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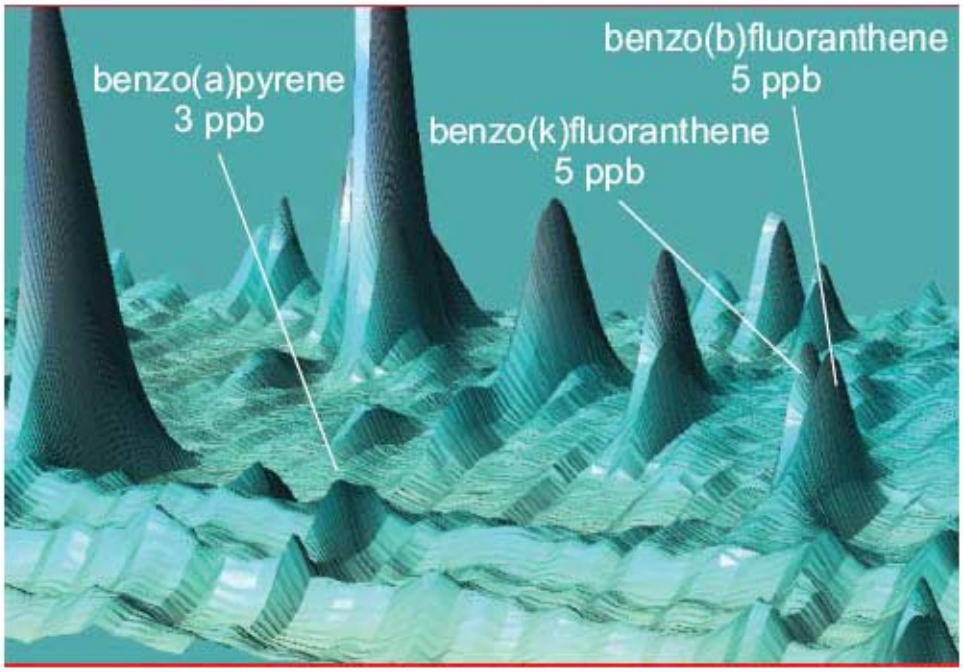

Comprehensive GC (GCXGC) as a Tool for Assessment of Remediation Potential of Oil Contaminated Soils

Master's Thesis in the International Master's Programme Applied Environmental Measurement Techniques

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Comprehensive GC (GC×GC) as a Tool for Assessment of Remediation Potential of Oil Contaminated Soils

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Abstract

This report presents the evaluation of two-dimensional gas chromatography (GC×GC) as a novel analytical tool for assessment of oil contaminated soils. The thesis project was carried out at VITO, Belgium and the results will be used in a soil remediation project by a petroleum company.

In previous oil polluted soil remediation projects, conventional GC-FID was normally used for characterisation of pollutants in soil samples. Different methods such as the TPH (total petroleum hydrocarbon) methods were developed for GC-FID. The results obtained from GC-FID are usually not sufficient to be used by environmental scientists for soil remediation projects. As a new analytical technique, GC×GC has been proved more powerful than conventional GC, especially for analysis of complex environmental samples.

Several series of experiments were carried out in order to find the optimal operating conditions. Three columns with different stationary phase (BP20, SolGelwax and BPX50) were tested as second dimension column. BPX50 was chosen as the preferred column because the other two were not stable at high temperature. The goal of the optimisation was to maximize the plate number in second dimension, and at the same time avoid the wrap-around and breakthrough problems. A compromise had to be made between analysis time, separation power and wrap-around of high boiling point compounds in second dimension to obtain the optimal condition.

In order to investigate the water solubility and volatility characteristics for pollutants in soil samples, several boiling point/LogKow matrices were built for both specific petroleum hydrocarbons and compounds in different organic groups. A good correlation between LogKow values and retention time coordinates in the boiling point/LowKow matrix was obtained for petroleum hydrocarbons. However, the same regularity could not be applied to compounds in different organic groups. BPX50 showed a comparable result compared to SolGelwax for petroleum hydrocarbons.

A six week biodegradation study for oil contaminated soil samples was also carried out in this project. Original extracts of the selected soil sample and the extracts after 2 weeks, 4 weeks and 6 weeks biodegradation were analysed by GC×GC. As a comparison, three window defining methods were developed to show biodegradation information. GC×GC gave more detailed information compared to conventional GC for biodegradation studies. GC×GC also gave the possibility to make a distinction between major chemical classes of (poly)aromatic hydrocarbons. The methods developed for GC×GC are faster, easier and more suitable for analysis of a large number of soil samples.

List of abbreviation

1. GC×GC: comprehensive two-dimensional gas chromatography
2. qMS: quadrupole mass spectrometer
3. DSQ: dual stage quadrupole mass spectrometer
4. TOF-MS: time of flight mass spectrometer
5. FID: flame ionisation detector
6. ECD: electron capture detector
7. ASE: accelerated solvent extraction
8. PTV: programmable temperature vaporizing
9. CT: constant temperature
10. PAHs: polyaromatic hydrocarbons
11. TPH: total petroleum hydrocarbons
12. TAL: total aliphatics
13. TAR: total aromatics
14. Rt1: retention time in first dimension
15. Rt2: retention time in second dimension
16. N: plate number

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1. Introduction

The analysis of complex mixtures such as petroleum samples, food samples, environmental and flavor samples has becoming more and more important in recent years. The most common instrument for characterisation of organic mixtures is conventional Gas Chromatography (GC) which is widely used in both scientific and commercial areas for chemical identification and quantification. However, it is impossible for conventional GC to separate all the individual components of such complex mixtures mentioned above. Even though there have been lots of improvements for GC technology such as connecting GC with Mass Spectrometry (GC-MS), the sufficient separation of complex mixtures is still not achieved.

In the early 1990s, the comprehensive two-dimensional GC was introduced in analytical chemistry. This technique can increase the separation power by over a factor of 10 compared to conventional GC. GC×GC uses a dual column system: small fractions eluting from a non-polar first column, where separation is mainly on the basis of the volatility of the analytes, are after refocusing transferred to a polar second column, where separation is mainly on the basis of polarity or molecular structure of the analytes. The first GC×GC instrument was build by Liu and Phillips in 1991. Since then, more and more laboratories and research groups began to do research work on GC×GC technology since it is considered as a very promising technology in analytical chemistry.

This thesis project is within a soil remediation project and was carried out at VITO (Flemish Institute for Technology Research, Belgium). It is a part of Applied Environmental Measurement Techniques master programme. The master thesis report was written under supervision at department of Water Environment Transport, Chalmers University of Technology. A GC×GC instrument was used as a tool to analyse petroleum hydrocarbons in soils. The results from the analysis will be used to define water solubility and biodegradability of the oil contaminants in the soil. Evaluation of GC×GC as a tool for assessing the remediation potential of contaminated soils is based on the GC×GC chromatograms from the experiments.

1.1 Background

VITO

VITO (The Flemish Institute for Technology Research) is an independent research center which is located in Boeretang, Mol, Belgium. The institute mainly focuses on customer oriented contract research and develops innovative products and processes in the field of energy, environment and materials. All the experimental work in this thesis project was conducted at the organic chemical analysis laboratory of VITO.

Oil characterisation in polluted soils

Soil pollution by petroleum hydrocarbons is gaining more and more emphasis by governments and companies because numerous sites polluted with petroleum hydrocarbons are being investigated yearly in the context of soil remediation projects. However, knowledge of the behavior of

petroleum hydrocarbons in soils is still rather poor. Regularly, in-situ soil remediations turn out to require more time than expected, remediation target values are not met and/or groundwater purification units turn out to be less efficient than expected. This is primarily a consequence of lack of an adequate method of analysis, which prevents access to the 'right' and over-all information about the oil pollution. The Total Petroleum Hydrocarbon Criteria Working Group in VITO developed an oil characterisation method based on a silica column separation of aromatics and aliphatics and followed by a GC-FID subdivision into equivalent-carbon fractions (EC) ('TPH-method') (Weisman, 1998). However, this method was mainly developed for assessing human risks of oil compounds. Ideally, a characterisation method should allow a complete characterisation of the oil in terms of composition, (human) risks, volatility, water solubility, plume behavior (migration velocities of the soluble components) and biodegradation potential. In order to achieve this, the adoption of GC×GC became an option considering its higher separation power.

Instrument

VITO disposes of a Thermo Electron Trace 2DGC coupled to a FID (Flame Ionization Detector) detector. A method has been developed for the total characterisation of petroleum hydrocarbon spills. The system is operating with the CO_2 Dual-Jet modulator, which is fully synchronized with the GC electronics and completely controlled by the HyperChrom system used for monitoring of the GC×GC system and data processing.

For the proper identification of compounds, the GC×GC has to be connected to a mass spectrometer. The first choice was a TOF-MS (Time of Flight Mass Spectrometer), but due to persisting instrumental shortcomings the idea of hyphenation with a TOF-MS had to be abandoned. Instead, the use of a rapidly scanning quadrupole MS like the Finnigan DSQ (Dual-stage quadrupole) was carried out. Nevertheless, the quadrupole is still too slow for quantification purposes, but makes identification possible. The GC×GC system available in the laboratory for organic analysis at VITO was coupled to a Finnigan DSQ.

GC×GC for oil characterisation in soil remediation project

In 2004 – 2005 VITO evaluated comprehensive GC as a tool for fast oil characterisation in the context of soil remediation projects. The column set was optimized for group-type separation: the first dimension was an apolar RTX-1 phase (separation based on boiling point) and the second dimension was a 50% phenyl phase (BPX50). Separation in the second dimension was based on aromatic character of the molecules because aromatic hydrocarbons have more affinity for the phenyl-groups in the BPX50 and exhibit stronger retention then the aliphatic hydrocarbons. The method allows a group-type characterisation of oil pollution and especially the aromatic fraction is described more in detail compared to other characterisation methods (Van De Weghe et al., 2005).
For the prediction of degradation potential and plume behavior it is preferable to incorporate water solubility since it is a very useful parameter for determining the fate of the organic pollutions. This was assumed to be better done by substitution of the BPX50 phase with a highly polar phase (BP20 or SolgelWax). The separation in the second dimension will then be based on polarity instead of aromaticity of the molecules, and the GC×GC chromatogram could be interpreted as a boiling point/LogKow matrix.

1.2 Aims and goals

The objective of this project was to evaluate GC×GC as a tool for assessing the remediation potential of polluted soils. The following specific goals had to be accomplished in the 3 months experiment period:

- To develop a suitable GC×GC method and find the optimal operating condition.
- To construct the boiling point/LogKow matrix in the retention time reference frame.
- To conduct a 6 week biodegradation experiment for assessment of the bioremediation potential of different petrochemical compounds in soil samples.

In order to achieve these goals, the following experiment phases were carried out:

(1) A series of experiments using different second dimension columns (BPX50, BP20 and Solgelwax) and under different operating conditions were implemented in order to find the optimal GC×GC method for this project.

(2) Several boiling point/LogKow matrices based on the selected second dimension column and operating condition were built and compared.

(3) The original extractions of the soil samples were analysed and the most interesting one was picked for biodegradation experiment. The extractions of this soil sample after 2 weeks, 4weeks and 6 weeks were respectively analysed by GC×GC to show the biodegradation potential of different chemical groups.

2. Theory

The central focus in this project is the GC×GC system. It is a relative young technique with only 14 years history, including 6 years as a commercial instrument. The GC×GC technology has developed very fast since it was introduced into analytical chemistry in 1991 by Phillips. The reason is that GC×GC system has many advantages compared to conventional GC, which are necessary in chemical characterisation especially for complex mixtures. This section gives a general description of the principle of GC×GC system, and the main advanced characteristics of GC×GC compared to conventional GC. A general introduction of water solubility and Kow is also presented in this section.

2.1 Principle of GC×GC

GC×GC uses a dual column system with two columns in series. Two GC separation based on fundamentally different separation mechanisms are applied to the entire sample in order to achieve orthogonal separation conditions (Phillips and Beens, 1999). The schematic description of the GC ×GC system is shown in Figure 1.

Columns

The non-polar first dimension column usually is a high resolution capillary GC column (typically a 15-30m×0.25-0.32mm I.D., d_f 0.1-1μm column) where separation is mainly on the basis of volatility of the analytes. The temperature programming of the first column usually with a heating rate no more than 1-5°C /min. The second dimension column is a much shorter and narrower column compared to the first one (typically a 1-2m×0.1mm I.D., d_f 0.1μm column). The separation in the second column is generally based only on the basis of the polarity or shape-selective nature of the analytes so as to achieve the required orthogonal separation conditions (Dallüge et al., 2003). Due to the much shorter, narrower column size and higher temperature condition, the separation in the second column is extremely fast and takes only 1-10s compared to 45-120min for the first dimension separation (Dallüge et al., 2003).

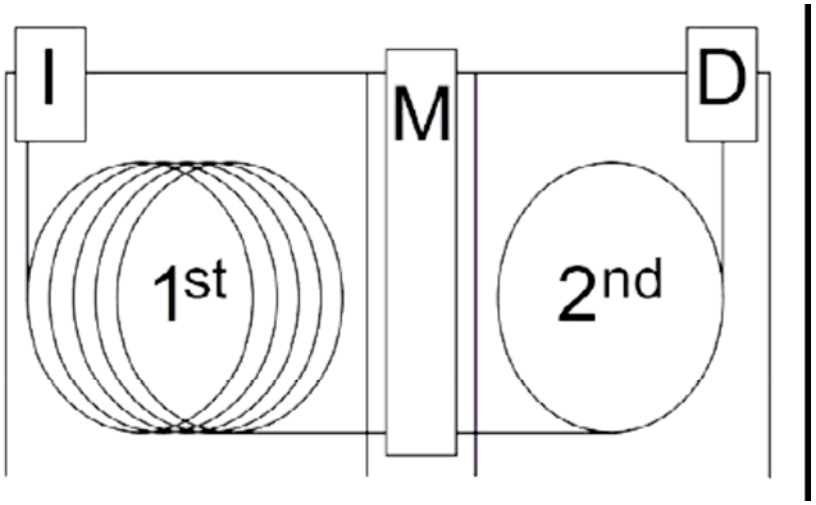

Figure 1. Schematic description of GC×GC system, I, injector; M, modulator; D, detector

Modulation

A modulator is used as an interfacing device to connect the two columns, and is the most important part of the GC×GC system. It has three functions: (1) Continuously accumulate or trap small fractions of effluent from the first column. (2) Refocus the trapped fractions either in time or in space. (3) Inject the refocused fractions as very narrow pulses into the second-dimension column (Dallüge et al., 2003).

Generally there are three types of modulators: valve, thermal and cryogenic. Valve modulator has the limitation of either requiring a substantial higher flow-rate through the second column or sending most of the sample to vent. Most of the fractions of the first column effluent are disregarded except of the very small and narrow ones (Phillips and Beens, 1999). So it is not practical for most applications. Thermal modulator is a common used modulation technique in GC. With manipulation of the temperature, almost all the volatile chemicals can be retained onto and desorbed from the stationary phase. The first commercially available thermal modulator is the so called “Sweeper” which was designed by Ledford and Phillips based on a moving heater technique. The working principle of the Sweeper is a separate heating element that moves over the modulator capillary and heats it locally. In this modulator, the heat mass is large enough to provide a stable and controlled temperature. But the main problem of this type of modulator is its limited temperature range (Phillips and Ledford, 1996). In recent years, cryogenic modulator became a new way to retain and desorb compounds from the stationary phase. There are different kinds of cryogenic modulators such as LMCS (Longitudinally Modulating Cryogenic System) modulator and dual-jet CO_2 modulator. Figure 2 is a schematic description of the dual-jet CO_2 modulator, and the principle of cryogenic modulation is shown in Figure 3.

The principle of cryogenic modulation: (1) The modulator (right-hand jet) retains part of a peak eluting from the first column. (2) The right-hand jet is switched off, the cold spot heats up very quickly and the analytes are released and launched for separation in the second column. Meanwhile, the left-hand jet is switched on to prevent material eluting from the first column to interfere with the focused fraction. (3) The right-hand jet is switched on again and the next modulation cycle is started (Dallüge et al., 2003).

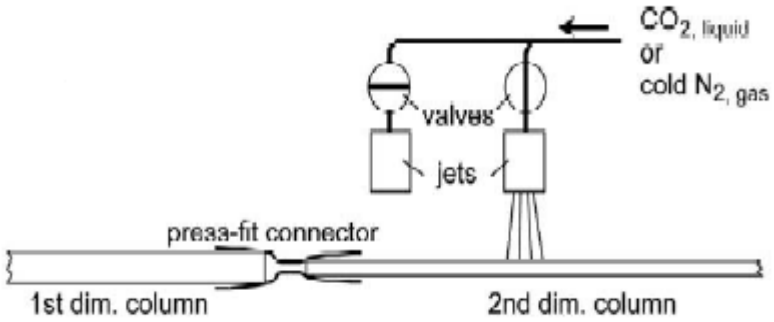

Figure 2. Schematic description of dual-jet CO_2 modulator

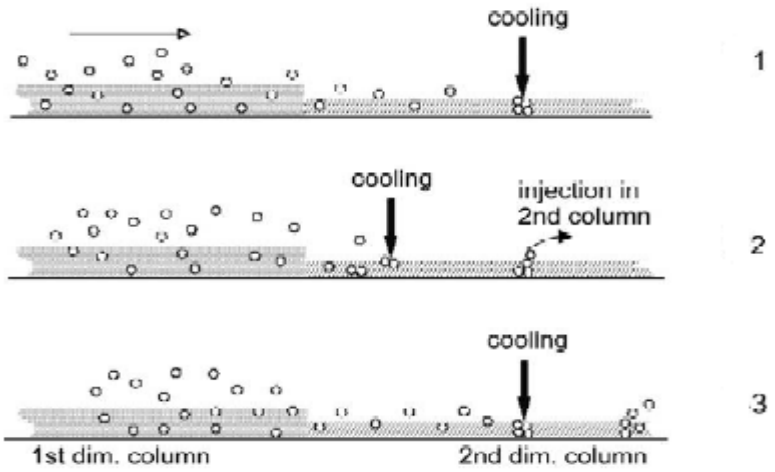

Figure 3. Principle of cryogenic modulation (Dallüge et al., 2003)

Detection

The very fast separation in the second dimension results in narrow peaks, which require fast detectors with a small internal volume, a short detector response time and a high data acquisition rate to ensure a proper reconstruction of the second-dimension chromatograms. The most commonly used detector is fast FID. Modern FIDs have a negligible internal volume and can acquire data at frequencies of 50-200 Hz. Today, μECD (micro electron capture detectors) are also adopted in some of the GC×GC experiments (Dallüge et al., 2003). The data acquisition frequency of a μECD is typically 50 Hz.

Recently, the combination of GC×GC and mass spectrometer (MS) has been more and more popular. Spectrometric detectors, specifically a mass spectrometer are very helpful to allow the identification of the numerous separated compounds. However, the problem of adopting MS is that few of them can catch the high speed chromatograms generated by the second-dimension column. Today, the most commonly used MS is TOF-MS since it can acquire 50 or more mass spectrum per second which is necessary for the proper reconstruction of GC×GC chromatograms and for quantification. However, since TOF-MS is not reliable and very expensive, it is not suitable for routine analysis. Instead, a DSQ-MS is preferred in routine applications due to its reliability, relative high speed and low price.

2.2 Advantages of GC×GC compared to conventional GC

The advantage of GC×GC versus conventional GC is mainly due to the following reasons:

1. High resolution and high peak capacity. The peak capacity of GC×GC is the multiply product of the two columns, which yields a distinctly improved separation power.
2. High sensitivity, which is usually 20-50 times than conventional GC (Beens et al., 1998; De Geus et al., 1998).
3. Less analysis time compared to conventional GC due to the easier separation of analytes.
4. Identification is more reliable than conventional GC since most of the target compounds can be baseline separated.

5. If proper orthogonal conditions are used, chemical related compounds show up as ordered structures, which is helpful for group-type analysis and classification of unknown compounds.

By virtue of these characteristics, GC×GC technique is becoming the most suitable solution for analysis of target compounds in complex matrices, as well as for detailed sample characterisation.

