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DISSERTATION

Characterisation and quantification of petroleum hydrocarbons by one- and two-dimensional gas chromatography

prepared for the purpose of obtaining the academic degree Doctor of Technical Science
(Doktor der technischen Wissenshaften)

under the supervision of

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Deutsche Kurzfassung

Im Rahmen dieser Arbeit wurden quantitative Methoden für die Analyse von Mineralöl-Kohlenwasserstoffen in Wasser- (und gegebenenfalls auch Boden-)Proben, basierend auf ein- und zweidimensionaler Gaschromatographie (GC×GC) entwickelt. Kohlenwasserstoff-Kontaminationen sind allgegenwärtig in unserer Umwelt, da diese unbeabsichtigt bei Produktion, Transport und Verwendung oder durch schwere Unfälle entweichen.

Die beiden derzeit meistverwendeten Standard-Methoden zur Bestimmung der (Gesamt-) Kohlenwasserstoffe beruhen auf gaschromatographischer Analyse des Extraktes der Kohlenwasserstoffe in einem leichtflüchtigen zyklischen oder linearen Alkan nach einer Aufreinigung Das Signal der Flammenionisationsdetektors (FID) wird in einem definierten Retentionszeitfenster (C10-C40) integriert, und das quantitative Ergebnis wird als "Kohlenwasserstoff-Index" (nach DIN ISO 9377:2000) bezeichnet. Alternativ zur externen Anreicherung des Extraktes, die zur Erreichung der festgelegten Nachweisgrenze erforderlich ist, ermöglicht die OSPAR (Kommission der Oslo-Paris-Konvention)-Methode die Verwendung eines GC mit einem Temperatur-programmierbaren Injektor für großvolumige Injektion (PTV-LVI) zu. Beide Methoden zur quantitativen Bestimmung von Mineralöl-Kontaminationen wurden miteinander verglichen. Es wurde dabei gezeigt, dass die Ergebnisse der beiden Verfahren korrelieren, obwohl signifikante methoden- und probenabhängige Unterschiede zwischen verschiedenen Öl-Typen bestehen. Da das OSPAR-Verfahren die Injektion großer Probenvolumina ermöglicht, wurde ein Modell für die Auswahl geeigneter Parameter für die großvolumige Injektion mit gleichzeitiger Lösungsmittelverdampfung entwickelt und experimentell validiert. Die Gültigkeit dieses Ansatzes wurde durch die erfolgreiche Übertragung der LVI-Parameter von einem zu einem anderen Probenlösungsmittel, nur auf Basis ihrer physikalisch-chemischen Eigenschaften, gezeigt. Beide, OSPAR und DIN ISO-Norm erreichen in der Praxis vergleichbare Nachweis- und Bestimmungsgrenzen (von ca. 0,02 und 0,06 mg/l).

Zweidimensionale Gaschromatographie (GC×GC) in der "Normalphasen"-Anordnung (d.h. mit einer apolaren 1D Säule und einer polaren 2D Säule) wurde für die umfassende Charakterisierung der verschiedenen Öle verwendet. Der Vorteil der GC×GC liegt nicht nur in der deutlich verbesserten Trennleistung, die in der klassischen (1D) GC unerreichbar ist, sondern darüber hinaus auch in der Möglichkeit, bestimmte Verbindungsklassen einfach anhand ihres Auftretens im zweidimensionalen Trennraum zuzuordnen. Dies ermöglicht eine einheitliche und universelle Kalibration, bei der die substanzklassenspezifischen Unterschiede in der Response dadurch ausgeglichen werden, dass den einzelnen Stoffklassen unterschiedliche Korrektur-Faktoren zugeordnet werden. Die Verwendung dieser Korrekturfaktoren ermöglicht es, substanzklassenspezifische Verluste, die im Clean-up auftreten, auszugleichen und dadurch zu einer einheitlichen Response für verschiedene Öle zu kommen. Dies ist ein großer Schritt in Richtung einer universellen Kalibration, die nicht nur zu Vergleichswerten für die Kohlenwasserstoff-Konzentration ("Kohlenwasserstoff-Index") führt, sondern den wahren Wert der Kohlenwasserstoff-Konzentration in der analysierten Probe liefert. Obwohl dieser Ansatz unter Verwendung von verbindungsklassenspezifischen Korrekturfaktoren im Prinzip gut funktioniert, ist eine weitere Verfeinerung erforderlich, um auf der Basis des Modells bessere quantitative Ergebnisse zu erzielen.

Abstract

In the frame of this work, quantitative methods for the analysis of petroleum hydrocarbons in water (and potentially soil) samples based on one- and two-dimensional gas chromatography (GC×GC) were developed. Petroleum hydrocarbon contaminations are ubiquitous in our environment since they inadvertently escape from production, transport and use, or through major accidents.

The two currently most widely employed standard methods for the determination of (total) petroleum hydrocarbons are based on gas chromatographic analysis of the extract of petroleum hydrocarbons in a highly volatile cyclic or linear alkane after purification by column chromatography on Florisil. The flame ionization detector (FID) signal is integrated in a defined retention time window (C10-C40), and the quantitative result is called 'hydrocarbon index' (DIN ISO 9377:2000). Alternatively to the external preconcentration of the extract, required to reach the stipulated detection limit the OSPAR (Commission of the Oslo-Paris Convention) method allows the use of a GC with a programmed-temperature vaporizing injector for large volume injection (PTV-LVI). Both methods for the determination of petroleum hydrocarbon contamination are compared in terms of quantitative analysis. It was demonstrated that the results of the two methods are correlated, although a significant method- and oil type-dependent bias becomes evident from the comparison of quantitative results for various oil types. Since the OSPAR method allows the injection of large sample volumes, a rational approach was designed and experimentally validated for the selection of suitable parameters for large volume-injection with concurrent solvent evaporation. The validity of this approach was proven by successfully transferring LVI parameters from one to another sample solvent, only based on its physico-chemical properties. Both, OSPAR and the DIN ISO standard in fact reach comparable detection and quantitation limits (of ca. 0.02 and 0.06 mg/l).

