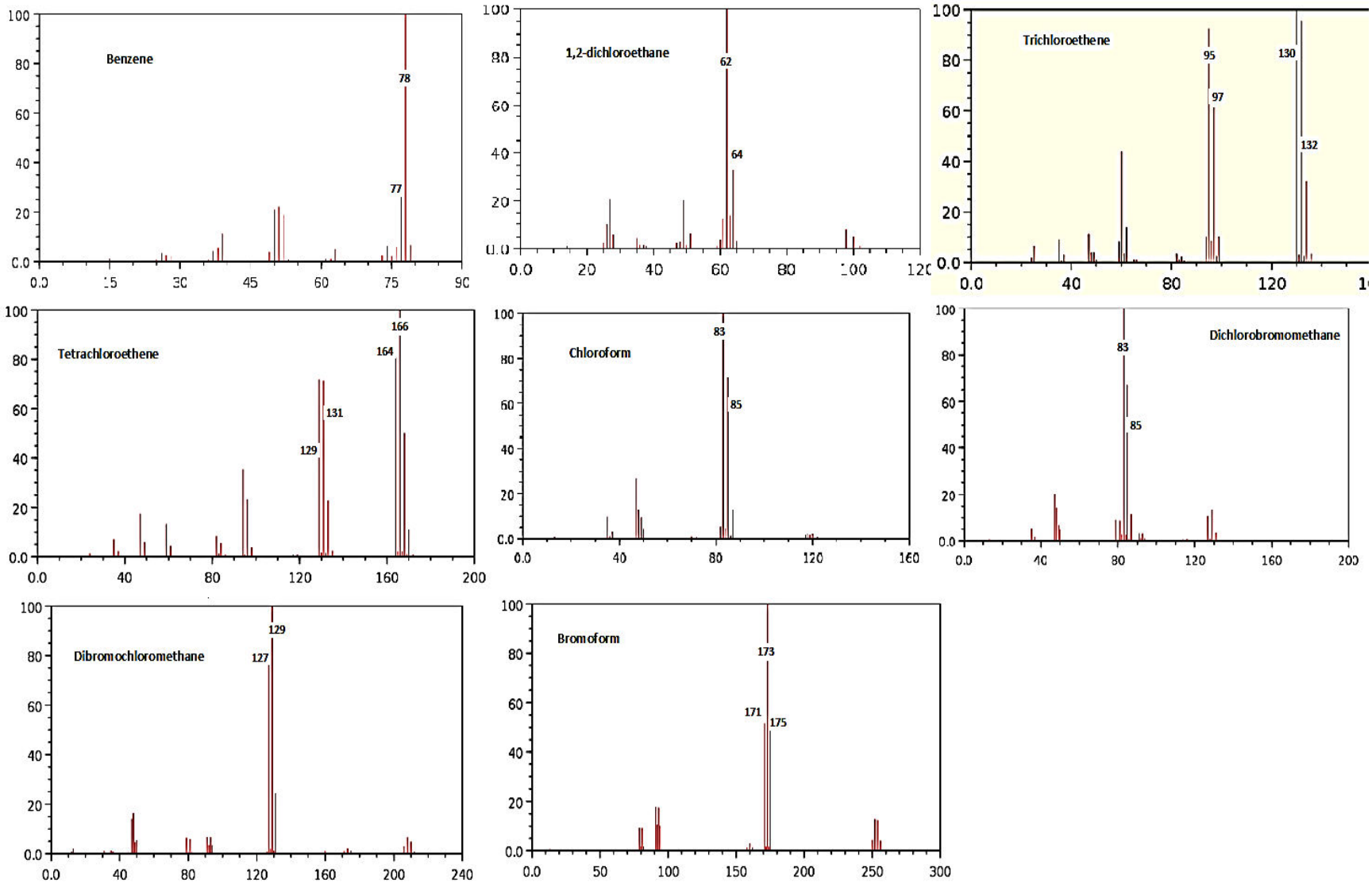
Experimental data	
x	y
1.0	0.29
2.0	0.98
4.0	1.99
6.0	3.10
8.0	4.21
10.0	5.20

Derived values			
Slope, m	m	0.542	SLOPE(y,x)
Intercept, b	b	-0.175	INTERCEPT(y,x)
Observations, n	n	6.000	COUNT(x)
Std error in estimate, S_{yx}	SYX	0.062	STEYX(y,x)
Average x	XAVG	5.167	AVERAGE(x)
SSX	SSX	60.833	DEVSQ(x)
t(α,df)	t	2.776	TINV(0.05,n-2)
Stnadrad deviation at intercept	Sa	0.0507499	
Standard deviation of the slope	Sb	0.0090321	