2.3 Water Solubility (S) and Kow

Water solubility is the maximum amount of a substance that can dissolve in water at equilibrium at a given temperature and pressure, usually 25°C and 1atm (USGS). It is one of the most important property concerning the fate of organic contaminants. The larger the value in mole per liter, the more likely a compound will stay in water rather than go into air, sediment or tissue.

Octanol-water partition coefficient (Kow) is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature (USGS). Units are (mole per liter of octanol) per (mole per liter of water). In many cases, the values are presented as LogKow. This parameter is used in many environmental studies to help determine the fate of chemicals in the environment. Kow is inversely proportion to the water solubility and indicates compound hydrophobicity. This is an important indication of whether a compound will bioaccumulate or not. Increasing hydrophobicity often lead to an increasing bioaccumulation.

These two parameters of pollutants characteristics are important for defining the right remediation method in soil remediation projects. A general trend is the larger the compound, the smaller the water solubility and the larger the Kow.

3. Development of GC×GC technology

The first part of this section gives an overview of the main application areas of the GC×GC technology, which have been applied successfully. In the next parts of the section, the recent development and a literature of review about the application of GC×GC in petrochemical characterization, and the hyphenation of qMS (quadrupole mass spectrometer) to GC×GC technology are presented since they are related to this thesis project.

3.1 Applications of GC×GC

Table 1 gives a list of application areas and show the developments of GC×GC technology. Classification is based on sample type, analytes and detection methods.

Table 1. Overview of GC×GC applications in recent years (Dallüge et al. 2003)

Sample type	Analytes	Detection Method
Petrochemical	Group-type characterisation	FID/ MS(DSQ)/TOF MS
	Biomarkers	FID
	Target compounds	FID
Essential oils, food extracts	Flavours	FID/TOF MS
Food (fish)	PCBs PCDDs PCDFs	ECD/FID
Food (vegetables)	Pesticides	TOF-MS
Biological oil	Fatty acids	FID
Blood plasma	Pesticides	FID
Fly ash	Micropollutants	TOF MS
Sediment	PAHs	TOF MS
(Surface) water	Volatiles BTEX	FID
	Microcontaminants	FID
Air/gaseous samples	Volatiles	FID/TOF MS
Cigarette smoke	General characterisation	TOF MS

From the table, it can be concluded that GC×GC is mainly used in samples which have very complex compositions. FID is the most commonly used detector for GC×GC instrument while TOF MS is the most commonly used MS in order to enhance the identification power of GC×GC.

3.2 GC×GC for petrochemical characterisation

Petrochemicals are the most common complex sample composed by 2-4 different groups of compounds. There could be 4×10^7 saturated hydrocarbon isomers existing within the range of C_{10}-C_{25} in petrochemical samples. Although only a small part of these hydrocarbons are in a

specific oil sample, it is still impossible for conventional GC to separate the compounds due to the low peak capacity.

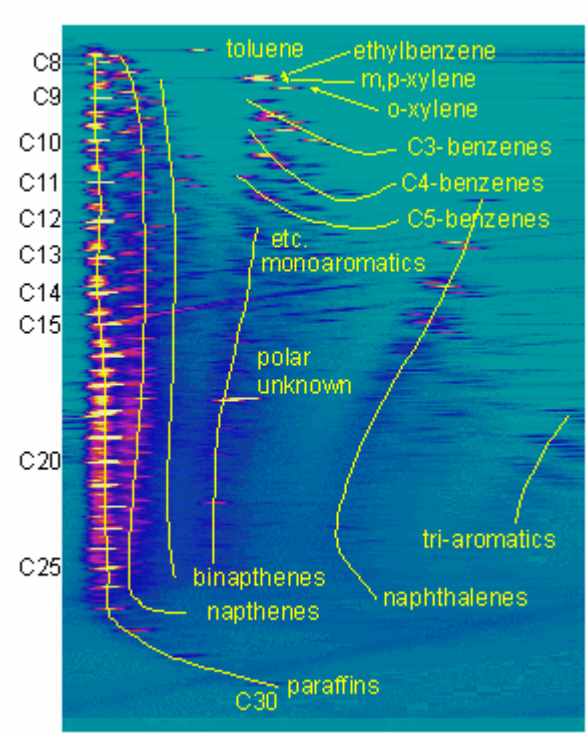

Figure 4. GC×GC chromatogram of diesel oil (Shell International Chemicals)

There have been many applications using GC×GC for petrochemical sample characterisation. Different kinds of oils have been separated and good separation results have been gained (Blomberg et al., 1997; Beens et al., 1998). Figure 4 is a GC×GC chromatogram of diesel oil. The chromatogram exhibits about 2000 peaks arranged in bands of compound classes, and sub-bands of structural isomers. Saturates, naphthene, mono-aromatics, bi-aromatics and tri-aromatics are all separated into obvious different regions in the chromatogram. The orderly peak arrangement simplifies interpretation. Only two meters of 2D-column were used in this analysis, which lasted 60 minutes.

Two dimensional chromatograms of kerosene, separated with different stationary phase combinations in thousands of components have been presented (Xu, 1997). Benzene, toluene, ethylbenzene, xylenes (BTEX) and total aromatics have been separated using GC×GC (Frysinger et al., 1999). GC×GC was also used as a very effective tool to identify spilled oil sources (Gaines, 1999). Pattern recognition of jet fuels was done using GC×GC (Johnson and Synovec, 2002). Comprehensive GC×GC was also coupled with Pressurized liquid extraction (PLE) technology for fast-screening of polycyclic aromatic hydrocarbons in soil (Ong et al., 2003). The experiment of fingerprinting as well as identification of N- and S- containing unknown compounds in petrochemical was carried out (Van Stee et al., 2003). Comprehensive GC×GC was also used for detailed analysis of petrochemical samples, and both qualitative and quantitative results demonstrated an improved resolution power compared to conventional GC (Vendeuvre et al., 2004)

3.3 Hyphenation of qMS to GC×GC

Combination of GC×GC with mass spectrometer is a very helpful method for further identification of target samples. The most commonly used mass spectrometer is TOF-MS due to its fast scan rate, which is necessary for coupling the very fast peak eluting speed of the second dimension column of GC×GC. Despite the strengths of GC × GC–TOFMS, cost including purchase and maintenance may be an obstacle for adopting the technique as a routine analytical method. Besides, the large data files generated by GC × GC–TOFMS at a data acquisition rates of 50 Hz (or higher) demand long data-processing time and large hard disk memory space for data handling which could also become a problem for adoption of TOFMS (Dalluge 2002, 2003). Considering this, a low-cost qMS with a shorter data processing time become an attractive option for many current and potential GC×GC users.

Marine diesel fuel was analysed with GC×GC connected to a qMS for identification purposes, although the scan speed of the MS was not quite suited for the fast second dimension peaks (Frysinger et al., 1999). Rapid scanning qMS was also coupled to GC×GC in a comprehensive two-dimensional gas chromatography-mass spectrometry analysis of Pelargonium graveolens essential oil and high quality mass spectra were obtained (Shellie and Marriott, 2003). Hyphenation of qMS to GC×GC for the analysis of suspected allergens was done. Although it was still time consuming for data processing, the co-elution problems was improved a lot compared to former GC-MS work (Debonneville et al., 2004a, b). Comprehensive GC×GC was also combined with qMS in the application of drug analysis (Song et al., 2004). With the creation of new “truncated” library, 75% of the drugs yielded matches of at least 90%. Comprehensive GC ×GC in combination with rapid scanning qMS was also applied in perfume analysis recently (Mondello et al., 2005). Quadrupole MS system supplied high quality mass spectrum to GC×GC which was very helpful for identification and quantification of perfume samples.

4. Experimental

The first part of this section gives the basic information of the analytes and samples used in this thesis project. The sample preparation procedures are also presented in this part. The instrumentation information of the GC×GC system including DSQ is presented in the second part. Identification and quantification methods used in this project are also presented in this section. The last part gives the initial condition of the GC×GC system including initial column combination and initial inlet pressure.

4.1 Analytes and samples

Four standard mixtures (PAH, Window, Alcket and Calibration. Made in VITO) were used in this thesis project (Table 2). PAHmix contains 13 polyaromatics and Windowmix contains the most common compounds exist in oil samples including aliphatics and aromatics. Both of these two samples were prepared and used in previous experiments (Van De Weghe et al., 2005). Alcketmix contains 16 compounds including representative alcohols and ketones as well as some polyaromatic compounds. Individual compounds were mixed and dissolved in acetone (MERCK, Darmstadt, Germany). The mixture was further dissolved in hexane (MERCK, Darmstadt, Germany) to make the final concentration of each compound within the range of 10-50mg/kg. Calibrationmix was used for quantitative calibration. It contains 11 representative compounds in petroleum hydrocarbons including both aliphatics and aromatics. PCB-128 was added into Calibrationmix as internal standard.

Table 2. Compounds names for PAHmix, Windowmix and Alcketmix

PAHmix	Windowmix	Alcketmix	Calibrationmix
Naphthalene	Alkanes+Alkenes C8-C40	1-Pentanol	n-C12
Acenaphthylene	Ethylbenzene	1-Hexanol	n-C14
Fluorene	m,p,o-Xylene	1-Octanol	n-C18
Phenanthrene	A9-aromatics	1-Heptanol	3-Ethyltoluene
Anthracene	A10-aromatics	1-Decanol	Diethylbenzene
Fluoranthene	> A10-aromatics	1-Benzofuran	1,2,4-Triethylbenzene
Pyrene	Cycloalkanes	Quinoline	Phenyloctane
Benzo(a)anthracene	**PAHmix**	2-Henanone	p-Xylene
Chrysene	C1-C4 Naphthalenes	2-Octanone	o-Xylene
Benzo(b)fluoranthene	Biphenyl	Phenol	Biphenyl
Benzo(k)fluoranthene	C1-C2 Biphenyls	2-Methylphenol	Dibenzothiophene
Benzo(a)pyrene	C1-C3 Fluorenes	1-Benzothiophene	PCB-128(int. std.)
	Dibenzothiophene	Indole	
	C1-C4 Phenanthrene	Dibenzofuran	
	C1-C3 Dibenzothiophenes	Carbazole	
	Indeno(123cd)pyrene	2-Pentadecanone	
	Dibenzo(ah)ahthracene		
	Benzo(ghi)perylene		

Soil samples (Marked as Extra1, Extra2 and Extra3) from three different sites in Belgium which were polluted by oil industries were taken by another department of VITO.

4.2 Sample preparation

4.2.1 ASE extraction

Approximate 10g or 15g soil sample was taken and mixed with 5g celite (MERCK, Darmstadt, Germany) for drying purpose. The mixture was well homogenized in a grinding bowl, then transferred into a 33ml metal cell and weighed. The same procedure was done for all three samples and their biodegraded samples. The weights of the selected sample (Extra 2) and its biodegraded samples used for ASE extraction are shown in Appendix 1.

Accelerated Solvent Extraction was carried out using an ASE200 Accelerated Solvent Extractor (DIONEX, Sunnyvale, USA) with an extraction temperature of 100°C. The extraction solvent was n-hexane/acetone (50/50) standard solvent. After ASE extraction, the extracts were kept in the vial and preserved in the refrigerator.

4.2.2 Biodegradation

The samples were kept wet in separate aluminum containers for 6 weeks with good ventilation conditions for aerobic biodegradation. ASE extraction was carried out for the original samples, samples after 2 weeks, 4 weeks and 6 weeks biodegradation. Each time when ASE extraction was carried out, approximate 5g soil samples were also taken and put in the oven for measurement of dry matter content. After GC-MS analysis of three original samples, the most interesting sample (sample with most petroleum hydrocarbons presenting) was picked for biodegradation analysis by GC×GC. Hexane was added into the selected soil extracts to make all the extract volumes to 50ml. Approximate 1ml was taken respectively and added with 30μl internal standard (PCB-128 Concentration: 1000mg/l). GC×GC analysis was then implemented for all the extracts including original sample, sample after 2 weeks, 4 weeks and 6 weeks biodegradation to make a comparison and show the bioremediation potential of the selected sample.

4.3 Instrumentation

The GC×GC system consisted of a Thermo Electron Trace 2DGC (Thermo Electron Corporation, Milan, Italy), which was equipped with a CTC-PAL Autosampler (CTC Analytics AG, Zwingen, Switzerland), a PTV (Programmable Temperature Vaporizing) Injector and a FID detector (275°C). CT (constant temperature) splitless mode with 300 °C injection temperature, 40ml/min total flow and 1 min splitless time was performed during the optimization of GC×GC system using BP20

column. PTV spliteless mode was performed at 50°C as a based temperature, and 330°C transfer temperature with 3 min spliteless time when using Solgelwax and BPX50 columns to hinder the discrimination of high boiling point compounds. Hydrogen was used as carrier gas.

The column-set used a 30m×0.32mm i.d.×0.25µm RTX-1 film (100% dimethyl polysiloxane) column (Restek, Bellefonte, PA, USA) as the first dimension column. Three different kinds of column were used as the second dimension column: a 0.1mm i.d.×0.1µm BP20 film (polyethylene glycol) column (SGE, Ringwood, Australia), a dedicated 0.1mm i.d.×0.1µm SolGelwax film (polyethylene glycol in a SolGel matrix) column (SGE, Ringwood, Australia) and a 0.1mm i.d.×0.1µm BPX50 film (50% Phenyl polysilphenylene-siloxane) column (SGE, Ringwood, Australia). The difference between BP20 and Solgelwax is that SolGelwax can stand a higher temperature (300°C) compared to BP20 (270°C) since the solid phase in SolGelwax is fixed in SolGel matrix. Different second dimension column lengths including 1m, 1.5m and 2m were tested in order to find an optimal running condition of the GC×GC system for this specific project. A CO_2 Dual-Jet cryogenic modulator was placed at the beginning of the second column. The HyperChrom (Version 2.3 β2.0, Thermo Electron Corporation, Milan, Italy) system was used for monitoring of the GC×GC system and data acquisition and processing.

A Finnigan Trace DSQ (Thermo Electron Corporation, Milan, Italy) was also configured for identification purpose and connected to GC×GC. The ion source of the DSQ was 250°C and the start scan time was 6 min in order to avoid solvent peak. A mass range of 30-400 m/z was chosen for the soil samples resulting in a scan rate of 10.989 scans/ sec. The chromatographic condition (temperature programme and carrier gas pressure) were the same as for the GC×GC-FID system when processing the same sample. Xcalibur (Version 1.4 SR1, Thermo Electron Corporation, Milan, Italy) system was used for monitoring the GC×GC-DSQ system and also for data processing.

4.4 Definition of component windows for identification

The Windowmix standard mixture was used as the “window defining” mixture for identification purpose because it contains all the most abundant compound classes existing in mineral oil. Identification of the compounds in the soil extracts was based on retention time matching with the individual compounds or compound groups in Windowmix. As mentioned in section 2.2, one of the advantages of comprehensive GC is the chemical logic (The groups that present in the chromatogram are in an ordered structure) behind the repeating groups in the chromatogram. As a consequence, it is not necessary to identify all the individual components in a group. The groups in the GC×GC chromatogram can be assigned with only one or two standard components. In the case no individual component was available, a tentative identification was done based solely on the chemical logic in the chromatogram (Van De Weghe et al., 2005).

4.5 Quantification method

The GC×GC chromatogram of the Windowmix was adopted to create a component table in the HyperChrom software by graphically enclosing the spots or groups of spots with a square box (polygon). When processing data of soil samples, all the peaks within the same elution window of a polygon are identified as target compounds and each peak area is added to obtain the total area for that compound. Quantification was performed using the internal standard method.

In the internal standard method, the following equation was used to calculate concentrations of target compounds:

$$\frac{C_j}{A_j R_j} = \frac{C_{is}}{A_{is}} \qquad (1)$$

Equation (1) can be written in another form: $C_j = \frac{A_j}{A_{is}} \times \frac{1}{R_j} \times C_{is}$ (2)

Where C_j is the concentration of target compound, A_j is the peak area of target compound, R_j is the relative response factor (RRF) of the target compound to internal standard compound, A_{is} is the peak area of internal standard compound and C_{is} is the weight concentration (mg/kg) of internal standard compound (PCB-128).

A_j and A_{is} can be easily integrated from the GC×GC chromatogram. To determine the average RRFs to internal standard (PCB-128) for both aliphatic and aromatic hydrocarbons, The Calibrationmix containing n-alkanes and (poly)aromatic hydrocarbons was injected to the GC×GC. Based on the individual RRFs of the aliphatic and aromatic compounds in the Calibrationmix (see Appendix 2), an average RRF of 4.5022 was assigned to aliphatic compounds while an average RRF of 4.5371 was assigned for aromatic compounds.

Weight concentration of internal standard can be calculated using the following equation:

$$C_{is} = C_{vis} \times V_{is} \times \frac{1}{M} \qquad (3)$$

Where C_{vis} is the original volume concentration of the PCB-128 (1000mg/l), V_{is} is the volume of PCB-128 which was added into the 1ml extract (30μl) for GC×GC analysis and M is the final weight of soil dry matter in 1ml extract. M can be calculated by the following equation:

$$M = m_s \times D \times \frac{m_{1ml}}{m_{50ml}} \qquad (4)$$

Where m_s is soil intake for ASE extraction, D is the dry matter percent of the soil, m_{1ml} is the weight of 1ml extract using for GC×GC analysis and m_{50ml} is the weight of the total 50ml extract. Weight concentrations of internal standard and values of the other factors of soil sample Extra2 are listed in Appendix 3. With the results of R_j and C_{is}, the weight concentrations of all the individual components or component groups can be calculated based on equation (2).

4.6 Initial condition of GC×GC system

4.6.1 Initial column

Simulations of several different GC×GC column-dimension combinations were done using the Microsoft Excel computer model. After the comparison of performance parameters such as plate number and modulation criterion for different combinations, a 30m×0.32mm i.d.×0.25μm film first dimension column and a 2m × 0.1mm i.d. × 0.1μm film second dimension column combination was chosen for the initial column option of the condition optimization (Beens et al., 2005). Figure 5 shows the relation between different parameters (inlet pressure, first dimension plate number, second dimension plate number and modulation criterion) under this column combination.

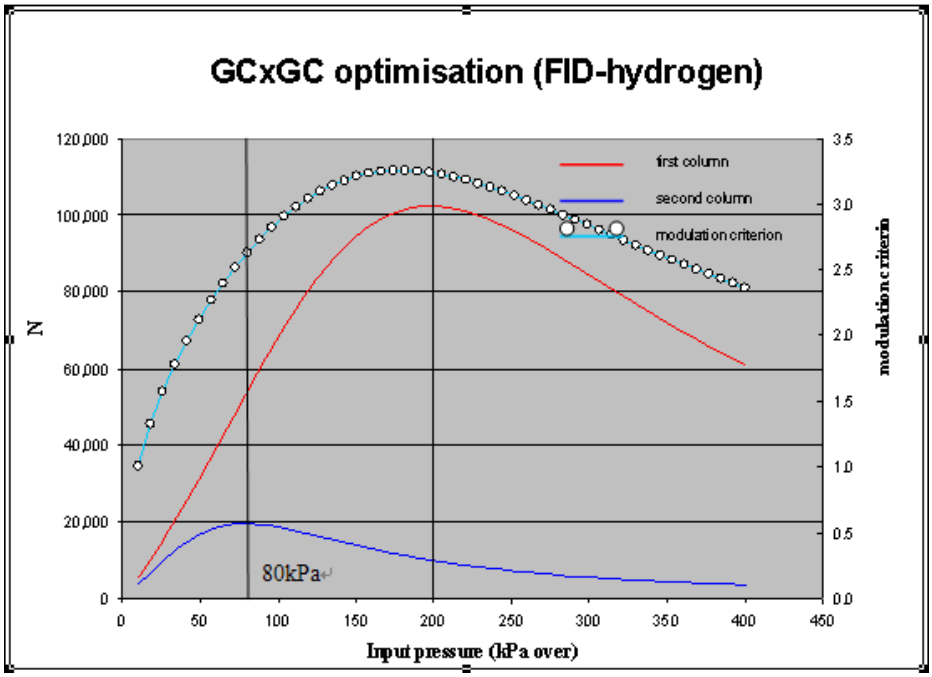

Figure 5. GC×GC optimization diagram (FID-hydrogen)

4.6.2 Initial inlet pressure

In this project, the highest resolution in the second dimension column is needed in order to better define water solubility of the compounds. To achieve this, a compromise had to be made to sacrifice some of the resolution power in the first column since it is not the most important factor in this project. As shown in Figure 5, when highest plate number is achieved in the second column (with inlet pressure of 80kPa), the resolution in the first column is far from its optimal condition (with inlet pressure of 200kPa).