Comprehensive two-dimensional gas chromatography (GC×GC) in the 'normal phase' set-up (apolar 1D column, polar 2D column) was used for the comprehensive characterization of various oils. The advantage of GC×GC not only lies in the significantly improved resolution, virtually unattainable in classical (1D) GC, but moreover in the ability to assign specific compound classes simply on the basis of the location of the analyte signal in the two-dimensional separation space. This ability is the basis for a compound-class specific calibration, in which different response (or correction) factors are assigned to individual substance classes. The use of these correction factors (for flame ionization detection, FID) allows to partially make up for the compound-class specific losses in the clean-up procedure and to produce a more uniform response for different types of oils. This is a step forward towards a universal calibration procedure that would not only produce an indicator value ('hydrocarbon index'), but instead a true value of the hydrocarbon concentration in the analysed sample. Although the approach using compound-class specific correction factors in principle works well, it requires further refinement to be able to better model the expected response.

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Abbreviations

1-D – first dimension
1D-GC – conventional one-dimensional gas chromatography
2-D – second dimension
AED –atomic emission detector
APC – advanced pressure control
API – American Petroleum Institute
APH – air-phase petroleum hydrocarbons
ATSDR – Agency for Toxic Substances and Diseases Registry
BAM – Bundesanstalt für Materialprüfung
BTEX – benzene, toluene, ethylbenzene and *o*-, *p*- and *m*-xylenes
DSI – direct sample introduction
ECD – electron capture detector
ECHA – European Chemicals Agency
FAME – fatty acid methyl esters
FID – flame ionisation detector
EPH – extractable petroleum hydrocarbons
FPD – flame-photometric detector
GC – gas chromatography
GC/MS – gas chromatography/mass spectrometry
GC×GC or 2D-GC – comprehensive two-dimensional gas chromatography
HPLC – high performance liquid chromatography
IL – oonic liquid
IR – infrared absorption
LC – liquid chromatography
LC-GC – two-dimensional liquid chromatography/gas chromatography
LC×LC – comprehensive two-dimensional liquid chromatography
LOQ – limit of quantification
LOD – limit of detection
LMCS – the longitudinally modulated cryogenic system
LVI – large volume injection
qMS – quadrupole mass spectrometer
(ToF)-MS – time-of-flight mass spectrometer
MA DEP – Massachusetts Department of Environmental Protection
OSHA – Occupational Safety and Health Administration
PAH – polycyclic aromatic hydrocarbons
PTV – programmed-temperature vaporizer
RfD – reference dose
RSD – relative standard deviation
SCD – sulfur-chemiluminescence detectors
S/N_{BP} – signal-to-noise ratio of the base peak
SVE – solvent vapor exit
SPE – solid-phase extraction
TPH – Total Petroleum Hydrocarbons
TPHCWG – Total Petroleum Hydrocarbons Criteria Working Group
US EPA – United States Environmental Protection Agency
UV – ultraviolet
VPH – volatile petroleum hydrocarbons
NCD – nitrogen-chemiluminescence detector

Publications

Part of the contents of the thesis was or will be published in the following form:

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Scope of the work

Contamination of water, soil and sediment samples by petroleum hydrocarbons is a common and severe environmental problem, caused by improper handling, storage, transport or use of petrochemical products or raw materials. Petroleum hydrocarbons represent a vast mixture of compounds, and some of them (e.g. benzene, or polycyclic aromatic hydrocarbons, PAHs) may exhibit carcinogenic and / or toxic properties. Their analytical determination is performed, according to the established ISO standard 9377-2:2000[[1]] by gas chromatography with flame ionization detection (GC-FID) where the sample is extracted by a low-boiling hydrocarbon solvent (e.g. hexane), cleaned up to remove potentially present non-petroleum hydrocarbons, and then analysed by GC-FID where a certain retention time window is defined as being indicative for petroleum hydrocarbon contamination which in that particular case is called "hydrocarbon index". Although in common use, this GC-FID method has (among several practical shortcomings) the disadvantage of being tedious, slow, and only laboratory-based.

At the Institute of Chemical Technologies and Analytics (at the Vienna University of Technology), an alternative method, ASTM Standard D7678 [[2]] for the determination of Oil in Water (OIW) has been developed in the Working Group "Process Analysis and Vibrational Spectroscopy" (headed by Prof. B. Lendl) in cooperation with the spin-off company QuantaRed Technologies (ranking among the participants in this year's Austrian National Prize for Innovation) which overcomes the main limitations of the tedious GC/FID method. It is based on the use of mid-infrared (IR) quantum cascade lasers (QCLs) to detect the minor absorbance of trace hydrocarbon contaminations in the extracts of water or soil samples. While the instrument has already demonstrated its ability to successfully measure low petroleum hydrocarbon concentrations in the field, and even under most demanding conditions, such as on offshore oil-drilling platforms, a very rigid method validation is required to ensure widest acceptance of this method, particularly by the standardising organisations such as ASTM and DIN/ISO. To this end, a joint project (funded by the FFG) has been initiated between the Institute of Chemical Technologies and Analytics, and QuantaRed Technologies which aims at the validation and potential improvement of the quantum cascade laser technology that QuantaRed technologies have implemented in their Eracheck instrument by establishing a GC-based reference analysis of highest accuracy and precision.

During the first project period, the reference analysis was established based on simple (one-dimensional) gas chromatography, as suggested in the DIN/ISO standard. The high precision and accuracy of the GC measurement allowed to detect systematic differences in the response of the GC and

[1] DIN ISO 9377-2:2000. Water Quality – Part 2, Method Using Solvent Extraction and Gas Chromatography. International Organization for Standardisation: Geneva, 2000.

[2] ASTM Standard D7678 – 11. Standard Test Method for Total Petroleum Hydrocarbons (TPH) in Water and Wastewater with Solvent Extraction using Mid-IR Laser Spectroscopy, DOI: 10.1520/D7678-11; ASTM International: West Conshohocken, PA, 2011.

the IR-QCL methods which can only be explained by the differences in the chemical composition of the petroleum products investigated. While the GC-FID response does not depend on the chemical structure of the petroleum hydrocarbons in first approximation, the IR measurement does to a certain degree. The only way to improve the accuracy of the prediction of the IR measurement appears thus to be a detailed analysis of the petroleum hydrocarbon mixture, based on individual chemical compound classes, e.g. linear and branched aliphatics, (poly-)aromatics, alkyl-substituted aromatics, heterocycles, etc., and the establishment of a more complex mathematical model for the correlation of GC-FID and IR response, based on the exact knowledge of the amounts of substance of each compound class in a given sample. Unfortunately, classical, that is, one-dimensional chromatography is not able to provide this information, even with more selective detection, such as mass spectrometry (MS). The reason for this is the chromatographic overlap of the countless compounds contained in the sample that does not allow to detect separately and identify the constituents of such a hydrocarbon sample.