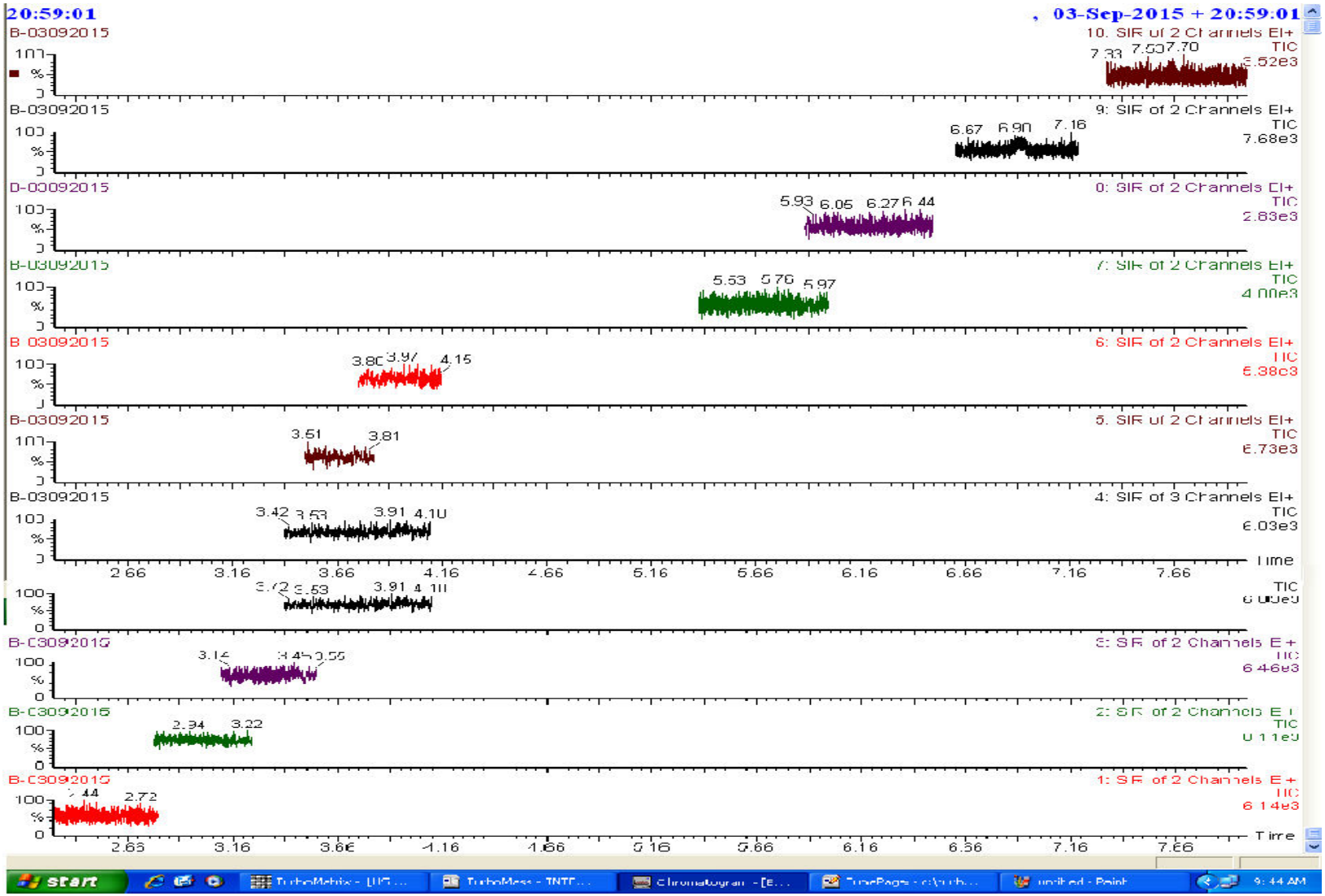
Regression line confidence interval			
x	CI	y+CI	y-CI
1	0.11	0.48	0.25
2	0.10	1.01	0.81
4	0.07	2.07	1.92
6	0.07	3.15	3.01
8	0.09	4.26	4.07
10	0.13	5.37	5.12



Annexure B



Total ion chromatograms: Major ions for all the compounds are labelled. The x-axis is m/z and the y-axis shows the realtive intensity of the ions.



An over laid SIM chromatogram of dilution water blank for all the VOCs.