As inlet pressure 80kPa was chosen for the start of system condition optimization. At this inlet pressure, the second-dimension column has the highest plate number which means best resolution.

The modulation criterion is 2.7 at this pressure which is a little higher than required 1.5 (Beens et al., 2005) but still acceptable.

5. Results and discussion

This section is divided into three parts, which based on the three aims and goals. The first part states the column optimization procedure. This part was most time consuming but very important since without an optimal operating condition, good analysis results can not be achieved. The other two parts give the results of Boiling point/LogKow matrix and biodegradation studies.

5.1 Operating condition optimization

Different operating conditions (temperature programming rate and inlet pressure) were tested in order to achieve the best separation in the second column and to avoid wrap-around problem at the end of the chromatogram. As mentioned in 4.6.2, an initial testing condition with an optimal inlet pressure of 80kPa was chosen with a dead time (the time a non-retained compound spends in the column) of 6.42min. The ideal temperature programming rate is 1.558°C/min correspondingly (ideal temperature programming rate=10/dead time).

Five different conditions were tested in the preliminary testing step (without switching on the modulator) and listed in Table 3. (PAHmix was used as standard mixture and BP20 was used as the second dimension column. Injection mode was CT splitless).

Table 4 presents the retention time and peak width of spiked compounds under different inlet carrier gas pressure and temperature programmes.

Comparing the analysis time and peak width variation after different inlet carrier gas pressure and temperature programmes, Condition 5 was chosen as the optimal condition for it has the shortest

Table 3. List of column conditions in the preliminary testing step

No.	Column condition
1	80kPa constant pressure, 40°C for 5min and 1.6°C/min to 270°C for 60min
2	120kPa constant pressure, 40°C for 5min and 1.6°C/min to 270°C for 60min
3	80kPa constant pressure, 40°C for 5min and 2°C/min to 270°C for 60min
4	80kPa constant pressure, 40°C for 5min and 2.5°C/min to 270°C for 60min
5	80kPa constant pressure for 80min then 1kPa/min to 120kPa for 40min, 40°C for 5min and 2.5°C/min to 270°C for 60min

Table 4. Retention time and peak width of spiked compounds in different column conditions (Rt: retention time; PW: peak width. Unit: min)

Spiked compounds	80kPa 1.6°C/min		120kPa 1.6°C/min		80kPa 2.0°C/min		80kPa 2.5°C/min		80-120kPa 2.5°C/min	
	Rt	PW	Rt	PW	Rt	PW	Rt	PW	Rt	PW
naphthalene	50.6	0.47	44.3	0.36	44.9	0.45	40	0.47	40	0.47
fluoranthene	117.3	0.64	109.7	0.47	99.1	0.56	83.9	0.55	83.8	0.51
Benzo(a)pyrene	161.1	0.87	150.8	0.59	136.7	0.97	117.2	0.95	111.8	0.7

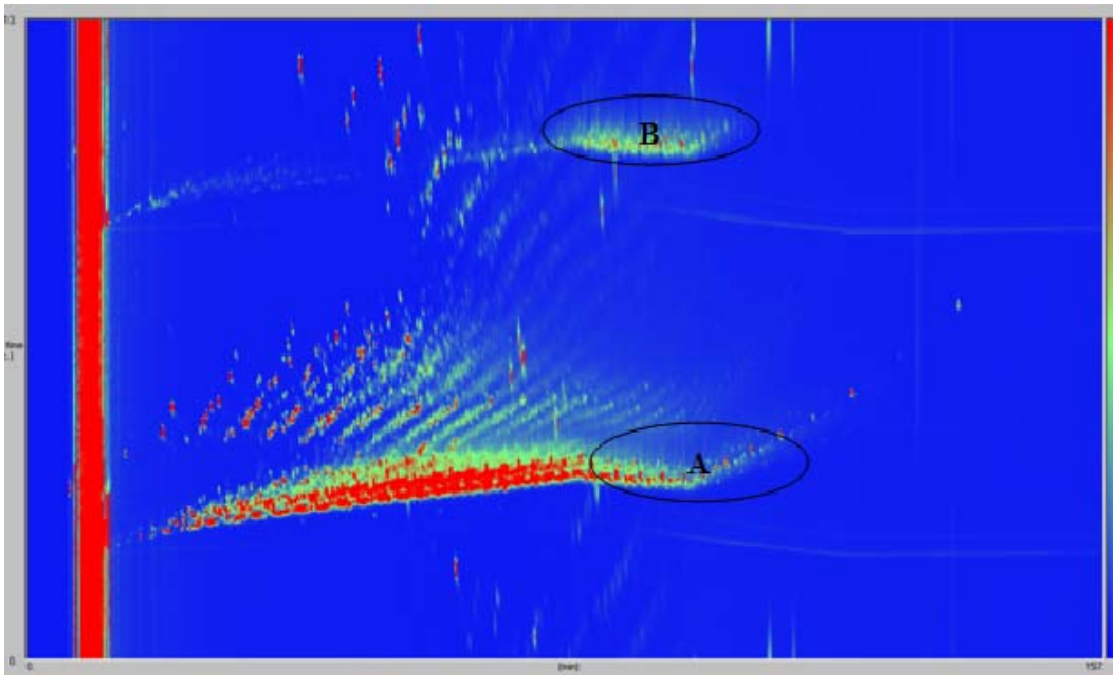

Figure 6. The GC×GC chromatogram of Windowmix under condition 5 (Interpretation of the 2D-GC color contour plots in this report: the X-axis (horizontal) is the retention time in first dimension and the unit is in minutes while the Y-axis (vertical) is the retention time in second dimension and the unit is in seconds. The background color is blue. The color varies from blue to red when the concentrations of components increase.).

analysis time (retention time of Benzo(a)pyrene is only 111.8min) and its peak width variation (0.7/0.47) is also smaller compared to other conditions.

The Windowmix was then used as a standard mixture under Condition 5. This time the modulator was switched on with a modulation time of 13s to show the GC×GC chromatogram (Figure 6).

Obvious wrap-around (compounds with the second dimension retention times exceed the modulation time) problem could be seen from Figure 6. At the end of the chromatogram (area A), there are two times shifts of the chromatogram shape. This was possibly caused by the pressure programming and temperature limitation. When the pressure began to programme, the compounds eluted faster at higher pressure and resulted in a shorter second-dimension retention time. But when the pressure programming was finished, and the temperature reached its maximum for BP20 column (270°C), the elution times of the high boiling point compounds were delayed. It is clearly presented by the GC×GC chromatogram in Figure 6. This shape shift could lead to a discrimination of the high boiling point compounds (Eric Jover et al., 2005). In area B, there is a phenomenon of break through, possibly caused by the problem of the modulator jets.

The severe wrap-around is unacceptable in this project because it not only excludes all the high boiling point compounds but also disturbs the other parts of the chromatogram. A faster temperature programme 4°C/min was tried in order to solve this problem, but there was no obvious improvement of wrap-around (Appendix 4).

The length of the second column had to be cut shorter in order to better solve this problem. Four different column length combinations were simulated using the Microsoft Excel computer model: 30m×1.5m, 30m×1m, 15m×1.5m and 15m×1m. Figure 7(A-D) shows the diagrams of different parameters in these four chosen column combinations.

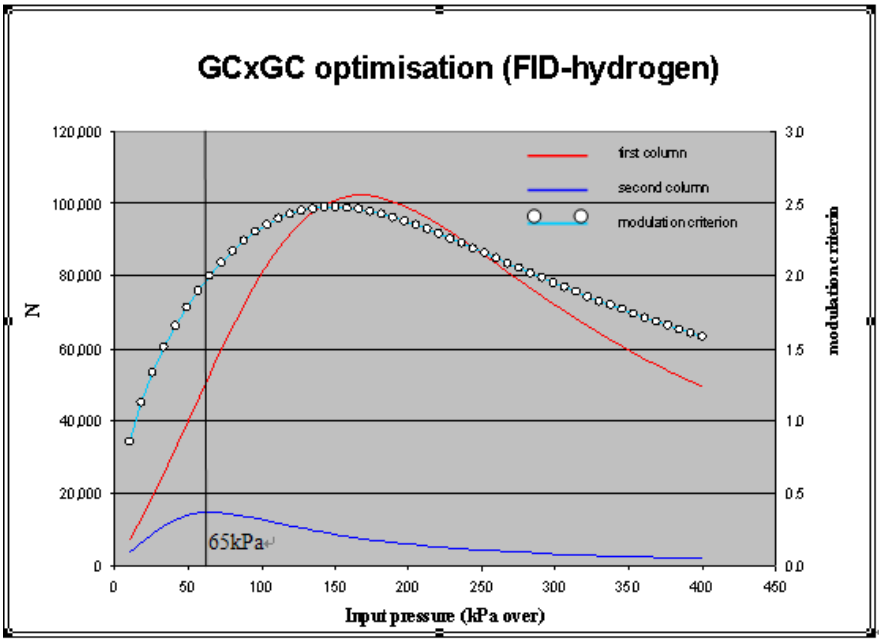

Figure 7(A). GC×GC optimization diagram (30m×1.5m).

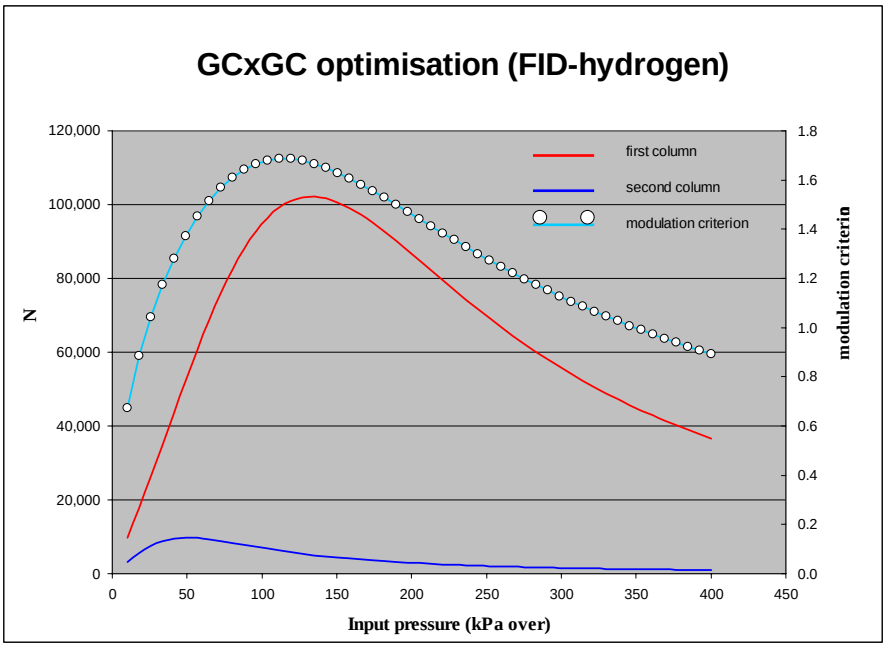

Figure 7(B). GC×GC optimization diagram (30m×1m).

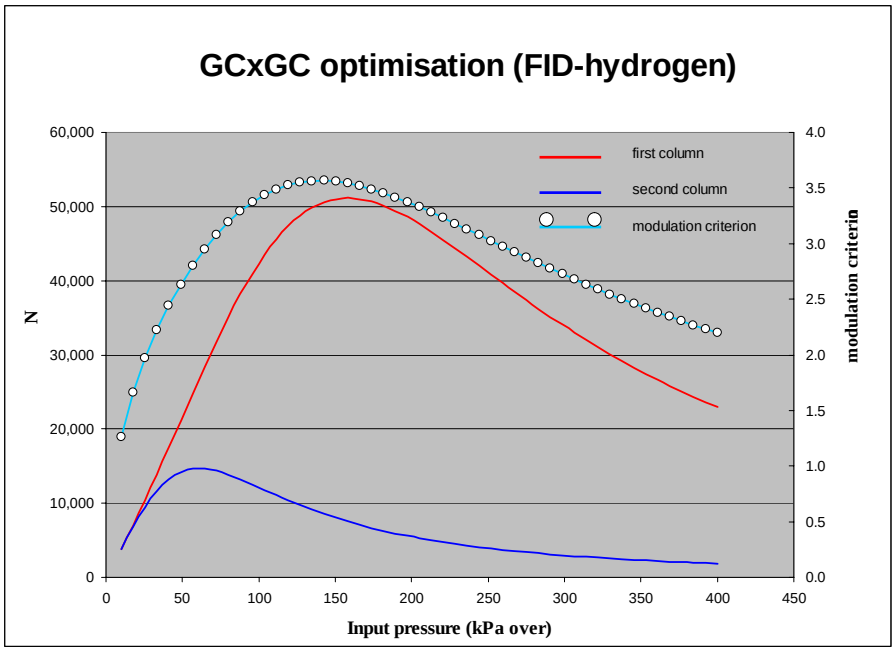

Figure 7(C). GC×GC optimization diagram (15m×1.5m).

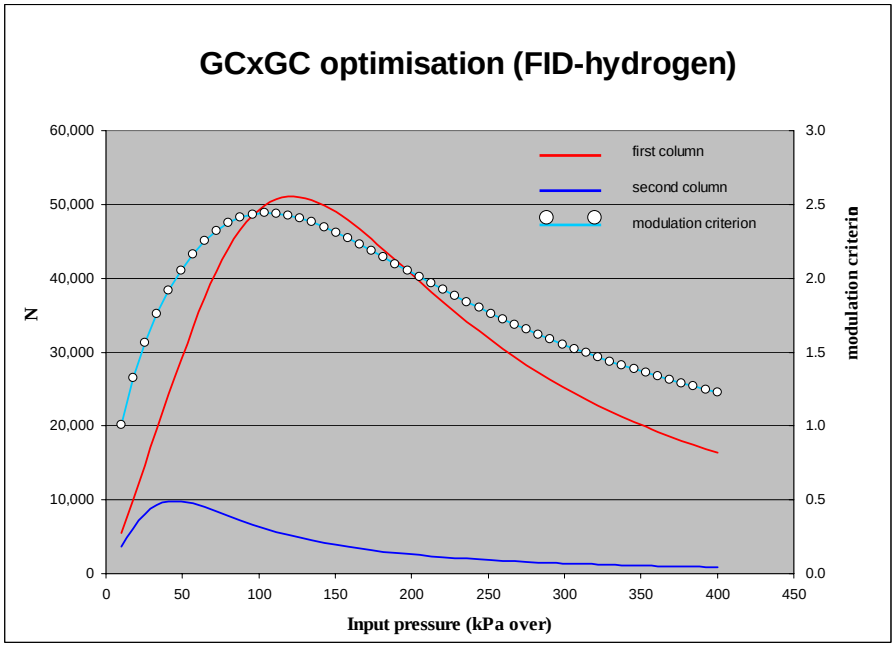

Figure 7(D). GC×GC optimization diagram (15m×1m).

In these four diagrams, A and C have higher plate number (approximate 15000) than B and D (approximate 10000). This is because the longer the second dimension column, the better the separation power. But when the second dimension column was under optimal condition, the modulation criterion was much better for combination A (approximate 1.8) compared to combination C (approximate 2.8). As a result, combination A (30m×1.5m) was selected as the initial column combination for next optimization step. Under this column combination, 70kPa and 2°C/min temperature programming rate were chosen as the initial condition, since at this condition the second column is near to its optimal separation (Figure 7(A)).

Table 5. List of column conditions for further optimization

No.	Column condition
1	1.5m second column length, 70kPa constant pressure, 40°C for 5min and 2°C/min to 270°C for 30min
2	1.5m second column length, 90kPa constant pressure, 40°C for 5min and 3°C/min to 270°C for 60min
3	1m second column length, 70kPa constant pressure, 40°C for 5min and 2.5°C/min to 270°C for 60min
4	1m second column length, 120kPa constant pressure, 40°C for 5min and 2.5°C/min to 270°C for 60min
5	1m second column length, 120kPa constant pressure, 40°C for 5min and 4°C/min to 270°C for 60min
6	1m second column length, 70kPa constant pressure, 40°C for 5min and 4°C/min to 270°C for 60min
7	1m second column length, 70kPa constant pressure, 40°C for 5min and 4°C/min to 280°C for 10min, then 4°C/min back to 270°C for 60min

In order to find the best compromise between separation power in the second dimension and wrap-around problem, a series of experiments were conducted under different column conditions (Windowmix was used as standard mixture and BP20 was used as the second-dimension column. Injection mode is CT splitless and modulation is 13s). Table 5 shows the list of column conditions which were adopted in this step. (The GC×GC chromatograms could be seen in Appendix 5)

Under condition 7, the temperature went up to 280°C, 10°C higher than routine temperature limitation of the BP20 column in order to solve the shape shift problem. For these 7 column conditions, condition 2 had the best separation in second dimension but with an obvious wrap-around problem. Compared to column condition 2, the wrap-around problem was best solved under column condition 7. However, the separation power in the second dimension was less than condition 2.

In this thesis project, the separation power in second dimension was the most important factor to be considered. Condition 2 was chosen as the best to be further tested. In order to solve the wrap-around problem, the modulation time has to be extended, meaning a further sacrifice of separation power in the first column. In order to solve the shape shift problem, a more temperature resistance SolGelwax column was adopted instead of BP20.

Another experiment was conducted using condition 2.1 (1.5m second column length, 90kPa constant pressure, 40°C for 5min and 3°C/min to 300°C for 60min). Compared to condition 2, the SolGelwax column which has a higher temperature limitation of 300°C was adopted, meanwhile the modulation time was doubled to 26s. Instead of 270°C, the final temperature was set up to 300°C. The other condition parameters were kept the same as condition 2. Windowmix was used as standard mixture and the injection mode was changed to PTV splitless in order to avoid discrimination of high boiling point compounds. The GC×GC chromatograms could be seen in Appendix 6.

Table 6. Second dimension retention times of selected compounds in repeatable injections

Injection number	Rentention time of naphthalene(s)	Retention time of acenaphthylene(s)
1	8.350	11.012
2	6.064	8.907
3	5.881	8.438
4	5.736	8.323
5	5.299	7.684
6	4.890	6.951
7	4.364	6.241

Compared to BP20 column under condition 2, the second dimension retention times are much shorter for all the compounds, e.g. the second dimension retention time of naphthalene is only 6.2s compared to 8.4s for BP20, while acenaphthylene is 8.0s compared to 11.1s for BP20. This caused the 2D chromatogram to be more compressed and the peak capacity was not efficiently used. The reason was probably that SolGelwax has less affinity to PAHs than BP20 since the phase is put in a special matrix. A slower temperature programme rate of 2°C/min was used to solve this problem. Meanwhile, the stability of SolGelwax column was also tested by comparison of the GC×GC chromatograms of repeatable injections under the same condition (1.5m second column length, 90kPa constant pressure, 40°C for 5min and 2°C/min to 300°C for 60min).

The second dimension retention times of selected compounds in repeatable injections are shown in Table 6. The second dimension retention time of naphthalene decrease gradually from 8.35s in injection1 to 4.36s in injection7, while acenaphthylene decrease from 11.01s in injection1 to 6.24s in injection7. The GC×GC chromatograms for all the injections are shown in Appendix 7.

The result indicates that Solgelwax column is not a stable column when staying a long time at high temperature of 300°C. Besides, the break through problem persisted existing which may be due to the long modulation time. At a modulation time of 26s, it takes some time for the jets to cool back the column from oven temperature. During this period, some compounds may break through into the second column without trapped by the right jet. Since there is no big difference for the boiling point/Kow matrices of petroleum hydrocarbons built using SolGelwax column and BPX50 column (this will be discussed in next section), BPX50 was regarded as a better second dimension column for soil sample analysis.