When staying in one dimension, chromatographic separation efficiency cannot be increased to that degree needed to resolve the several thousand compounds contained in a typical petroleum hydrocarbon sample. However, if two-dimensional chromatography is applied, then the separation efficiency of the system increases drastically since it is the product of the separation efficiencies of the columns in each dimension.

A system for two-dimensional gas chromatography (GC×GC or 2D-GC) was installed at the Institute of Chemical Technologies and Analytics and was used for the detailed analysis of total petroleum hydrocarbons (TPH). The use of GC×GC with MS detection (GC×GC/MS) is expected to not only allow the separation of the various constituents of complex TPH samples, but also to identify the compound-classes and individual resolved compounds based on MS detection.

This is supported by the unique ability of GC×GC to produce two-dimensional-chromatograms in which a certain class of compounds (a series of ‘homologues’) is forming a very distinct, clearly identifiable pattern in the two-dimensional space of the GC×GC separation. This will be essential for correlating the QCL-IR response with individual compound classes in the TPH sample, and is expected to provide a more accurate calibration model than currently possible.

1. Introduction

1.1. Petroleum Hydrocarbon Contaminations

1.1.1. Petroleum hydrocarbons and their environmental relevance

Oil and gas resources are organic compounds, formed by the effects of heat and pressure on sediments trapped beneath the earth's surface over millions of years. The remains of animals and plants that lived millions of years ago in a marine environment were covered by layers of sand and silt over years. Heat and pressure from these layers helped the remains turn into crude oil or petroleum [1]. The word "petroleum" means "rock oil" (from Greek: petra (rock) + Latin: oleum (oil) [2]) or "oil from the earth." While ancient societies made some use of these resources, the modern petroleum age began less than a century and a half ago, when in 1859 Colonel Drake discovered oil in Oil Creek in Titusville, PA, USA. From that time on, the world's demand for fossil fuel and the production of oil have continuously increased. From the 1980s, that is, in particular after the second oil crisis of 1979, the petroleum business has developed into a high-technology industry. Advances in technology have greatly improved the ability to find and extract oil and gas and to convert them to efficient fuels and useful consumer products. About 100 countries produce crude oil. Russia, Saudi Arabia, United States, Iran and China are the top five producing countries in 2009 (**Table 1.1** and **Table 1.2**, data taken from [3]). In the U.S.A. the oil and gas industry employs 1.4 million people and generates about 4 percent of U.S. economic activity. It is larger than the domestic automobile industry and larger than education and social services, the computer industry, and the steel industry combined [4]. At a refinery, different fractions of the crude oil are separated into useable petroleum products. Various sources of information provide a good overview of the different processes in petroleum refining [5,6,7]. Petroleum products are used worldwide for energy production, fuel for transport, and as raw material for many chemical processes. The United States of America are the biggest consumer of oil in the world (**Table 1.1** and **Table 1.2**). Although there exist well-developed alternatives to the use of oil (particularly for energy production and transportation), our societies are still strongly dependant on oil which is an environmental burden, an economic problem and a political hazard. However, at the current time the economic situation still favours the use of petroleum and petroleum products for these applications rather than its alternatives which at the moment are not competitive from an economic point of view.

Table 1.1 The annual production and consumption of oil by the top ten industrial nations (*bbl*: barrel. 1 bbl ≈ ca. 159 litres), data taken from [3].

Oil production by country					***Oil consumption by country in the world***				
Rank	**Countries**	**Amount**	**Date**	**Percentage**	**Rank**	**Countries**	**Amount**	**Date**	**Percentage**
		bbl/day		*%*			*bbl/day*		*%*
1	Russia	10120000	2010	11.9	1	United States	18690000	2009	22.6
2	Saudi Arabia	9764000	2009	11.5	2	China	8200000	2009	9.9
3	United States	9056000	2009	10.7	3	Japan	4363000	2009	5.3
4	Iran	4172000	2009	4.9	4	India	2980000	2009	3.6
5	China	3991000	2009	4.7	5	Russia	2740000	2010	3.3
6	Canada	3289000	2009	3.9	6	Brazil	2460000	2009	3.0
7	Mexico	3001000	2009	3.5	7	Germany	2437000	2009	2.9
8	United Arab Emirates	2798000	2009	3.3	8	Saudi Arabia	2430000	2009	2.9
9	Brazil	2572000	2009	3.0	9	Korea, South	2185000	2010	2.6
10	Kuwait	2494000	2009	2.9	10	Canada	2151000	2009	2.6
Total:		84764555			**Total:**		82769370		

Table 1.2 The annual production and consumption of oil by the top ten countries in the EU (*bbl*: barrel. 1 bbl ≈ ca. 159 litres), data taken from [3].

Oil production by EU member states					***Oil consumption by EU member states***				
Rank	**Countries**	**Amount** *bbl/day*	**Date**	**Percentage** *%*	**Rank**	**Countries**	**Amount** *bbl/day*	**Date**	**Percentage** *%*
1	United Kingdom	1502000	2009	60.4	1	Germany	2437000	2009	16.2
2	Denmark	262100	2009	10.5	2	France	1875000	2009	12.5
3	Germany	156800	2009	6.3	3	United Kingdom	1669000	2009	11.1
4	Italy	146500	2009	5.9	4	Italy	1537000	2009	10.2
5	Romania	117000	2009	4.7	5	Spain	1482000	2009	9.9
6	France	70820	2009	2.8	6	Hungary	1373000	2009	9.1
7	Netherlands	57190	2009	2.3	7	Netherlands	922800	2009	6.1
8	Poland	34140	2009	1.4	8	Belgium	608200	2009	4.1
9	Spain	27230	2009	1.1	9	Poland	545400	2009	3.6
10	Austria	21880	2009	0.9	10	Greece	414400	2009	2.8
Total (EU 27 countries):		2485550	2009		**Total (EU 27 countries):**		15012050		
	Norway	2350000	2009			Norway	204100		
	Turkey	52980	2009			Turkey	579500		

Petroleum poses a range of environmental risks when it is released into the environment (whether by catastrophic spills or through chronic discharges). In addition to the physical impact of large spills, the toxicity of many of the individual compounds contained in crude oils or petroleum products is significant. Information on how petroleum hydrocarbons enter and diffuse in the environment is abundant [8,9]. The sources of petroleum input to the environment, particularly to the sea, are diverse. They can be categorized effectively into four major groups, namely natural seeps, petroleum extraction, petroleum transportation, and petroleum consumption.