A 1.5m BPX50 column was connected with the 30m first dimension RTX-1 column. Based on the Microsoft Excel computer model of this column combination, which is shown in Figure 7(A), the inlet pressure of 65kPa was used at 100°C since at this inlet pressure the second column has the highest plate number. In order to make the run time shorter, a 0.4ml/min constant flow mode was used instead of constant pressure mode.

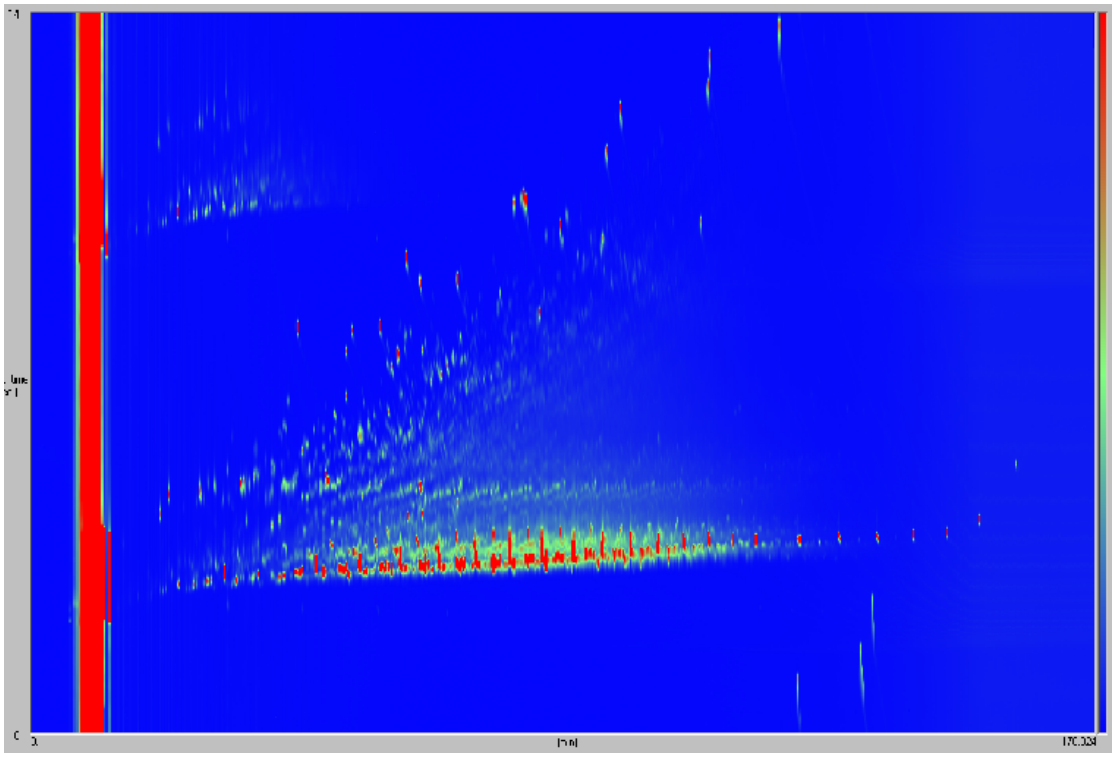

Figure 8. GC×GC chromatogram of Windowmix using 1.5m BPX50 column (Temperature programme: 40°C for 5min and 2°C/min to 330°C for 60min. Modulation time is 14s).

Figure 8 shows the GC×GC chromatogram of Windowmix using BPX50 column. It has a nice separation in the second dimension and only a little break through for low boiling point components. Besides, high boiling point hydrocarbons until C40 could be very well identified. Finally this column combination and operating condition was chosen as an optimal condition to be adopted in biodegradation studies.

5.2 Boiling point/LogKow matrix

This section shows the boiling point/LogKow matrices that were built based on the GC×GC chromatograms. Regularity of the compounds distribution in the boiling point/LogKow matrix is also discussed. The first part of this section gives the boiling point/LogKow matrix for petroleum hydrocarbons while the second part shows the boiling point/LogKow matrix for more polar compounds in order to show the applicability of the regularity for compounds in different organic groups

5.2.1 Boiling point/LogKow matrix for petroleum hydrocarbons

The Windowmix standard was used as the analyte to show the boiling point/LowKow matrix of petroleum hydrocarbons. Column condition 2.1 was used to build a boiling point/LowKow matrix for the SolGelwax column. Instead of FID, the GC×GC system was coupled to a DSQ for identification of the compounds (Appendix 8).

Representative compounds were selected and identified by the DSQ. Retention times and LogKow values of the selected compounds used for building the boiling point/Kow matrix are listed in Appendix 9. Water solubility and LogKow values were searched using Wskowwin v1.41 software (EPIWIN, Meylan, W&Howard.P).

Based on the data in Appendix 9, a boiling point/LogKow matrix could be built in a retention time reference frame as shown in Figure 9. In order to better relate to the GC×GC chromatogram and to give a direct image, the data in Figure 9 was correlated with the original GC×GC-DSQ color contour plot (Figure 10). The 32 selected compounds were identified and related to points the in boiling point/LogKow matrix.

A good correlation between LogKow values and coordinates defined by the first and second dimension retention times could be seen in Figure 9. The compounds with the same LogKow value range were always located in the same area in the retention time reference frame. If Rt1 (first dimension retention time) is the same, the value of LogKow decreases when Rt2 (second dimension retention time) increases. In contrast to this, if Rt2 is the same, the value of LogKow increases when Rt1 increases. In general, the value of LogKow increases following the direction of the arrow.

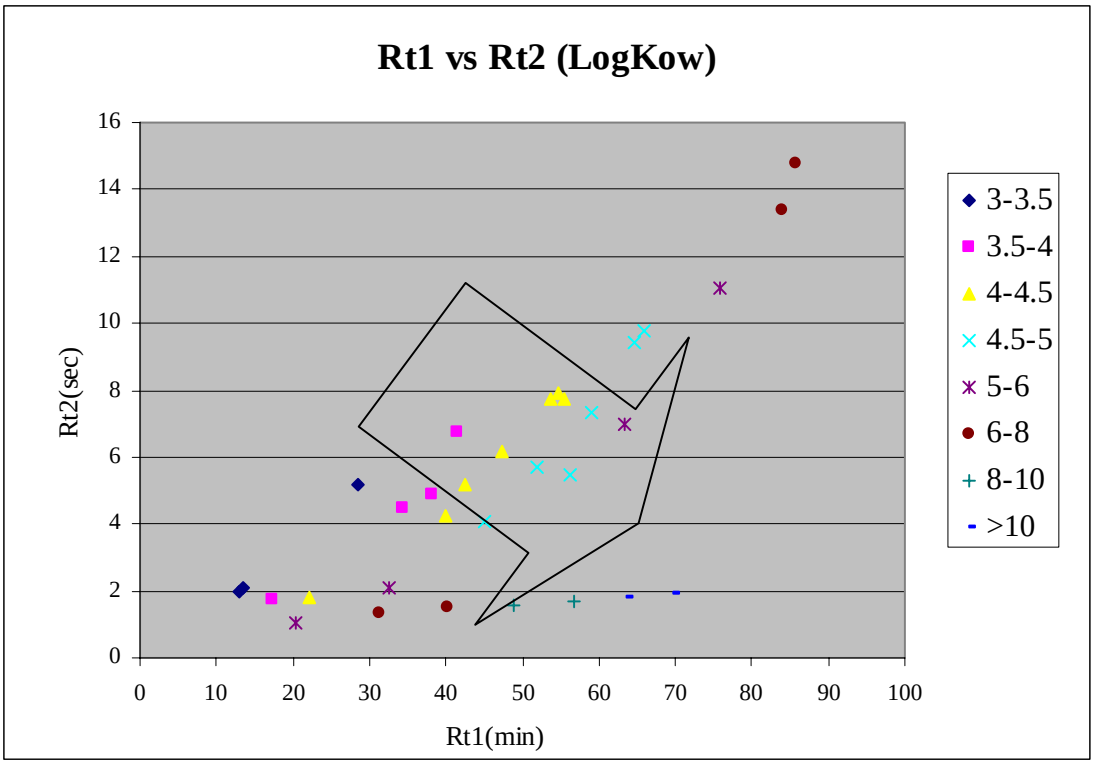

Figure 9. Boiling point/LogKow matrix of the representative compounds from petroleum hydrocarbons (SolGelwax column). The points within different LogKow value ranges are represented by different colors).

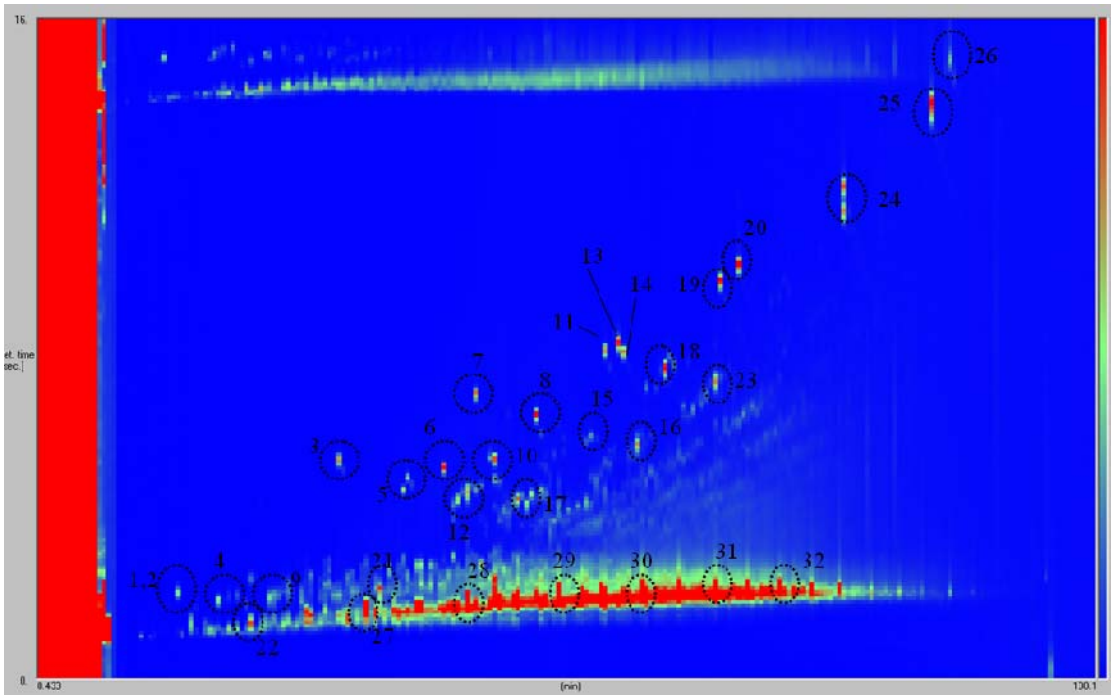

Figure 10. GC×GC-DSQ chromatogram with 100min first dimension retention time (x-axis) and 16s second dimension retention time (y-axis). (SolGelwax column under column condition 2.1)

This regularity could be used to build a standard boiling point/LogKow matrix for petroleum hydrocarbons. If enough petroleum hydrocarbon compounds with LogKow values were identified as points in the boiling point/LogKow matrix, the isoLogKow lines can be defined by a special accessory software in HyperChrom. Points on the same isoLogKow line have the same LogKow value. The 2D matrix can then be divided into different areas that the boundaries of the areas are defined by boiling point lines and isoLogKow lines. The oil polluted soil extracts will be analysed by GC×GC under the same operating condition. The compounds elute within a certain area in the boiling point/LogKow matrix could be regarded as having the boiling point and LogKow within a certain range. This is a much easier way to define the boiling point and water solubility information for the pollutants in soils compared to the conventional methods.

In Figure 11, a simulation example using exponential trend lines instead of isoLogKow lines is presented. The exponential trend lines are approximated as the isoLogKow lines with the average values of the ranges, e.g. exponential trend line of LogKow3-3.5 is assumed as the isoLogKow line of LogKow3.25. Alkanes with different carbon numbers are used to define boiling point boundaries as boiling point lines. The green area (Figure 11) that is defined by the exponential lines of LogKow4-4.5 and LogKow4.5-5 and boiling point lines of C16 and C18 could be regarded having the boiling range from C16 to C18 and the LogKow range from 4.25 to 4.75. All compounds in polluted soil samples that elute within this area will have their boiling point and LogKow value within this range.

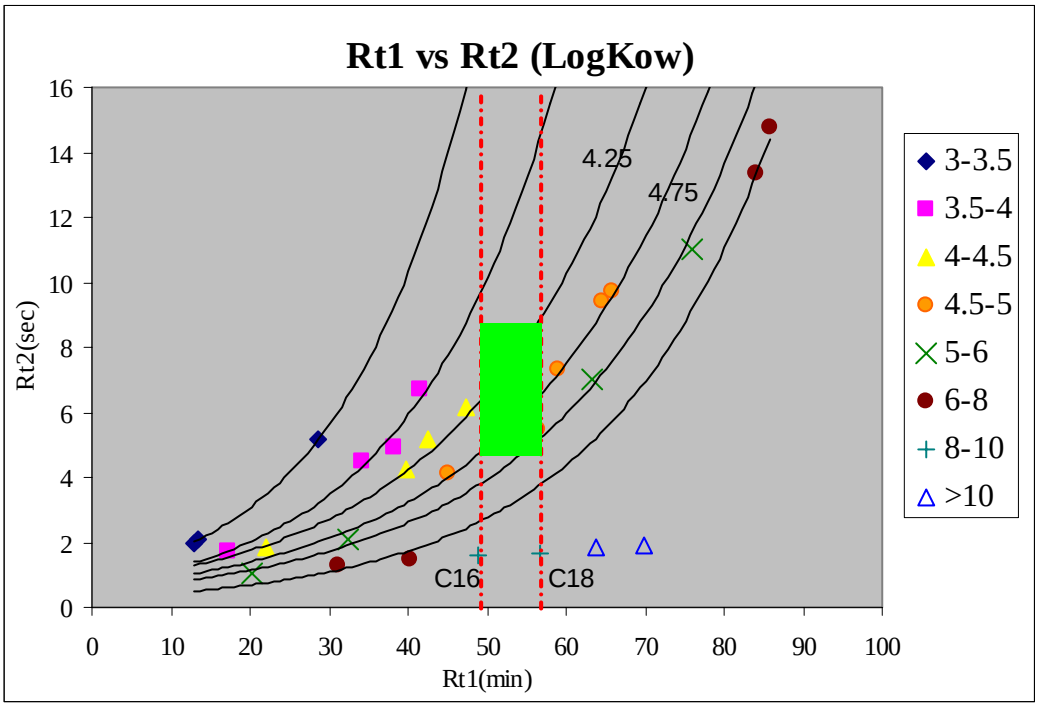

Figure 11. Boiling point/LogKow matrix of the representative compounds from petroleum hydrocarbons (SolGelwax column).

The boiling point/LogKow matrix for petroleum hydrocarbons built using SolGelwax column shows a very good correlation between LogKow values and coordinates defined by the 2D retention times. In order to make a comparison with other type of columns, another boiling point/LogKow matrix for petroleum hydrocarbons was built using BPX50 column. The same compounds as for the matrix built with SolGelwax column were selected for comparison. The compounds together with their retention times were taken from a previous experiment “27okt2004 Windowmix”. The retention times and LogKow values of the selected compounds are listed in Appendix 10.

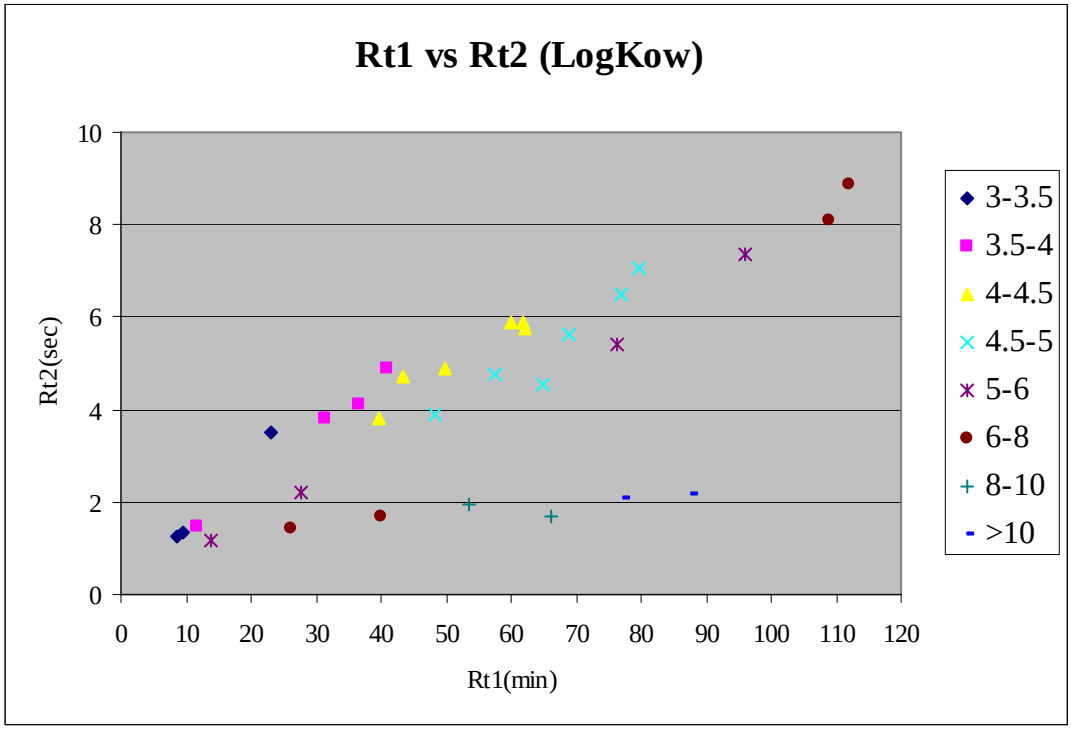

Figure 12. Boiling point/LogKow matrix of the representative compounds from petroleum hydrocarbons (BPX50 column)

The relative positions of the compounds in Figure 12 were almost the same compared to the positions in Figure 9, although some points have a little shift. It means that for petroleum hydrocarbon polluted soil samples, SolGelwax and BPX50 columns are both acceptable to be used for building boiling point/LogKow matrix.

5.2.2 Boiling point/LogKow matrix for compounds in different organic groups

In order to see if the regularity in the boiling point/LogKow matrix of petroleum hydrocarbons was also applicable for compounds in other organic groups, compounds from two previous analysis under the same condition as “27okt2004 Windowmix” were added into the matrix. The retention times, water solubility and LogKow values of the compounds are listed in Appendix 10.

A boiling point/LogKow matrix was built in the retention time reference frame based on the data in Appendix 10 and presented in Figure 13.