Natural seeps are frequently encountered phenomena that occur when crude oil seeps from the geologic strata beneath the seafloor to the overlying water column as a natural process [10]. Recognized by geologists for decades as indicating the existence of potentially exploitable reserves of petroleum, these seeps release vast amounts of crude oil annually. Yet these large volumes are released at a rate low enough that the surrounding ecosystem can adapt and even thrive in their presence, what is not true in case of the catastrophic and accidental impact of a tanker or oil well spill. Natural processes are therefore, responsible for over 45 percent of the petroleum entering the marine environment worldwide (**Table 1.3**, data taken from [11]).

Table 1.3 Petroleum input to the sea, data taken from [11].

The source of input	**North America**		**Worldwide**	
	tons	%	tons	%
Natural seeps	160000	61	600000	46
Petroleum Extraction	3000	1	38000	3
Petroleum Transportation	9100	4	150000	12
Petroleum Consumption	84000	32	480000	37
Other	3900	2	32000	2
Total input:	**260000 tons**		**1300000 tons**	

As result of human activities about 700 000 tons of petroleum are released annually into the sea worldwide. Processes such as petroleum extraction, transportation and consumption can cause soil and groundwater contamination in case of equipment failure or operation errors and other reasons. Petroleum extraction can result in release of both crude oil and refined products as a result of human activities associated with efforts to explore and produce petroleum. The nature and size of these releases is highly variable – see **Table 1.4** for the largest oil spills observed until 2010 [12] –, and can include accidental spills of crude oil from platforms and blowouts such as that of the oil rig "Deepwater Horizon" in the Gulf of Mexico, in April 2010, or slow chronic releases of water produced from oil or gas-bearing formations during extraction. Under current industry practices, this "produced water" is treated to separate from crude oil and either injected back into the reservoir or discharged overboard. Produced

water is the largest single wastewater stream in oil and gas production. The amount of produced water from a reservoir varies widely and increases over time as the reservoir is depleted. Petroleum transportation can result also in releases of dramatically varying sizes of petroleum products (not just crude oil), from major incidents - mostly from tankers - such as the one in 1979 off the coast of Tobago when two tankers collided and one of these, the "Atlantic Empress", sank, loosing all its freight, to relatively small operational releases that occur regularly like from pipelines.

Releases that occur during the consumption of petroleum, whether by individual car and boat owners, not-tank vessels, or runoff from urban or industrial areas are typically small but frequent and widespread, and are responsible for the vast majority (70%) of petroleum introduced to the environment through human activity.

Because crude oil and petroleum products are a complex and highly variable mixture of hundreds to thousands of individual hydrocarbon compounds, however, characterizing the risks posed by petroleum-contaminated soil and water has proven to be difficult and inexact. It is very important to have an understanding of the toxicology, analytical science, environmental fate and behavior, risk and technological implications of petroleum hydrocarbons, in order to interpret, evaluate the risk and make decision about the hazard effect and ensure the appropriate protection of the environment.

Table 1.4 Top ten oil spills in the world for 2010, data taken from [12].

	Incident	Location	Year	Type of incident	Magnitude of oil spill, [gallons]
1	Gulf War	Kuwait	1991	Oil spill due to war action and sabotage of oil drilling stations and pipelines, encompassing also the dumping of the charge of several oil tankers into the Persian Gulf by Iraqi troops during the Gulf War.	520 000 000
2	Deepwater Horizon	Gulf of Mexico	2010	Oil spill as a consequence of a methane blowout (which could not be prevented due to a technical problem) at the oil rig *Deepwater Horizon* which caused an explosion and its subsequent loss of the oil drilling polatform. The well continued to leak for over 100 days.	172 000 000
3	Ixtoc I	Mexico	1979	After an unexpected blowout at the offshore oil rig Ixtoc 1 in the Gulf of Mexico, the platform exploded and collapsed. Oil escaped freely from the well for almost one year until the well could be capped.	138 000 000
4	Atlantic Empress/Aegean Captain	Trinidad and Tobago	1979	Collision of two ships, the Aegean Captain and the supertanker *Atlantic Empress*, during a heavy storm in the Caribbean Sea. The Atlantic Empress, exploded, sank and lost its freight.	90 000 000
5	Fergana Valley/Mingbulak	Russia	1992	Technical failure of an oil well in the Fergana Valley located between Kyrgyzstan and Uzbekistan from which oil blew out for a period of 8 months.	88 000 000

Table 1.4 Top ten oil spills in the world for 2010, data taken from [12] (continued).

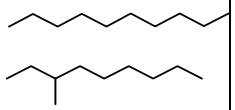
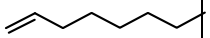
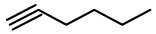
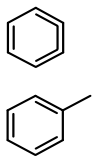
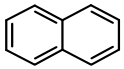
	Incident	Location	Year	Type of incident	Magnitude of oil spill, [gallons]
6	Nowruz Oil Field	Persian Gulf	1983	Collision of an oil tanker with an oil platform at the Nowruz Oil Field during the Iran-Iraq-War. After the oil drilling platform collapsed, the wellhead was destroyed, and leaked oil into the Persian Gulf for more than six months before being capped. A similar event at the same oilfield resulted directly form war action.	80 000 000
7	Castillo de Bellver	South Africa	1983	A fire at the tanker *Castillo de Bellver* caused the ship to drift and then break into two separate pieces. Relatively little damage was done to the South African coastline since the oil may have sunk into the sea or burned during the fire.	79 000 000
8	The Amoco Cadiz	France	1978	The crude oil carrier *Amoco Cadiz* ran aground off the French Atlantic coast, and finally spilt into halves, whereby it lost its complete freight which contaminated 200 km of the French coastline.	69 000 000
9	ABT Summer	Angola	1991	Following a fire aboard the oil tanker *ABT Summer*, it sank and all its freight either leaked to the sea or sank to the ground about 900 miles from the coast of Angola.	51 000 000
10	The MT Haven	Genova, Italy	1991	After unloading the oil tanker *MT Haven*, a fire broke out, followed by explosions after which the ship sank and continued to leak oil for 12 years.	45 000 000