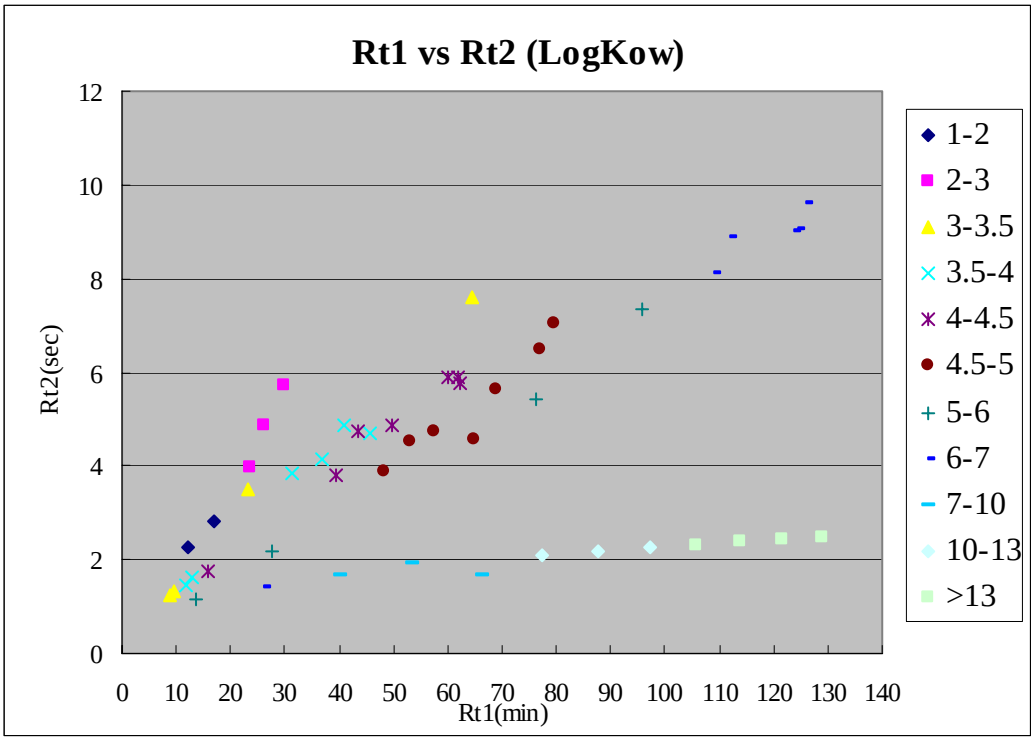

Figure 13. Boiling point/LogKow matrix of the compounds in different organic groups selected from previous experiments (BPX50 column).

The distribution of the points with different LogKow values in the retention time reference frame in Figure 13 does not seem to have regularity. Especially for low boiling point compounds, the points within different LogKow ranges mix together with each other.

The Alcketmix standard was used to further validate the applicability of the regularity since it contains compounds in different organic groups including alcohols, ketones and heterocyclic compounds. Retention times of the compounds were from the GC×GC-DSQ chromatogram of the Alcketmix and the LogKow values from searching in Wskowwin v1.41 software. Figure 14 shows the boiling point/LogKow matrix of these compounds. The related GC×GC-DSQ chromatogram is shown in Appendix 11 (Column condition: 1m BP20 second dimension column, 70kPa constant pressure, 40°C for 5min and 4°C/min to 280°C for 10min, then 4°C/min back to 270°C for 60min).

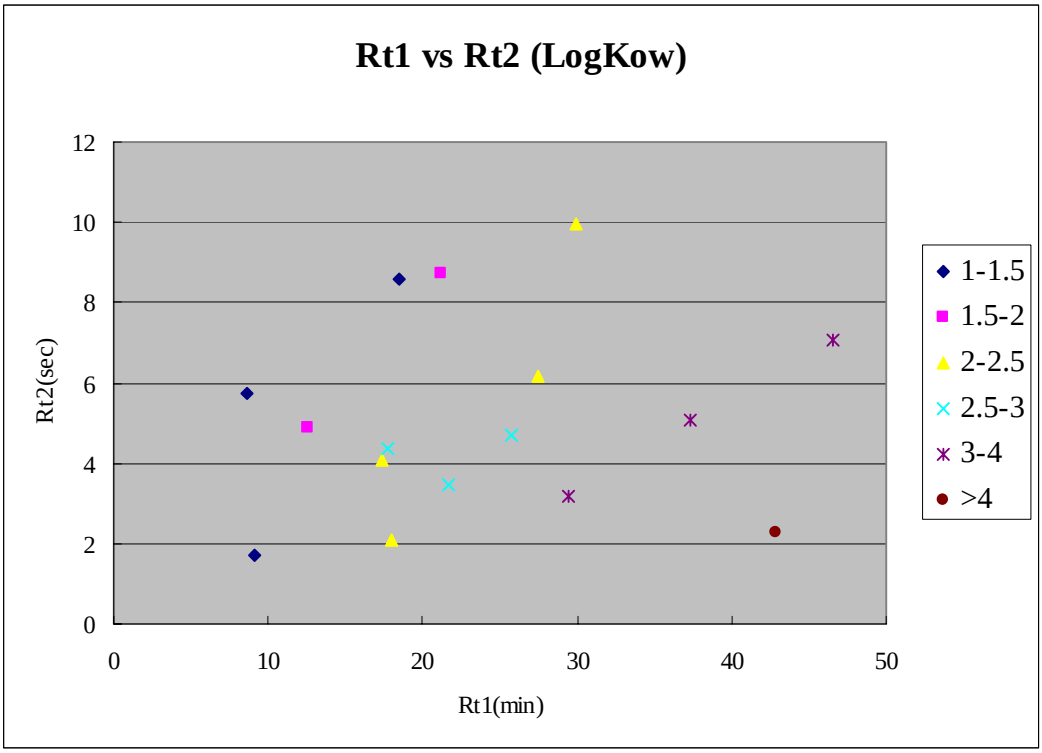

Figure 14. Boiling point/LogKow matrix of the polar compounds in the Alcketmix (SolGelwax column)

LogKow value and compounds distribution in the matrix did not show the same correlation as in petroleum hydrocarbon matrix. Compounds in different organic groups mix together in this matrix. Figure13 and Figure14 shows that the good correlation between LogKow and 2D retention time coordinates for petroleum hydrocarbons could not be applied to compounds in different organic groups. This indicates that for compounds not in the same organic groups, there could be other factors affecting the second dimension elution time besides polarity.

5.3 Biodegradation studies for soil samples

GC-MS was used to analyse the three original sample extracts in order to pick the most interesting sample for biodegradation studies. Soil sample Extra2 was chosen as the most interesting sample since it contained more petroleum hydrocarbons than the other two samples. The second dimension column was a 1.5m BPX50 column with 0.4ml/min constant flow mode and the temperature programme is 40°C for 5min and 2°C/min to 330°C for 20min.

5.3.1 Methods for biodegradation studies

Three window defining methods were developed for the biodegradation studies using Windowmix under this column condition: Group Method, TPH Method and CH Method (see Figure 15). Group Method divided the representative petrochemical compounds in Windowmix into different groups.

It shows a detailed biodegradation result for individual components or component groups. The TPH Method is based on a TPH method developed and tested earlier. The difference is that GC×GC uses “Window defining” to define the aliphatic and aromatic groups which is much easier compared to a common GC-FID (Figure 15(B)). It gives the biodegradation information of aliphatic and aromatic compounds respectively. The CH Method shows the biodegradation information of individual n-alkanes from C9 to C40.

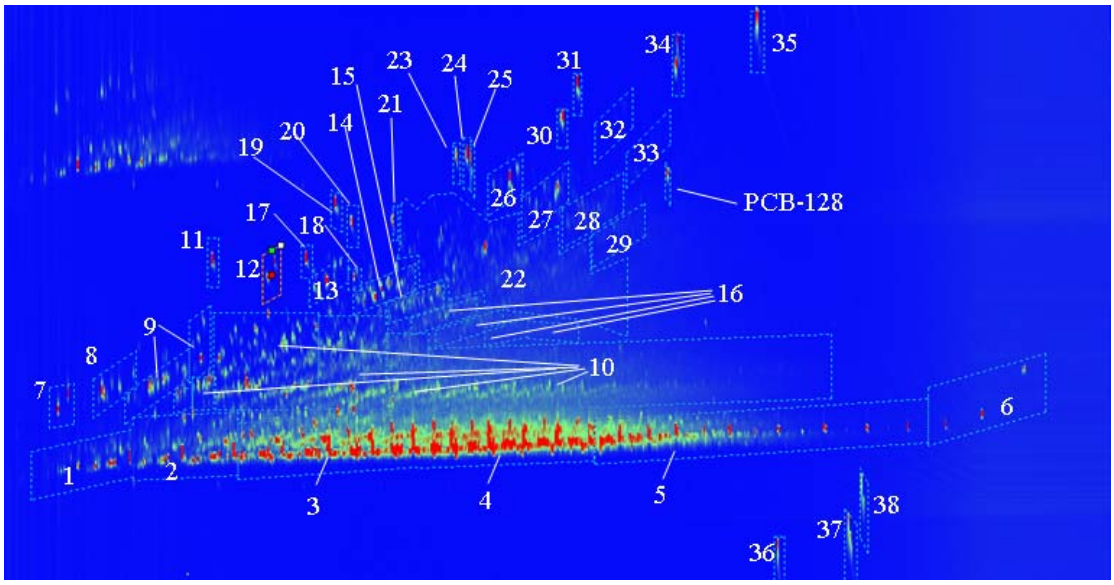

Figure 15(A). Defining windows of Group Method

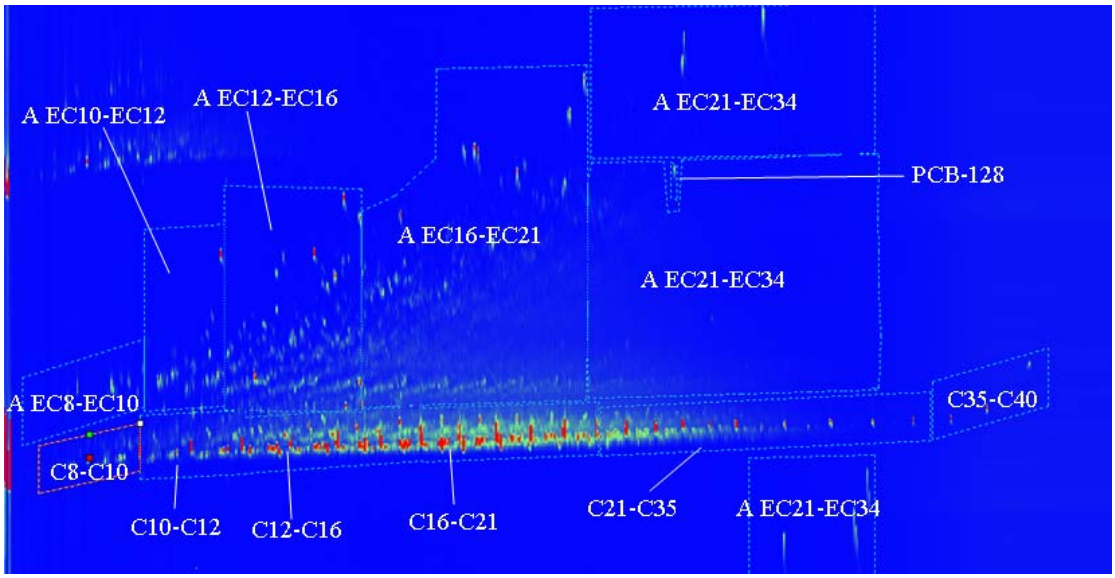

Figure 15(B). Defining windows of TPH Method

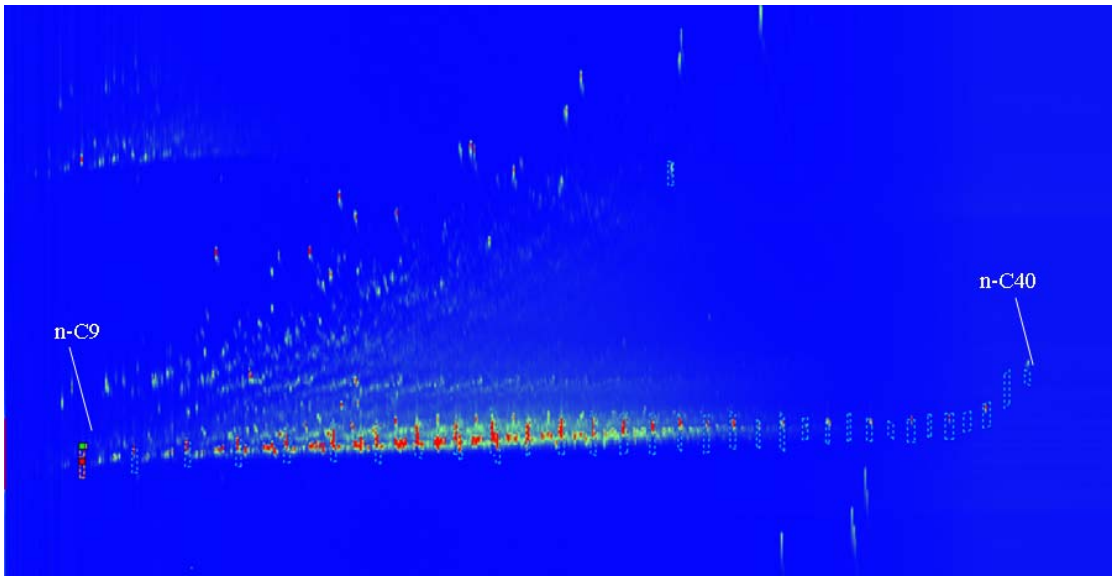

Figure 15(C). Defining windows of CH Method

Component descriptions of the windows in Group Method and TPH Method are listed in Table 7 and Table 8 respectively.

Table 7. Classification of individual components or component groups in Windowmix divided by Group Method

Nr.	Description	Abbreviation	Nr.	Description	Abbreviation
1	Aliphatics n-C8-nC10	C8-C10	20	Acenaphthene	Ace
2	Aliphatics n-C10-nC12	C10-C12	21	Fluorene	Flu
3	Aliphatics n-C12-nC16	C12-C16	22	Alkanefluorenes	CnFlu
4	Aliphatics n-C16-nC21	C16-C21	23	Dibenzothiophene	DBT
5	Aliphatics n-C21-nC35	C21-C35	24	Phenanthrene	Phe
6	Aliphatics n-C35-nC40	C35-C40	25	Anthracene	Ant
7	m/p/o-xylenes	xylenes	26	C1-phenanthrene	C1Phe
8	A9-monoaromatics	A9	27	C2-phenanthrene	C2Phe
9	A10-monoaromatics	A10	28	C3-phenanthrene	C3Phe
10	> A10-monoaromatics	>A10	29	C4-phenanthrene	C4Phe
11	Naphthalene	N	30	Fluoranthene	Fla
12	C1-naphthalenes	C1N	31	Pyrene	Pyr
13	C2-naphthalenes	C2N	32	C1-Pyrene	C1Pyr
14	C3-naphthalenes	C3N	33	C2-Pyrene	C2Pyr
15	C4-naphthalenes	C4N	34	Benzo(a)anthracene +chrysene	B(a)A+Chr
16	>C4-naphthalenes	>C4N	35	Benzo(b)fluoranthene + Benzo(k)fluoranthene	B(b+k)fla
17	Biphenyl	Bif	36	Benzo(a)pyrene	B(a)pyr
18	C1-biphenyls	C1Bif	37	Indole(1,2,3-cd)pyrene +Dibenzo(a,h)anthracene	Ind+Dba
19	Acenaphthylene	Acy	38	Benzo(g,h,i)perylene	B(ghi)per

Table 8. Classification of aliphatic and aromatic groups in Windowmix divided by TPH Methond

Nr.	Description	Abbreviation	Nr.	Description	Abbreviation
1	Aliphatics n-C8-nC10	C8-C10	7	Aromatic equivalent carbon C8-C10	A EC8-EC10
2	Aliphatics n-C10-nC12	C10-C12	8	Aromatic equivalent carbon C10-C12	A EC10-EC12
3	Aliphatics n-C12-nC16	C12-C16	9	Aromatic equivalent carbon C12-C16	A EC12-EC16
4	Aliphatics n-C16-nC21	C16-C21	10	Aromatic equivalent carbon C16-C21	A EC16-EC21
5	Aliphatics n-C21-nC35	C21-C35	11	Aromatic equivalent carbon C21-C34	A EC21-EC34
6	Aliphatics n-C35-nC40	C35-C40			

5.3.2 Biodegradation analysis of soil samples by GC×GC

Original extract of Extra2 and its biodegraded extracts were analysed by GC×GC. The three window defining methods were applied on the GC×GC chromatograms respectively. Equation (2) was used to calculate the concentrations of individual components and component groups defined by windows. The results were listed below. Spots that are not assigned to one of the identified groups are summed as aromatic groups and reported as “not identified”.

Table 9. Results of GC×GC analysis using Group Method (NID: not identified; TAL: total aliphatics; TAR: total aromatics; TPH: total petroleum hydrocarbons)

	concentration (mg/kg)					concentration (mg/kg)			
peak name	original	2weeks	4weeks	6weeks	peak name	original	2weeks	4weeks	6weeks
C8-C10	12	10	3	2	CnFlu	1701	1319	1443	1281
C10-C12	356	344	206	112	DBT	9	0	0	1
C12-C16	4211	3465	3257	2211	Phe	29	0	0	0
C16-C21	7671	6792	6765	4586	Ant	9	0	0	0
C21-C35	4845	3663	4294	3057	C1Phe	82	25	7	5
C35-C40	13	7	18	17	C2Phe	154	108	91	75
Xylenes	1	0	0	0	C3Phe	179	147	46	48
A9	-	-	-	-	C4Phe	141	116	36	44
A10	52	15	4	1	Fla	12	2	1	1
>A10	8931	7677	7549	6659	Pyr	5	4	3	3
N	1	0	0	0	C1Pyr	42	17	12	14
C1N	31	0	0	0	C2Pyr	43	47	18	24
C2N	179	15	3	2	B(a)A+Chr	2	1	3	1
C3N	295	122	51	49	B(b+k)fla	0	0	0	0
C4N	290	199	141	141	B(a)pyr	0	0	0	0
>C4N	683	666	691	622	Ind+Dba	0	0	0	0
Bif	1	0	0	0	B(ghi)per	1	1	1	0
C1Bif	14	1	0	1	NID	2021	1541	1346	1500
Acy	0	0	0	0					
Ace	3	1	0	0	TAL	**17108**	**14281**	**14543**	**9986**
Flu	23	12	0	9	TAR	**14935**	**12037**	**11449**	**10483**
					TPH	**32043**	**26318**	**25991**	**20469**

Table 10. Results of GC×GC analysis using TPH Method

	concentration (mg/kg)			
peak name	original	2weeks	4weeks	6weeks
C8-C10	12	8	3	1
C10-C12	348	282	190	110
C12-C16	4154	3412	3035	2196
C16-C21	7752	6267	6635	5210
C21-C35	4931	3831	4336	3348
C35-C40	-	-	-	-
Total aliphatics	**17199**	**13800**	**14200**	**10865**
A EC8-EC10	-	-	-	-
A EC10-EC12	141	57	21	9
A EC12-EC16	2302	1411	1232	933
A EC16-EC21	7702	6368	5759	5009
A EC21-EC34	4264	3897	3765	3512
Total aromatics	**14408**	**11733**	**10777**	**9464**
TPH	**31607**	**25533**	**24977**	**20329**

Table 11. Results of GC×GC analysis using CH Method

Peak name	concentration (mg/kg)					concentration (mg/kg)			
	original	2weeks	4weeks	6weeks	peak name	original	2weeks	4weeks	6weeks
n-C9	0	-	0	-	n-C26	151	70	85	25
n-C10	1	2	0	-	n-C27	78	46	66	15
n-C11	15	8	1	0	n-C28	45	36	47	12
n-C12	66	36	11	2	n-C29	35	23	25	5
n-C13	139	88	30	2	n-C30	22	11	14	4
n-C14	223	159	51	8	n-C31	13	6	10	2
n-C15	270	118	84	13	n-C32	5	2	4	1
n-C16	347	208	109	16	n-C33	3	2	2	1
n-C17	400	238	101	27	n-C34	1	1	1	0
n-C18	486	227	250	36	n-C35	1	1	1	0
n-C19	322	493	246	36	n-C36	0	0	0	0
n-C20	548	376	274	34	n-C37	0	0	0	0
n-C21	481	324	159	41	n-C38	0	0	0	0
n-C22	272	306	196	34	n-C39	-	0	0	0
n-C23	311	269	131	34	n-C40	1	-	0	0
n-C24	214	237	113	39	pristane	**242**	**222**	**234**	**125**
n-C25	197	147	143	15	phytane	**295**	**173**	**261**	**184**

It can be easily seen from Table 9 that the most abundant petrochemical pollutants exist in Extra2 are aliphatics C12-C35 and > A10-monoaromatics. About 25%-50% of these compounds have been biodegraded after 6 weeks. 30 percent of the aromatics have disappeared compared to 42 percent of the apliphatics. The biodegradation of total petroleum hydrocarbons is 36%. Within the same type of compounds (aliphatics or aromatics), the high boiling point compounds seem more difficult to biodegrade, which means the higher of the boiling point the lower of the biodegradation speed, e.g. after 6 weeks biodegradation, C2-naphthalenes almost disappeared while >C4-naphthalenes almost stayed stable. This regularity is more obvious in the TPH Method. Figure 16 shows the biodegradation percent of original concentrations for both aliphatic and aromatic compounds using the TPH Method. For aliphatic compounds, low volatility alkanes (C8-C10) had almost 90% biodegraded while high boiling point alkanes (C21-C35) had only 32% disappeared. The difference is clearer for aromatic compounds where low boiling point aromatics (A EC10-EC12) had more than 90% biodegradation which is much more biodegradable than high boiling point aromatics (A EC21-EC34) with less than 20% biodegradation.

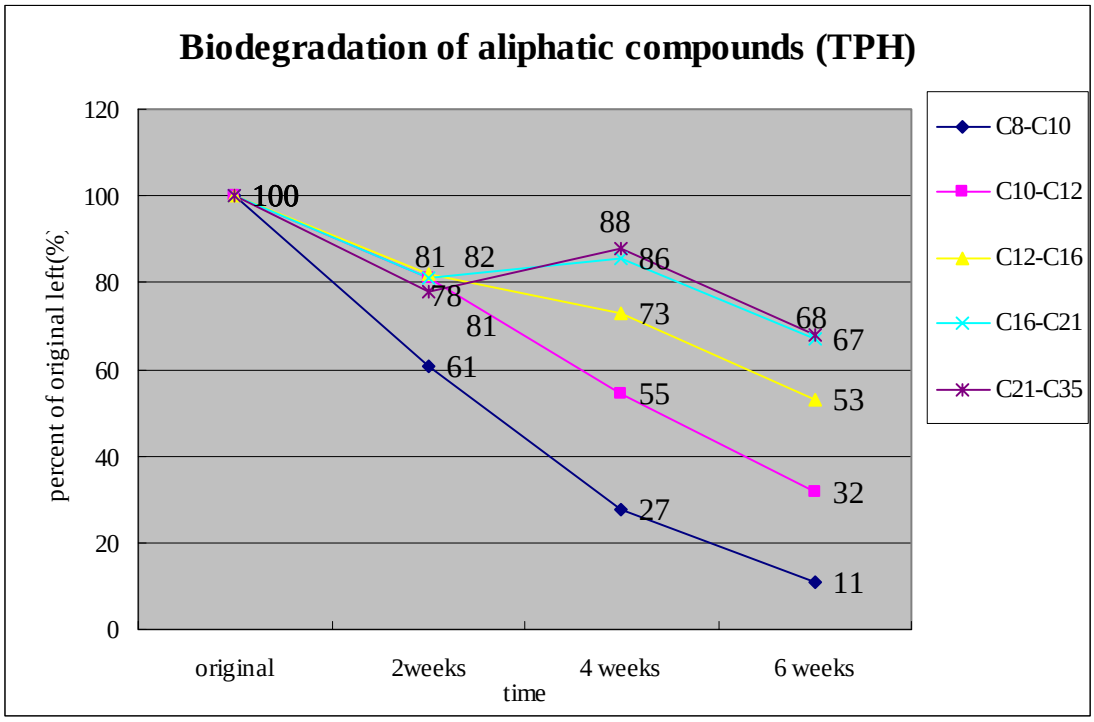

Figure 16(A). Biodegradation percent of original concentration for aliphatic compounds using TPH Method

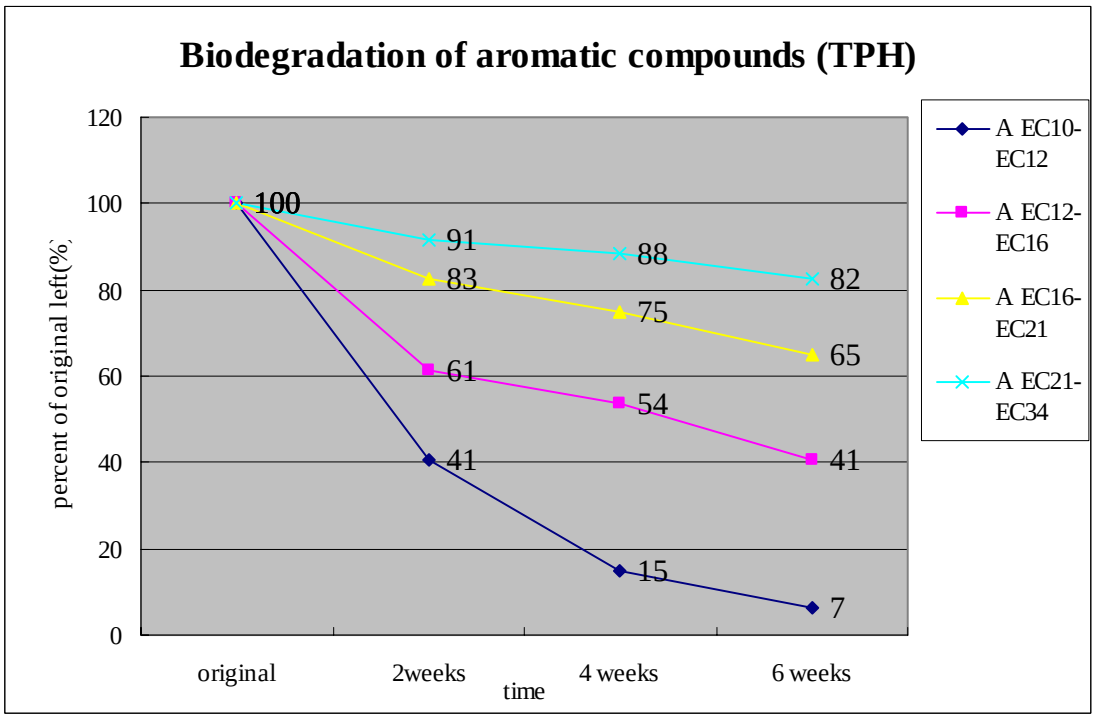

Figure 16(B). Biodegradation percent of original concentration for aromatic compounds using TPH Method

Table 12 shows the comparison of the results from the Group Method and the TPH Method for total aliphatics, total aromatics and total petroleum hydrocarbons biodegradation results. The results from the two methods match very well with each other. The advantage of the Group

Method is clearly showed in Table 9 and Table 10. With the TPH Method only a boiling point distribution of aromatics was obtained while with Group Method, it gives a more detailed result and allows to make a distinction between major chemical classes of (poly)aromatic hydrocarbons.

Table 12. Biodegradation results of total aliphatics, total aromatics and total petroleum hydrocarbons for both Group Method and TPH Method

Group Method	Concentration (mg/kg)				TPH Method	Concentration (mg/kg)			
	original	2weeks	4weeks	6weeks		original	2weeks	4weeks	6weeks
TAL	17108	14281	14543	9986	**TAL**	17199	13800	14200	10865
TAR	14935	12037	11449	10483	**TAR**	14408	11733	10777	9464
TPH	32043	26318	25991	20469	**TPH**	31607	25533	24977	20329

Figure 17 shows the biodegradation results of individual n-alkanes using the CH Method. It can be seen from Figure 17 that the most abundant n-alkanes exist in the soil as pollutants are from C13-C27. About 74%-99% of the n-alkanes had been biodegraded after 6 weeks. Compared to n-alkanes, the biomarkers (pristane and phytane) were only 38% and 48% biodegraded respectively. This indicates the fact that linear n-alkanes are more biodegradable than alkanes with branches. For C19, the concentration after 2 weeks biodegradation is even higher than original concentration which may be due to a little shift of C19 in the original chromatogram. This could cause C19 less integrated in the original extracts and led to a lower concentration. The strange concentration jump for phytane and pristane between 2 weeks and 4 weeks was due to the fact that the individual peaks were not fully separated in the 1st dimension from the adjacent n-alkanes (Some 1D separation was sacrificed by choosing a long modulation time to have better 2D separation) and in this way the peak integration and quantification of the individual phytane and pristane was compromised and less reproducible.

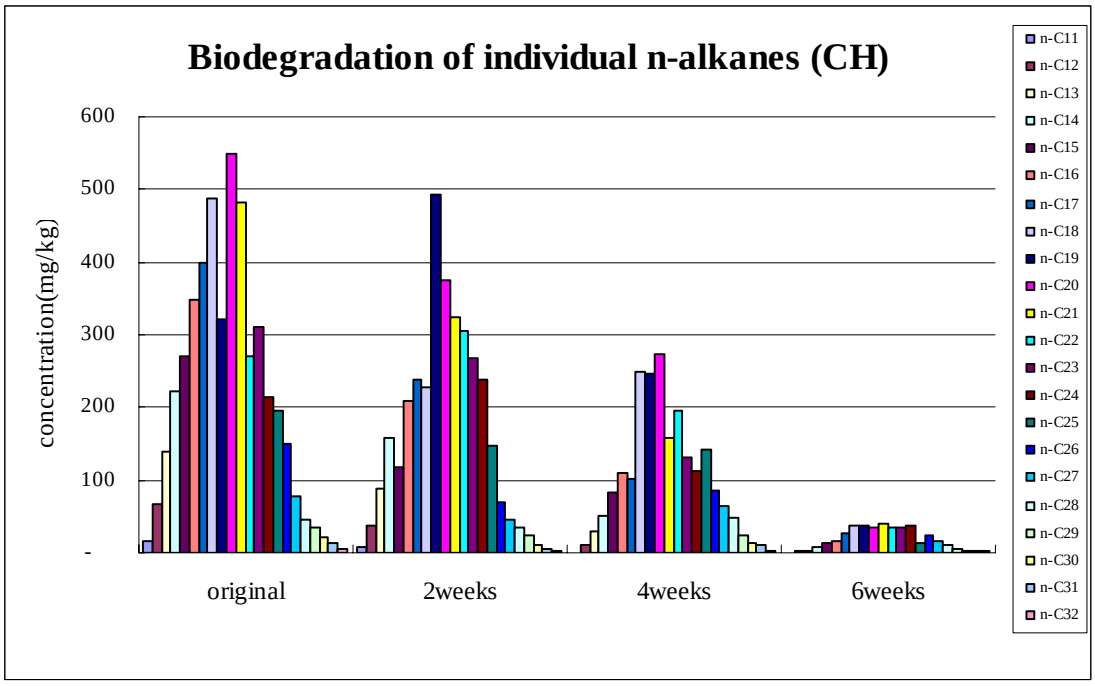

Figure 17. Biodegradation of individual n-alkanes (From C11-C32) using CH Method

6. Conclusions

In this thesis project, comprehensive two-dimensional gas chromatography was evaluated as a novel analytical tool for characterisation of polluted soils. Under the optimal column combination and column condition, GC×GC shows more powerful and gives much more information about the pollutants in contaminated soils compared conventional GC. This information could be regarded as very useful information in soil remediation projects.

SolGelwax column was supposed to be better as second-dimensional column compared to BPX50 for investigating water solubility of pollutants in contaminated soils. But due to its instability at high temperature, BPX50 column was preferred to be used in this project. It showed comparable results as SolGelwax column for building the boiling point/LowKow matrix for petrochemical pollutants. A compromise had to be made between separation power and wrap-around problem of high boiling point compounds in second dimension in order to find an optimal operating condition.

There was a good correlation between LogKow values and the retention time coordinates in the boiling point/LowKow matrix for petroleum hydrocarbons. IsoLogKow lines could be drawn and the 2D chromatogram will be divided into different areas with different boiling points and LogKow values. The components from petrochemical contaminated soil samples elute in the same area will have the same boiling point and LogKow range. This regularity could be very useful in defining water solubility and volatility of the petrochemical pollutants in soils. For more polar hydrocarbons, the regularity was not obtained.

For biodegradation studies, GC×GC was more powerful than conventional GC. The advantage of GC×GC was that it gave a more detailed results and obtained the possibility to make a distinction between major chemical classes of (poly)aromatic hydrocarbons. The methods developed by GC×GC are faster, easier and more suitable for analysis of large number of soil samples.

To further investigate GC×GC as a tool for contaminated soil characterisation, more work need to be done in the future. More points could be added into the boiling point/LowKow matrix in order to draw the isoLowKow lines. A high temperature resistant second dimension column needs to be developed with the separation is based on polarity. It may lead to better result in the boiling point/LowKow matrix. For soil extractions, extraction recovery needs to be considered. It means adding internal standard directly into the soil sample to avoid components loss during extraction. In future biodegradation studies, the fraction of organic carbon (f_{oc}) could be obtained by ignition of soil samples, then the soil/water partition coefficient (Kp) could be calculated by multiply Kow with $f_{oc.}$

As a relative new technique, GC×GC shows a very promising future in environmental analysis. With improvement of the modulator and second dimension column, GC×GC will have application potential not only in soil characterisation but also in the other environmental areas.

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Appendix

Appendix 1

Soil sample (Extra 2) weights for ASE extraction

Sample	Soil weight	Total weight with drying agent	Total Intake ASE	Soil Intake ASE
Extra 2 original	10.00	15.00	13.95	9.30
Extra 2 2 weeks	15.35	20.24	20.06	15.21
Extra 2 4 weeks	10.93	15.92	15.83	10.87
Extra 2 6 weeks	15.22	20.27	20.17	15.14

Appendix 2

Relative response factors of compounds in Calibrationmix

	µg/ml	µg/g	Area	RRF
n-C12	25.42	39.10	28253280	4.42
n-C14	25.96	39.94	28668490	4.39
n-C18	26.33	40.51	31012778	4.69
3-ethyltolueen	19.05	29.31	20634790	4.31
diethylbenzenes	19.33	29.74	18033270	3.71
1,2,4-triethylbenzeen	17.95	27.62	21852750	4.84
phenyloctaan	19.97	30.72	26177120	5.22
p-xyleen	18.69	28.75	21223574	4.52
o-xyleen	21.80	33.54	23683056	4.32
som o+p-Xyleen	40.49	62.29	44906630	4.41
bifenyl	17.86	27.48	21683880	4.83
dibenzothiofeen	17.22	26.49	17296080	4.00
int. std.	30.0		7537863	

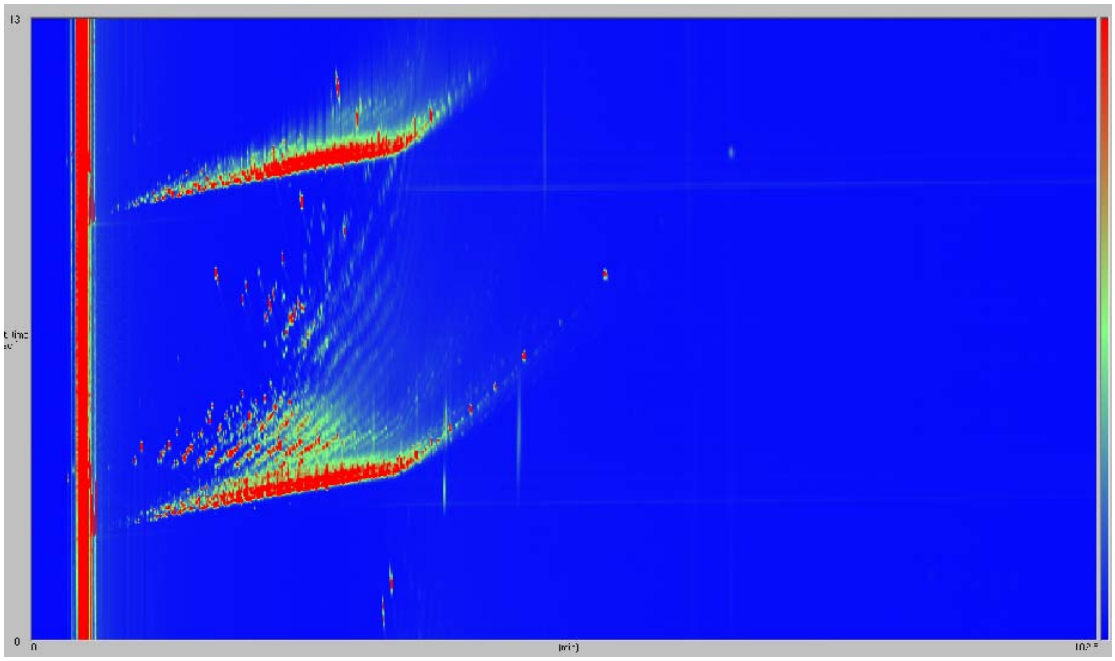
Appendix 3

Weight concentrations of PCB-128 and the other factor values of Extra2

Sample	m_s (g)	D (%)	m_{50ml} (g)	m_{1ml} (g)	C_{is} (mg/kg)
Extra 2 original	9.30	82.88	33.72	0.682	192.43
Extra 2 2 weeks	15.21	92.90	35.45	0.694	108.43
Extra 2 4 weeks	10.87	88.95	35.21	0.701	155.86
Extra 2 6 weeks	15.14	86.74	36.24	0.705	117.39

Appendix 4

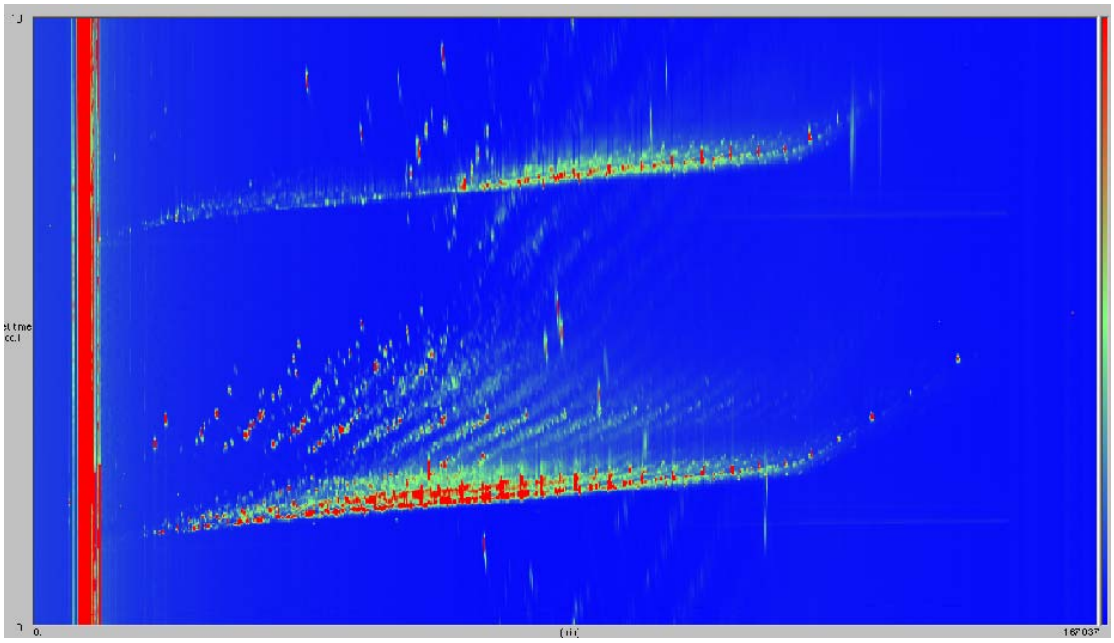
Column condition:
2m second column length, 80 kPa Hydrogen constant pressure, 40 °C for 5 min; then 4 °C/min to 270 for 120min.