1.1.2. General chemical composition features of crude oils and petroleum products

Crude oil is an extremely complex mixture of several thousands of different compounds, its compositions and physical properties vary widely depending on the source from which the oils are produced, the geologic environment and location in which they migrated and from which they are extracted. The nature of the refining processes has an effect on crude oil compositions as well. As indicated in **Table 1.5**, petroleum and petroleum products contain primarily hydrocarbons, heteroatom compounds, and relatively small concentrations of (organo-)metallic constituents [13,14]. The complexity of petroleum and petroleum products increases with carbon number of its constituents, so it is impossible to identify all components. Petroleum and petroleum products are typically characterized in terms of boiling range and approximate carbon number. Raw petroleum is usually dark brown or almost black although some fields deliver a greenish or sometimes yellow petroleum. Depending upon the oil field and the way the petroleum composition was formed, the crude oil will also differ in viscosity. The composition of crude oil impacts certain physical properties of the oil, and it is these physical properties (e.g., density or viscosity) by which crude oils are generally characterized, classified, and traded. These physical properties can be used to classify crude oils as light, medium or heavy. The API (American Petroleum Institute) gravity [15] is a measure of the specific gravity of a petroleum liquid compared to water (API 10). Light oils are defined as having an API < 22.3, heavy oils are those with API > 31.1 and medium oils have an API gravity between 22.3 API and 31.1 API.

Regardless of the complexity, petroleum compounds can be separated into two major categories: hydrocarbons and non-hydrocarbons. Hydrocarbons (compounds composed solely of carbon and hydrogen) comprise the majority of the components in most petroleum products and are the compounds that are primarily (but not always) measured as Total Petroleum Hydrocarbons (TPH) [16]. The nonhydrocarbon components are heterocyclic hydrocarbons (compounds containing heteroatoms such as sulfur, nitrogen, or oxygen in addition to carbon and hydrogen). These heterocyclic hydrocarbons are typically present in oils at relatively low concentrations and can be found in most refined motor fuels as they are concentrated in the heavier fractions and residues during refining. Most organic nitrogen hydrocarbons in crude oils are present as alkylated aromatic heterocycles mostly with a pyrrolic structure. Crude oils also contain small amounts of organometallic compounds (of nickel, vanadium and other metals up to atomic number 42 with the exception of rubidium and niobium) and inorganic salts. Although - depending on the analytical method - sulphur-, oxygen- and nitrogen containing compounds are sometimes included in the value reported as “total petroleum hydrocarbon” (TPH) concentration, they do not fall under the definition of petroleum hydrocarbons in the strict sense [16].

Table 1.5 Main constituents of petroleum hydrocarbons and representative examples.

PETROLEUM HYDROCARBON COMPOUNDS						
ALIPHATICS / ALICYCLICS				**AROMATICS**		**HETEROCYCLIC COMPOUNDS**
Saturated Hydrocarbons		**Unsaturated Hydrocarbons**		**Benzene and Alkylbenzenes (BTEX)**	**Polynuclear Aromatics (PAH)**	
Alkanes (Paraffins)	Cycloalkanes	Alkenes (Olefins)	Alkynes (Acetylenes)			
Single carbon bonds, straight and branched structure	Straight and cyclic structure	One or more double carbon bonds, straight, branched or cyclic	One or more triple carbon bonds, straight, branched or cyclic	Single aromatic ring or with attached functional group	Two or more aromatic rings fused together, can be with attached functional group	Aromatic ring structures with one or more heteroatoms (N, S, O) in the ring
C_nH_{2n+2}	C_nH_{2n}	C_nH_{2n}	C_nH_{2n-2}			
						
n-Decane	Cyclohexane	1-Octene	1-Hexyne	Benzol	Naphtalene	Pyrrole

Depending on the structure of petroleum hydrocarbons, the individual compounds are grouped into aliphatic (saturated and unsaturated) hydrocarbons and aromatics. Saturated hydrocarbons are the major class of compounds found in crude oil. The common names of these types of compounds are alkanes and isoalkanes or as used in petroleum industry, paraffins and isoparaffins, respectively. Unsaturated hydrocarbons have at least one multiple bond (double bond: alkenes or triple bond: alkynes) and they are typically not present in crude oil, but can be formed during the cracking process. Aromatic hydrocarbons are based on the benzene ring structure, and are further categorised depending on the number of rings. Benzene rings are very stable, and therefore persistent in the environment, and particularly the mono- and polycyclic aromatic compounds can have toxic effects on organisms. Aromatic hydrocarbons with one benzene ring and with one ore more side chains are alkyl benzenes and include benzene, toluene, ethylbenzene and *o*-, *p*- and *m*-xylenes (BTEX). This class of compounds has significant water solubility and is more mobile in the environment. Polycyclic aromatic hydrocarbons (PAH) are aromatic compounds with two or more fused aromatic rings. Occurrence of PAH compounds in oils is dominated almost completely by the C1 to C4-alkylated homologues of the parent PAH, in particular, for naphthalene, phenanthrene, dibenzothiophene (a sulphur-containing aromatic heterocycle), fluorene and chrysene. These alkylated PAH homologues form the basis of chemical characterization and identification of oil spills [17,18]. A typical crude oil may contain 0.2 percent to more than 7 percent total PAHs. Of the hydrocarbon compounds common in petroleum, PAHs appear to pose the greatest toxicity to the environment.

Different crude oil sources usually have a unique hydrocarbon composition [19,20]. The actual overall properties of each different petroleum source are defined by the percentage of the main hydrocarbons found within petroleum as part of the petroleum composition. The percentages for these hydrocarbons can vary greatly. It gives the crude oil a quite specific compound personality depending on geographic region. The typical percentage of hydrocarbons (although covering very wide ranges) is as follows: paraffins (15% - 60%), naphthenes (30% - 60%), aromatics (3% to 30%), with asphaltenes making up the remainder. Furthermore, due to differences in refining technologies and refinery operating conditions, each refining process has a distinct impact on the hydrocarbon composition of the product.