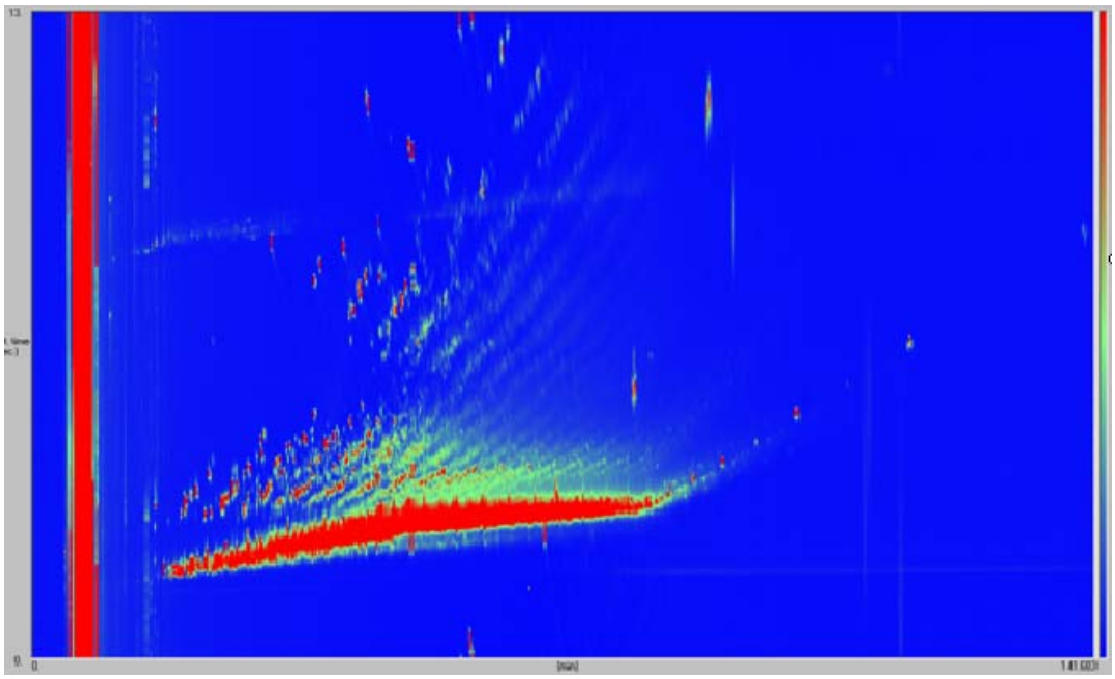
Appendix 5

2D chromatograms of further optimization

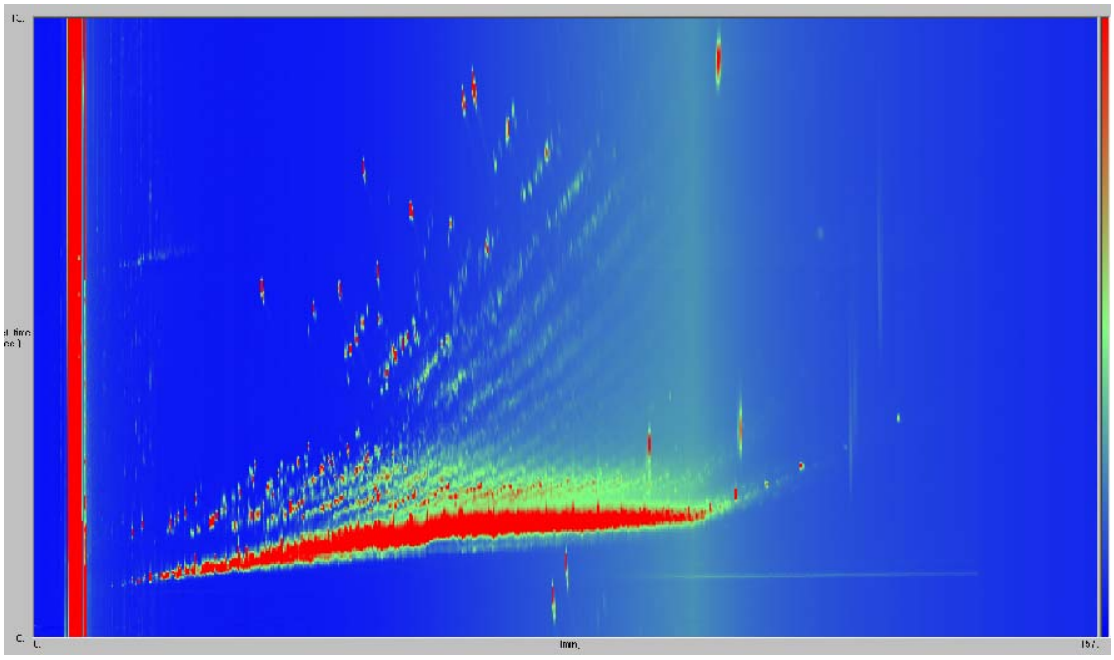
Condition 1:



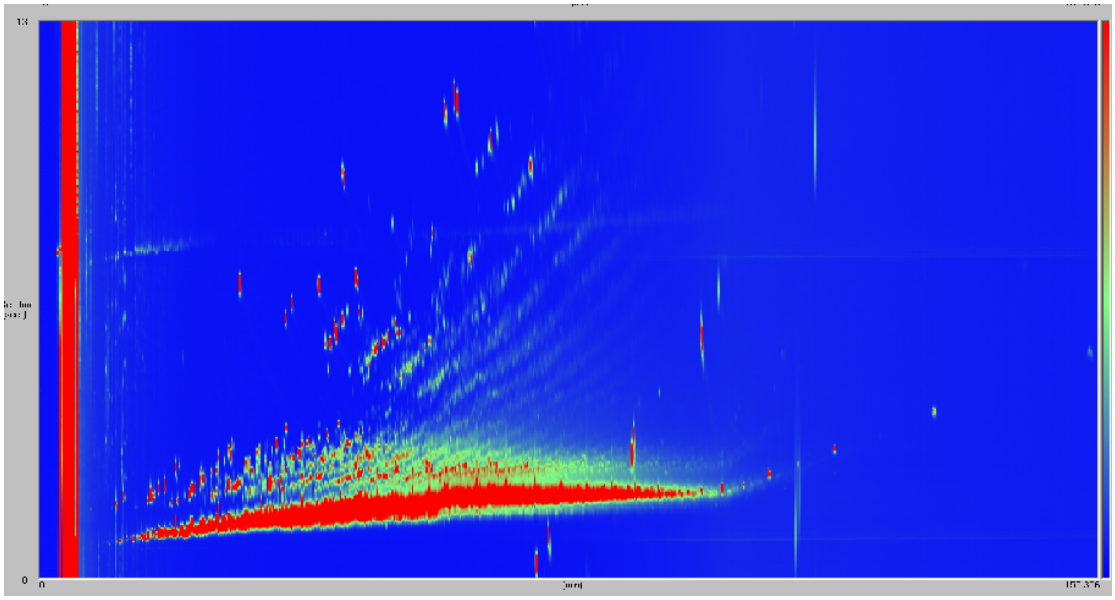
Condition 2:



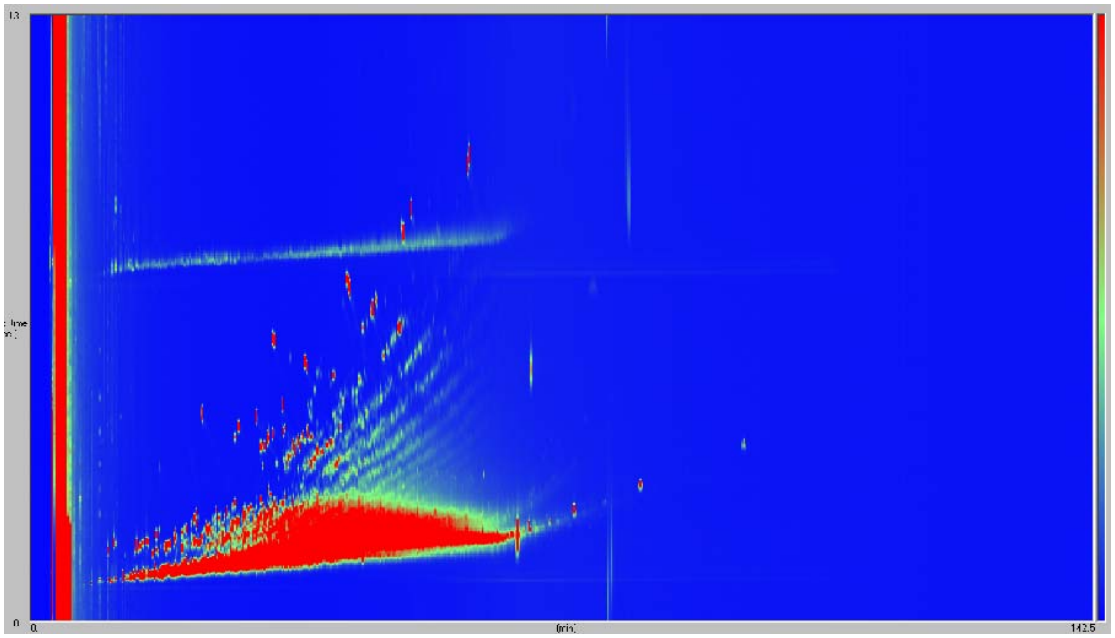
Condition 3:



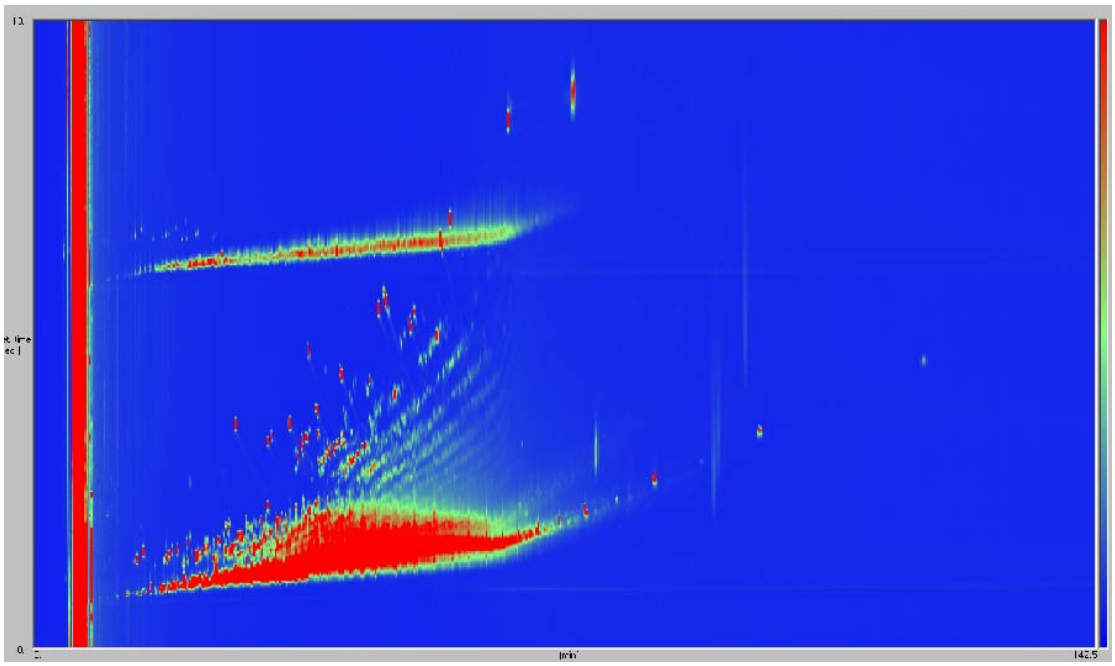
Condition 4:



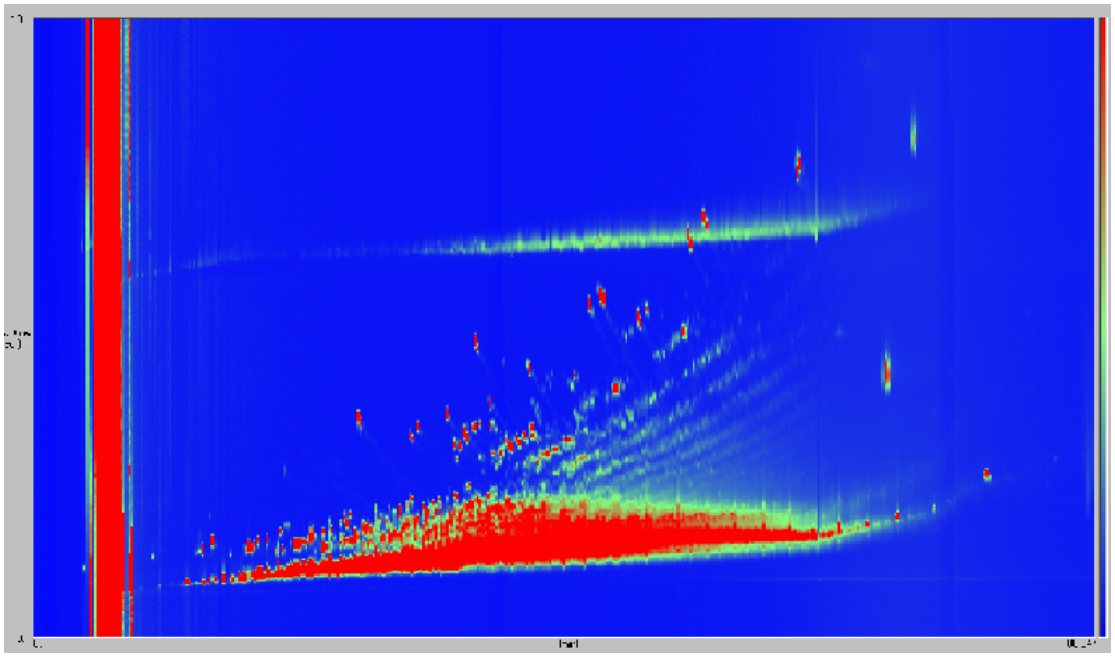
Condition 5:



Condition 6:

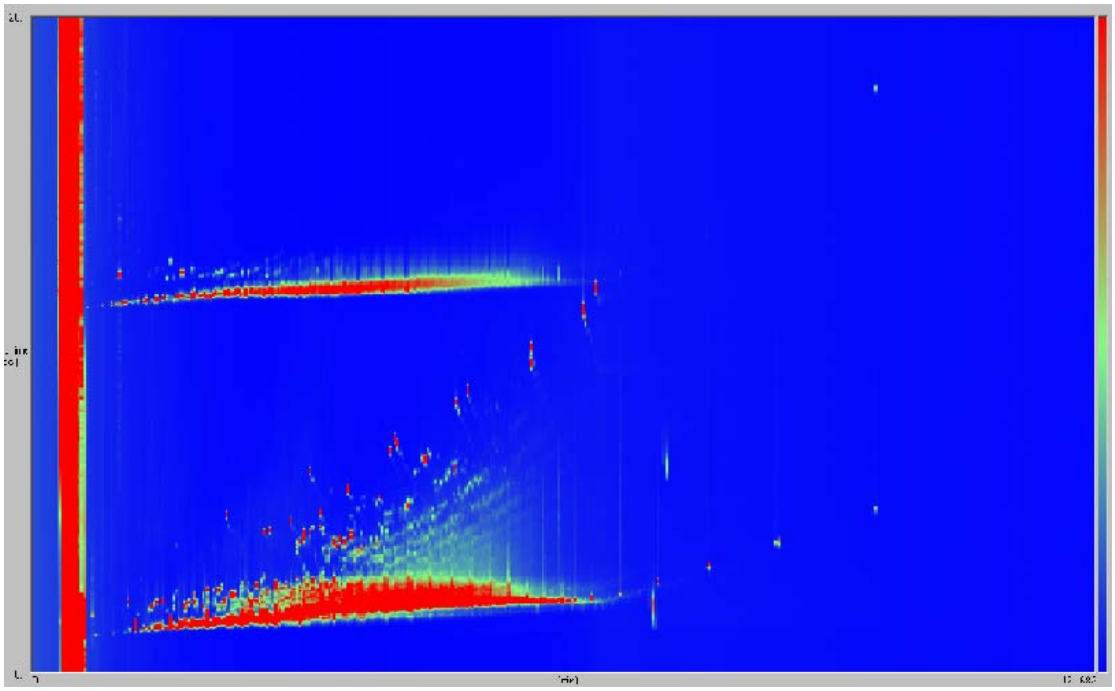


Condition 7:



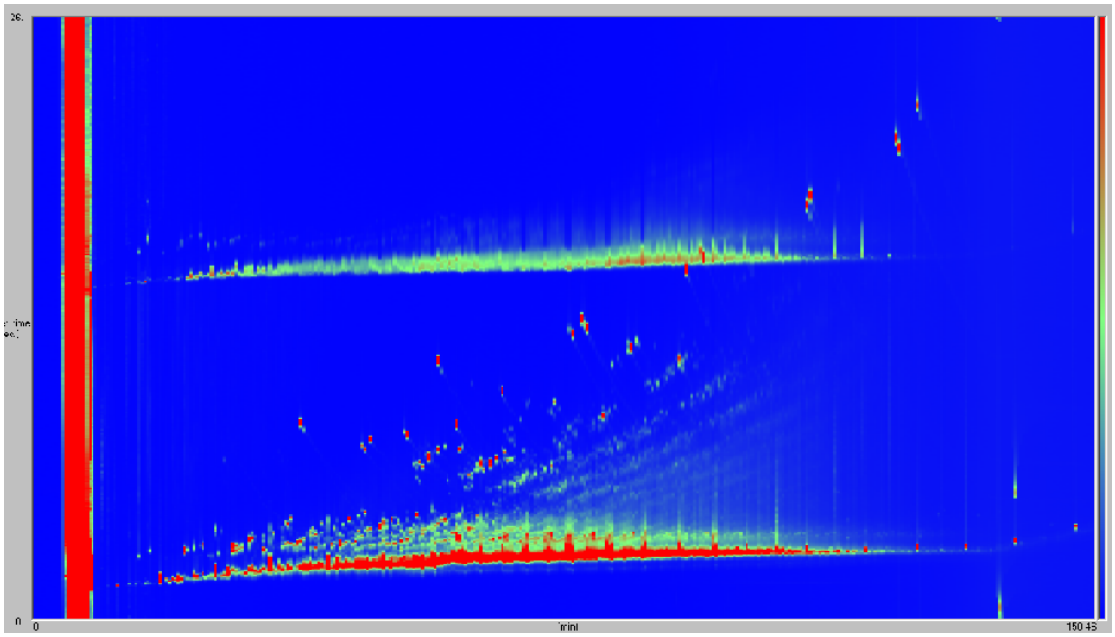
Appendix 6

GC×GC chromatogram under condition 2.1

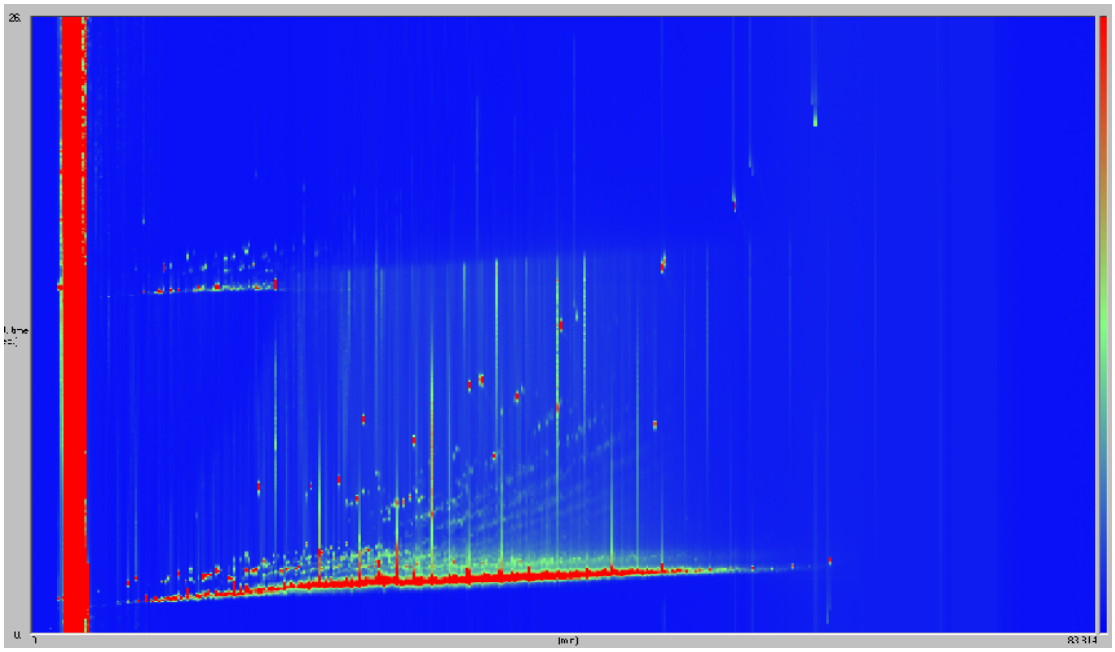


Appendix 7

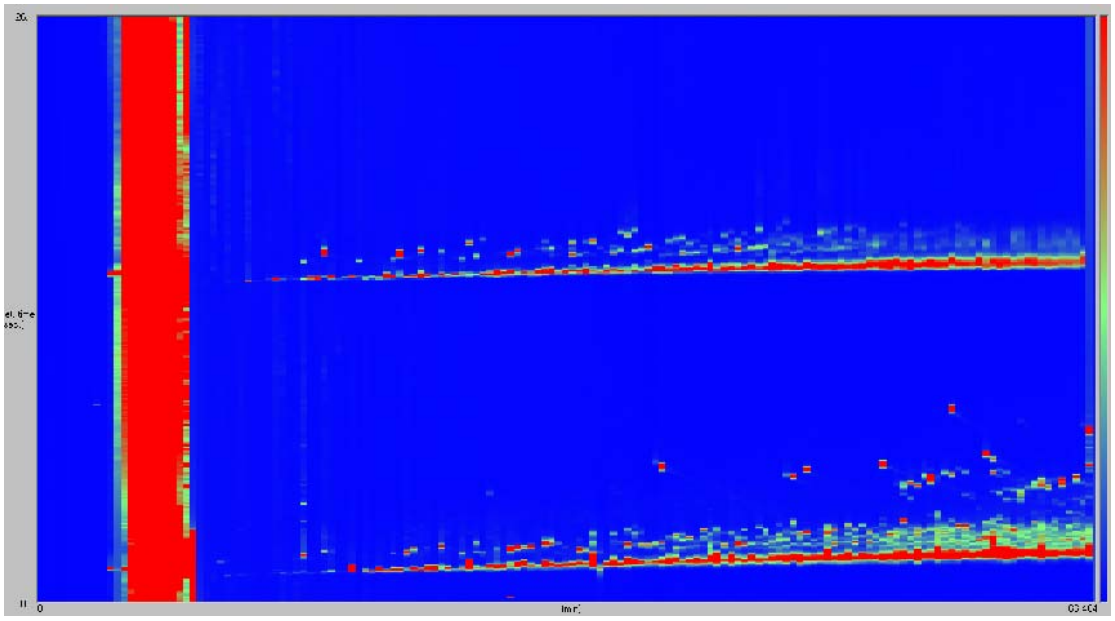
Injection 1:



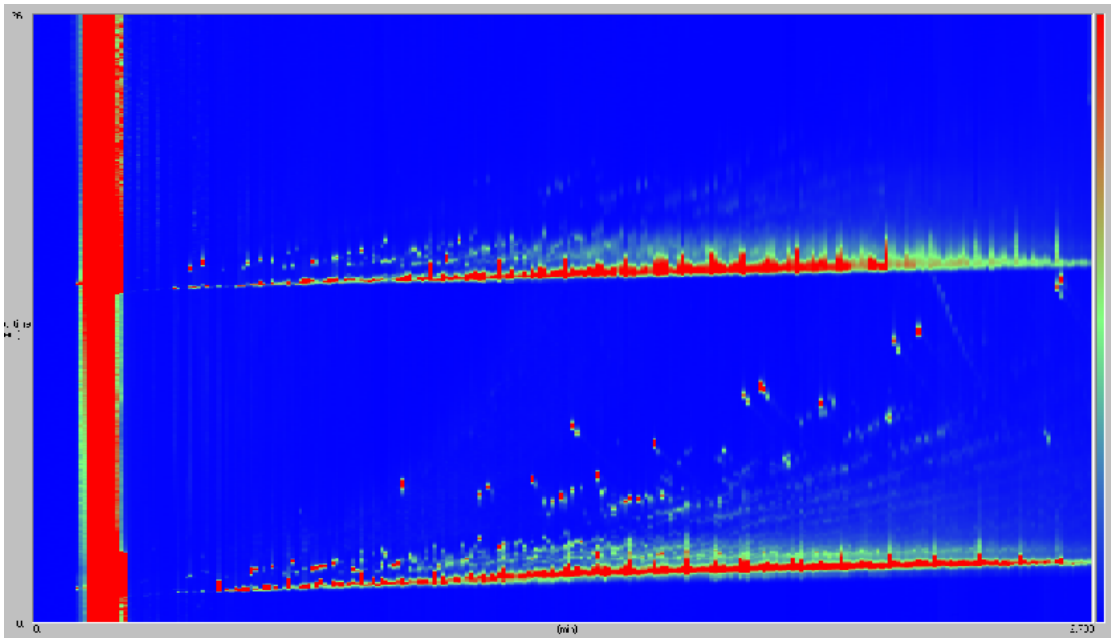
Injection 2:



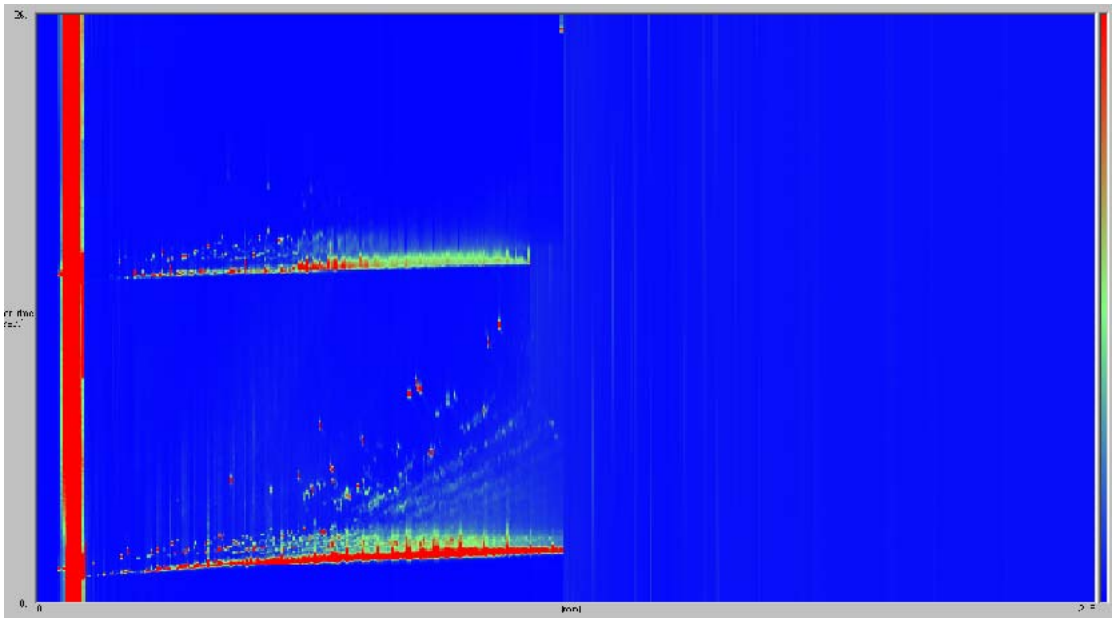
Injection 3:



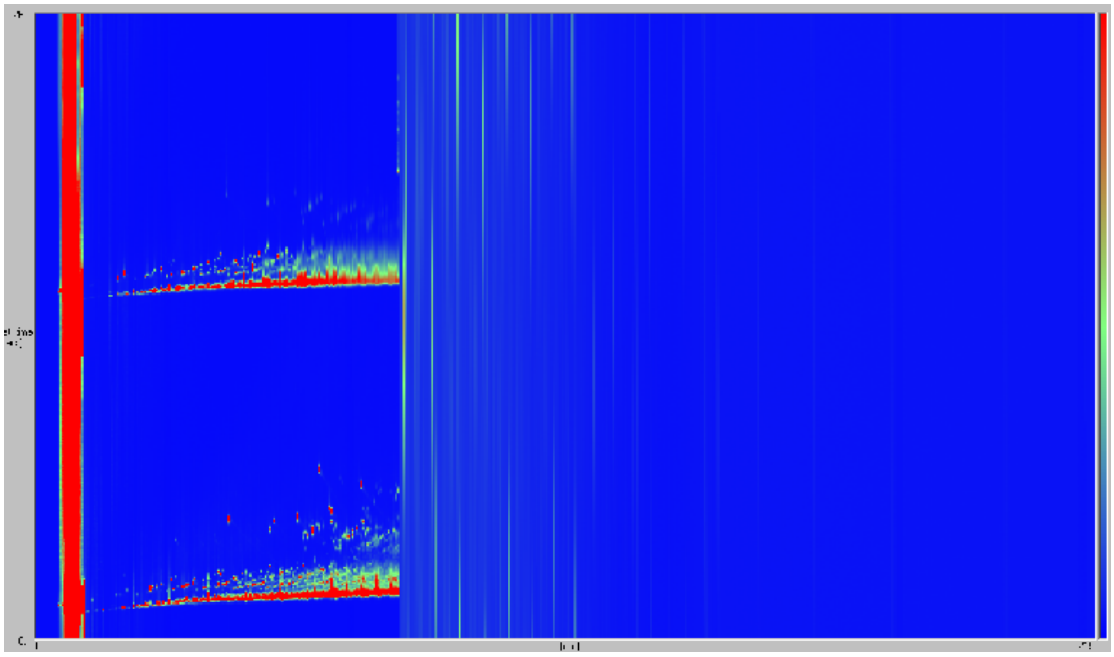
Injection 4:



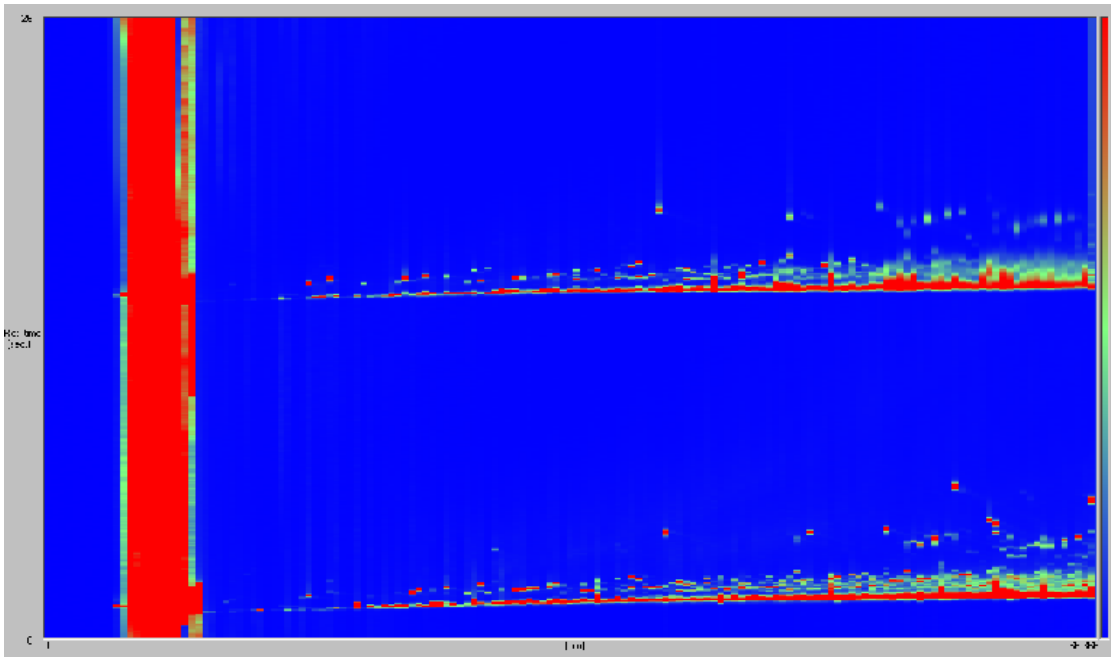
Injection 5:



Injection 6:

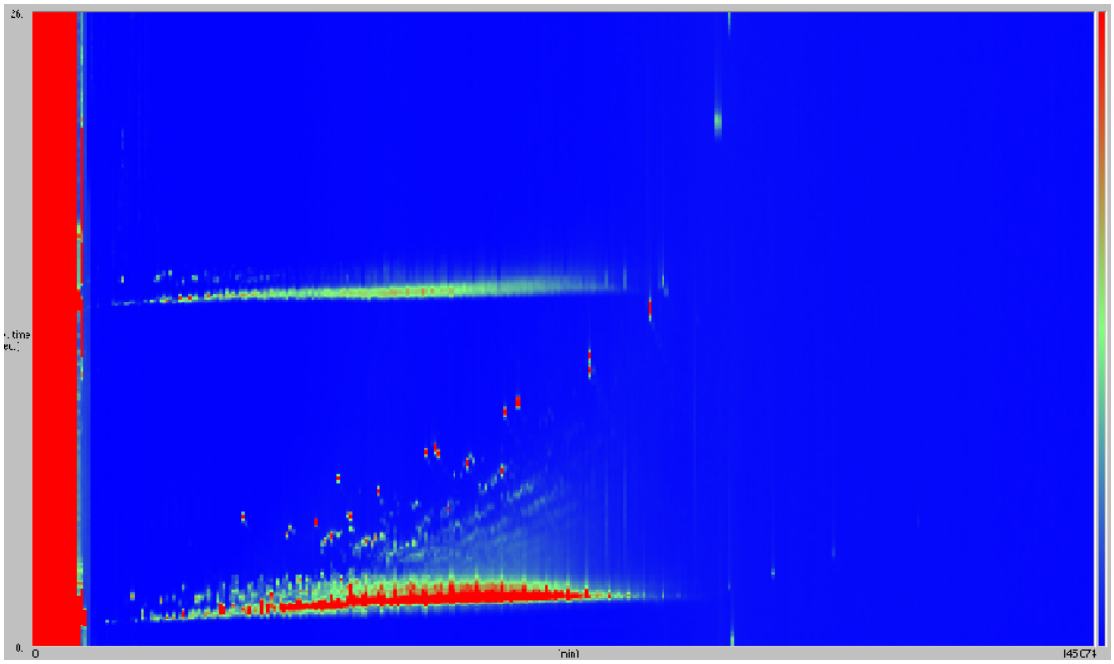


Injection 7:



Appendix 8

GC×GC-DSQ color contour plot under condition 2.1



Appendix 9.

Water solubility, LogKow and retention time of the representative compounds from petroleum hydrocarbons (SolGelwax column under Column condition 2.1)

Nr.	Name	Water solubility	LogKow (theoretical)	Ret1	Ret2
1	1,4-xylene	228.6	3.09	13.4333	2.086
2	1,2-xylene	242.4	3.09	13	1.989
3	naphthalene	142.1	3.17	28.6	5.163
4	ethyltoluene	96.88	3.58	17.3333	1.752
5	methylnaphthalene	40.62	3.72	34.2333	4.498
6	biphenyl	29.01	3.76	38.1333	4.915
7	acenaphthylene	2.487	3.94	41.6	6.738
8	fluorene	1.339	4.02	47.2333	6.156
9	diethylbenzenes	10.85	4.07	22.1	1.824
10	acenaphthene	2.534	4.15	42.4667	5.171
11	dibenzothiophene	0.7405	4.17	53.7333	7.752
12	dimethylnaphthalene	11.96	4.26	39.8667	4.225
13	phenanthrene	0.677	4.34	54.6	7.908
14	anthracene	0.6905	4.34	55.4667	7.719
15	methylfluorene	0.2428	4.56	52	5.687
16	dimethylfluorene	0.3537	4.7	56.3333	5.465
17	trimethylnaphthalene	4.777	4.81	45.0667	4.093

18	methylphenanthrene	0.1706	4.89	58.9333	7.308
19	fluoranthene	0.1297	4.93	64.5667	9.406
20	pyrene	0.2249	4.93	65.8667	9.746
21	triethylbenzenes	2.901	5.11	32.5	2.081
22	Decane	1.252	5.25	20.3667	1.075
23	dimethylphenanthrene	0.07133	5.44	63.2667	6.986
24	chrysene	0.02635	5.52	75.8333	11.033
25	benzofluoranthene	0.02065	6.11	84.0667	13.377
26	Benzopyrene	0.01038	6.11	85.8	14.759
27	Dodecane	0.1099	6.23	31.2	1.32
28	tetradecane	0.009192	7.22	40.3	1.498
29	C16	0.0009193	8.2	48.9667	1.582
30	C18	9.36E-05	9.18	56.7667	1.664
31	C20	9.40E-06	10.16	63.7	1.828
32	C22	9.37E-07	11.15	69.7667	1.911

Appendix 10.

Water solubility, LogKow and Retention time of the compounds from different organic groups

Nr.	Name	Water solubility	LogKow (theoretical)	Ret1	Ret2
1	phenol	26160	1.46	12.1333	2.255
2	methylphenol	9066	1.95	16.8	2.824
3	indole	1529	2.05	30	5.713
4	quinoline	1711	2.14	26.2667	4.886
5	benzothiophene	191.6	2.99	23.6	3.973
6	1,4-xylene	228.6	3.09	9.4667	1.342
7	1,2-xylene	242.4	3.09	8.6667	1.235
8	naphthalene	142.1	3.17	23.2	3.522
9	carbazole	3.274	3.23	64.5333	7.581
10	propylbenzene	70.73	3.52	12.9333	1.621
11	ethyltoluene	96.88	3.58	11.7333	1.461
12	dibenzofuran	1.475	3.71	45.7333	4.693
13	methylnaphthalene	40.62	3.72	31.333	3.823
14	biphenyl	29.01	3.76	36.6667	4.134
15	acenaphthylene	2.487	3.94	40.9333	4.875
16	fluorene	1.339	4.02	49.8667	4.875
17	diethylbenzenes	10.85	4.07	15.7333	1.754
18	acenaphthene	2.534	4.15	43.4667	4.725
19	dibenzothiophene	0.7405	4.17	60	5.874
20	dimethylnaphthalene	11.96	4.26	39.6	3.812
21	phenanthrene	0.677	4.34	61.7333	5.895

22	anthracene	0.6905	4.34	62.2667	5.766
23	methylfluorene	0.2428	4.56	57.6	4.746
24	methydibenzofuran	0.4949	4.6	53.2	4.532
25	dimethylfluorene	0.3537	4.7	64.8	4.553
26	trimethylnaphthalene	4.777	4.81	48.4	3.877
27	methylphenanthrene	0.1706	4.89	68.8	5.616
28	fluoranthene	0.1297	4.93	76.9333	6.497
29	pyrene	0.2249	4.93	79.6	7.044
30	triethylbenzenes	2.901	5.11	27.6	2.194
31	Decane	1.252	5.25	13.7333	1.153
32	dimethylphenanthrene	0.07133	5.44	76.2667	5.423
33	chrysene	0.02635	5.52	95.8667	7.356
34	benzopyrene	0.01038	6.11	112	8.871
35	benzofluoranthene	0.02065	6.11	108.9333	8.112
36	Dodecane	0.1099	6.23	26	1.421
37	benzoperylene	0.002842	6.7	126	9.614
38	dibenzoanthracene	0.003304	6.7	124.4	9.068
39	Indenopyrene	0.001176	6.99	123.8667	9.02
40	tetradecane	0.009192	7.22	40.1333	1.673
41	C16	0.0009193	8.2	53.6	1.94
42	C18	9.36E-05	9.18	66.1333	1.673
43	C20	9.40E-06	10.16	77.2	2.082
44	C22	9.37E-07	11.15	87.6	2.168
45	C24	9.25E-08	12.13	97.2	2.252
46	C26	9.07E-09	13.11	105.8667	2.315
47	C28	8.84E-10	14.09	114	2.376
48	C30	8.58E-11	15.07	121.7333	2.432
49	C32	8.28E-12	16.06	128.9333	2.475

Appendix 11

GC×GC-DSQ color contour plot of Alcketmix