Refined petroleum products are primarily produced through distillation processes that separate fractions from crude oil according to their boiling ranges. Production processes may also be directed to increase the yield of low molecular weight fractions, reduce the concentration of undesirable sulfur and nitrogen components, and incorporate performance enhancing additives. Therefore, each petroleum product has its unique, product-specific hydrocarbon pattern. The petroleum products are comprised of both aliphatic and aromatic hydrocarbons in a range of molecules that include C6 and greater. The different classes of compounds contained in various petroleum products are summarised in **Table 1.6** (data taken from [20,21]). Main products are gasoline (benzene), naphtha/solvents, jet fuels, kerosene, diesel fuel, and lubricating (motor) oils. Due to the variety of components in petroleum, they are typically characterised using the boiling range of the mixture and the carbon number rather than individual

components. For example, diesel is a fraction with boiling points between 200-325°C and is represented as C10-C22.

Table 1.6 Overview of petroleum products with respect to boiling point ranges, approximate carbon number and average percentage amount of aliphatic and aromatic compounds, data taken from [20,21].

		Fractions				**Hydrocarbons**	
	Boiling range	**<C7 (%w/w)**	**C7 - C10 (%w/w)**	**C10 - C40 (%w/w)**	**>C40 (%w/w)**	**Aliphatic**	**Aromatic**
Statfjord C (39.1)[a]		11.6	18.1	56.6	13.7		
Crude Oil (API 18.7) Grane[a]		0.9	3.0	63.2	32.8		
Normal benzine[b]	40-200°C	~100 (C5-C12)				~70%	20-50%
Jet fuel[b]	150-300°C		~100 (C6-C14,C16)			80-90%	10-20%
Kerosene[b]	150-300°C		~100 (C6,C9-C16)			60-80%	5-20%
Diesel[b]	200-325°C		~100 (C10-C22)			60-90%	30-40%
Light heating oil[b]	200-325°C		~100 (C10-C22)				
Lubricant or Motor oil[b]	325-600°C			~100 (C20-C40)		70-90%	10-30%
Heavy heating oil[b]	325-600°C			~100 (C20-C50)			

a: [20], b: [21]

While a physical property such as boiling range may establish the initial product specification, other finer specifications define their ultimate use in certain applications. A lighter, less dense raw petroleum composition with a composition that contains higher percentages of hydrocarbons is much more profitable as a fuel source. Whereas other, denser petroleum composition with a less flammable level of hydrocarbons and containing higher levels of sulphur are expensive to refine into a fuel and are therefore more suitable for plastics manufacturing and other uses. In contrast to the ever increasing demand, the world's reserves of light petroleum (light crude oil) are severely depleted and refineries are forced to refine and process more and more heavy crude oil and bitumen.

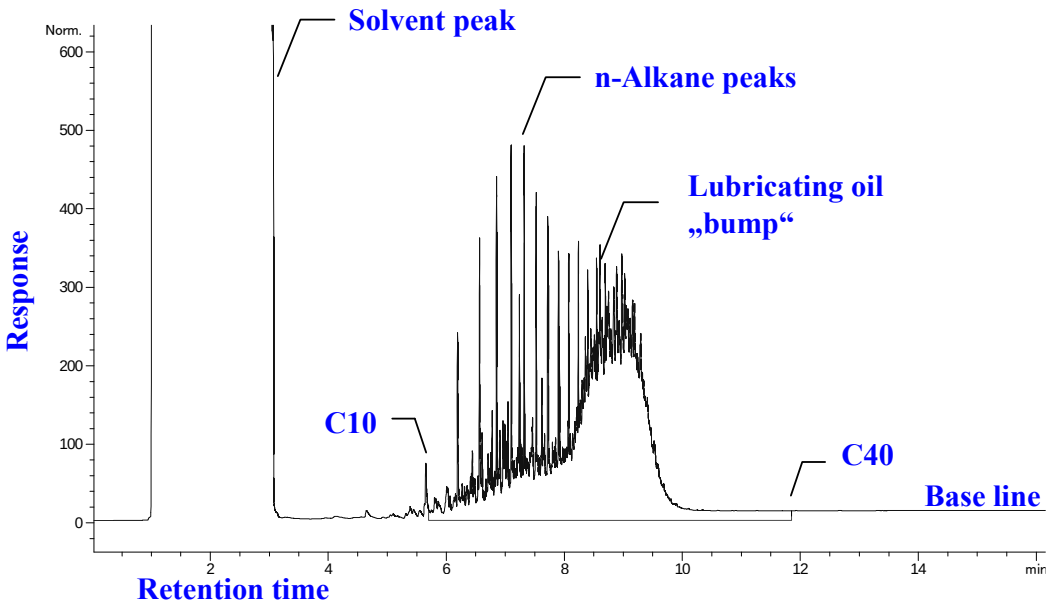


Figure 1.1 Chromatogram of a mixture of petroleum products (diesel and lubricating oil, 1:1), obtained by GC with flame ionisation detector (FID).

Petroleum fractions are among the most complex samples an analyst can face in terms of the number of compounds present. The characterisation of petroleum fractions is typically done by gas chromatography (GC). As can be seen in **Figure 1.1**, the petroleum products contain such a large number of hydrocarbon constituents that complete chromatographic separation is not possible. Even then, gas chromatography remains the most informative analytical technique, providing both quantitative information (deduced from the total signal recorded in a chromatogram), and qualitative information which derives form the fact that the retention times in the chromatograms can be correlated with the boiling points of the compounds contained in the petroleum. To illustrate the complexity of chemical composition of petroleum products **Figure 1.2** shows the chromatograms for 6 different petroleum products including a Crude Oil with API 18.7 and the BAM (Bundesanstalt für Materialprüfung, Berlin) petroleum hydrocarbon standard. The BAM standard K-010 is a certified reference material for the determination of mineral hydrocarbons, which is a synthetic mixture of a diesel and a lubricating oil. It is evident that these six samples are very different according to their carbon ranges. The difference is clearly seen from the comparison of their chromatograms. The volatile fuel with a higher content of hydrocarbons with less then C10 atoms (benzine and premium gasoline) has the majority of its constituents at the beginning of chromatogram (**Figure 1.2c**). The peaks in the chromatogram of diesel are shifted to the retention time window where hydrocarbons from C10 to C22 are eluted (**Figure 1.2d**). In turn the chromatogram of motor oil shows a characteristic ‘bump’ (because the fraction of saturated alkanes is very small) situated in the region where heavier hydrocarbons C20-C40 are eluted (**Figure 1.2e**). Thus, GC-based methods provide important qualitative information which in the ideal case even allows the assignment of the source of contamination. This is proven by the comparison of chromatograms of different oil samples (**Figure 1.2**).

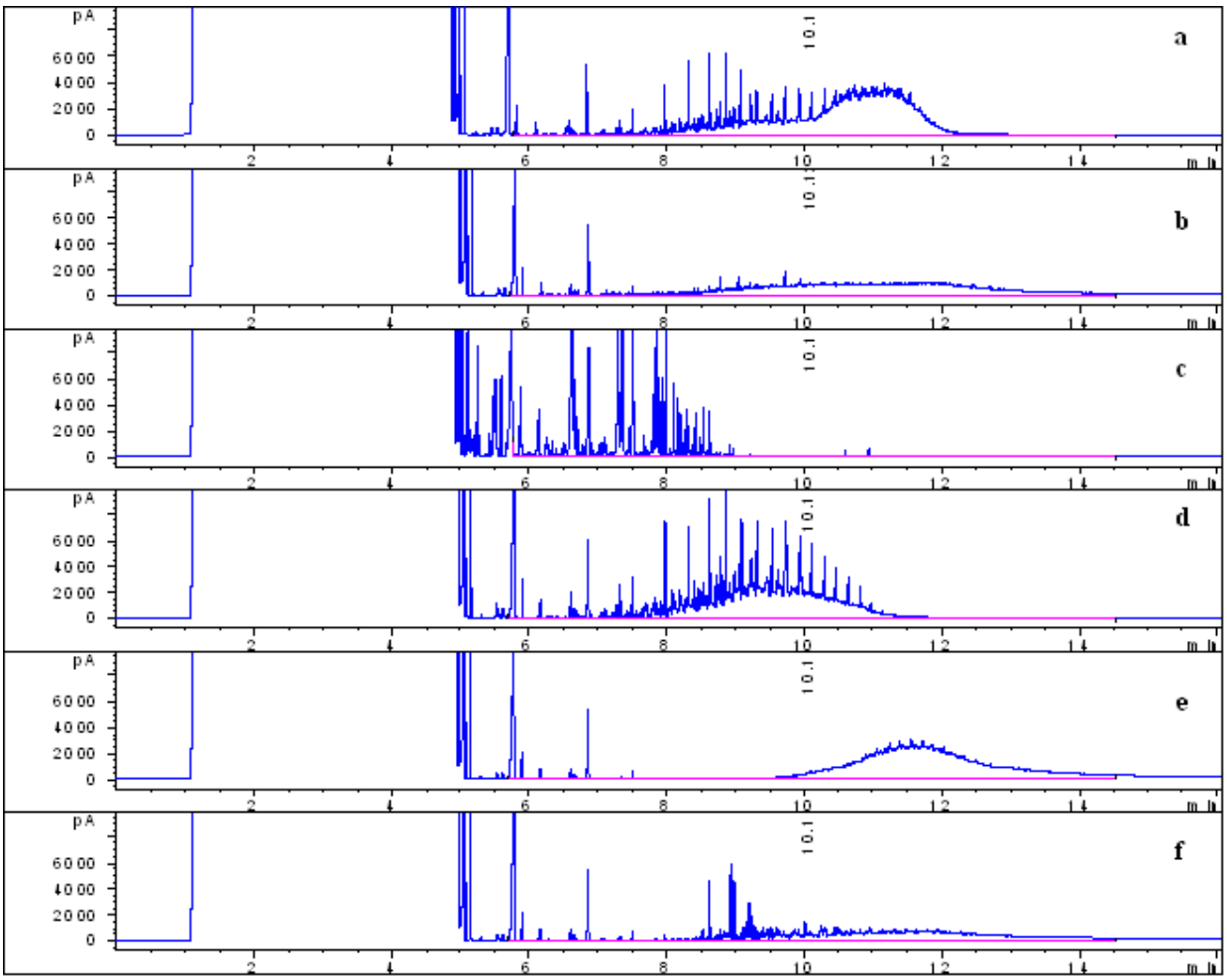


Figure 1.2 Comparison of chromatograms of different oil samples: *a)* BAM, *b)* Crude Oil (API 18.7), *c)* Gasoline, *d)* Diesel, *e)* Motor oil, *f)* Heavy heating oil with the same concentration (20 ppm oil in water) obtained by GC-FID method.

1.1.3. Fate of petroleum hydrocarbons in the environment

The effects of petroleum hydrocarbons entering the environment are a complex function of the magnitude and the rate of release, the nature of the released petroleum (its physico-chemical properties, and in particular the amount of toxic compounds it may contain), and the affected geographical, hydrogeological, and biological ecosystem. The fate of petroleum type pollutants in the environment has been investigated in many studies [22]. Complex transformation and degradation processes of oil in the environment start from its first contact with the atmosphere, seawater and soil. They depend on the physical properties (volatility, solubility etc.) as well as on the chemical properties (chemical composition) of the oil. While the former are responsible for transport, or diffusion of the petroleum hydrocarbons in the environment, the latter are responsible for their chemical, photo- and microbial degradation. The main processes affecting the environmental fate of petroleum hydrocarbons after their release to the environment are thus their volatilization, dissolution/dispersion and emulsification in water, adsorption to soil, oxidation, destruction and biodegradation [23,24]. In addition to the parameters that

characterise the oil's composition, reactivity and toxicity, the environmental conditions, i.e. the meteorological and hydrological factors also play an important role in the fate of petroleum hydrocarbons.

When petroleum hydrocarbons are released to the water column, certain fractions will float on top and form thin surface films. This process is controlled by the viscosity of the oil and the surface tension of water. A spill of 1 ton of oil can disperse over a radius of 50 m in 10 minutes, forming a slick 10 mm thick. Later it spreads, gets thinner and covers an area of up to 12 km^2 [11]. It should be pointed out that much of the environmental and ecological damage caused by oil spills actually is due to this oil film that covers the surface of the sea, or the coastline, thus physically impairing birds and other animals, and causing suffocation of fish as oxygen will not permeate the oil layers to a sufficient degree anymore. In the first days after the spill the volatile compounds from oil evaporate. Only a small proportion of the hydrocarbon constituents of petroleum products are significantly soluble in water. Dissolution takes more time compared with evaporation, considering that most oil components are soluble in water only to a limited degree (although the degradation products typically are more polar and thus more soluble). Other heavier fractions (up to 10-30%) will accumulate in the sediment at the bottom of the water, which may affect bottom-feeding fish and organisms. This mainly happens in the narrow coastal zone and shallow waters, where water is intensively mixing.

Crude oil released to the soil may percolate and reach the groundwater. Because petroleum has a lower specific gravity than water, free (undissolved) product and most dissolved contamination is usually concentrated near the top of the groundwater [25]. This may then lead to a fractionation of the original complex mixture, depending on the chemical properties of the compound. Some of these compounds will evaporate while others will dissolve into the groundwater and be diffused from the release area. Other compounds will adsorb to soil or sediments and will remain there for a long period of time, while others will be metabolised by organisms found in the soil [26,27].

While evaporation and dissolution redistribute the oil, photochemical oxidation and bacterial degradation transform it. Where crude oil is exposed to sunlight and oxygen in the environment, both photooxidation and aerobic microbial oxidation take place. The photochemical oxidation of hydrocarbons is dependent upon ultraviolet (UV) radiation and will therefore only occur in the upper surface layers. The aromatic hydrocarbons absorb UV radiation with high efficiency and are mainly transformed into hydrogen peroxides. Alkanes are much less efficient in absorbing UV radiation and only small quantities are transformed by this process. The final products of oxidation (hydroperoxides, phenols, carboxylic acids, ketones, aldehydes, and others) usually have increased water solubility and toxicity. Where oxygen and sunlight are excluded in anoxic environments, anaerobic microbial oxidation takes place [28,29].

Generally saturated alkanes are more quickly degraded by microorganisms than aromatic compounds; alkanes and smaller-sized aromatics are degraded before branched alkanes, multi-ring and substituted aromatics, and cyclic compounds [30,31]. Polar petroleum compounds such as sulfur- and nitrogen-containing species are the most resistant to microbial degradation. Complex structures (e.g., branched methyl groups) and the stability of hydrocarbons decrease the rates of mineralization, which are

likely a consequence of the greater stability of carbon-carbon bonds in aromatic rings than in straight chain compounds. Emulsification also provides greater surface area for microorganisms to attach.

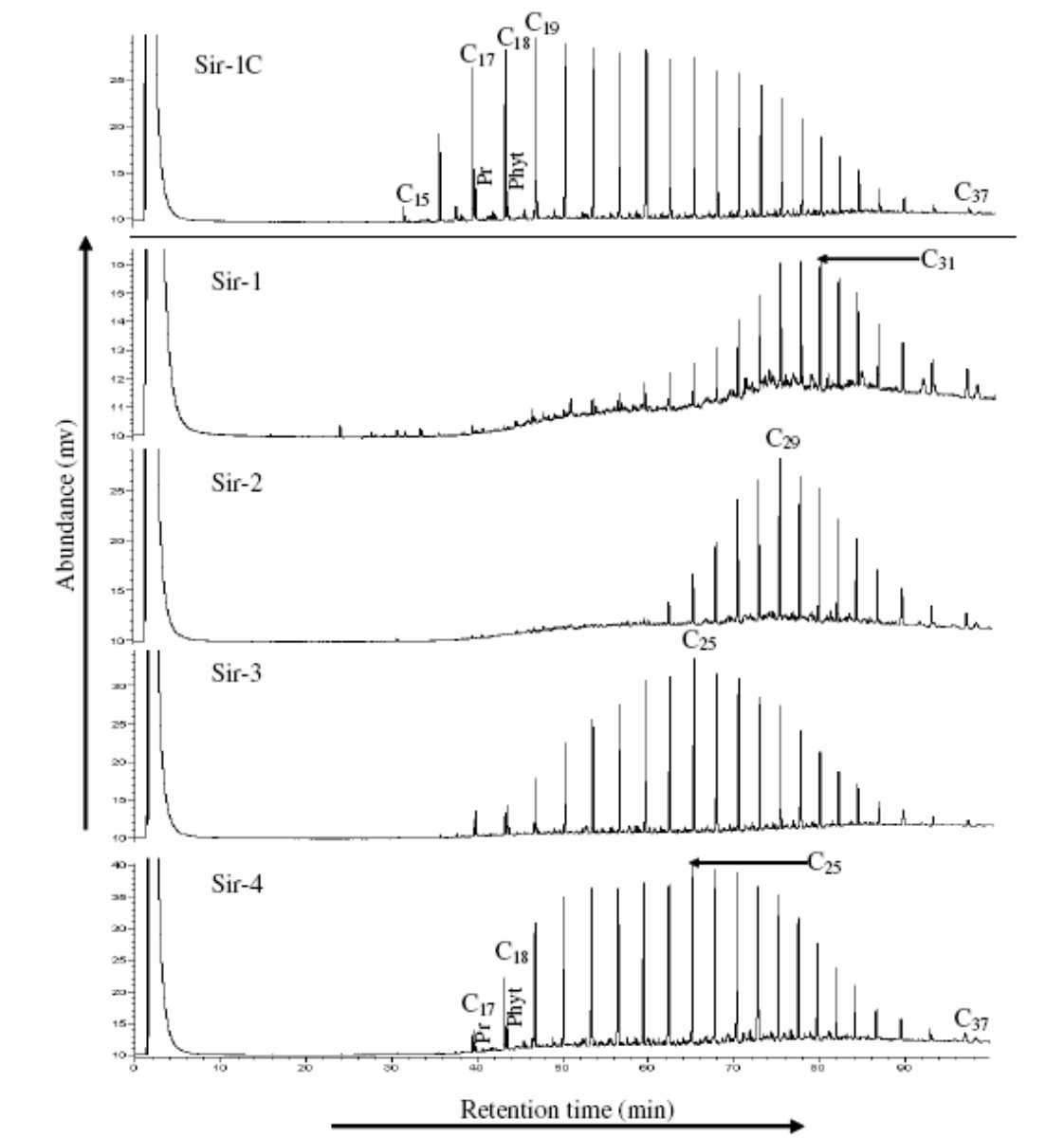


Figure 1.3 Gas chromatograms of the alkane fractions derived from crude oil Sirakovo (Sir, paraffinic type) after 90 days of simulated biodegradation with *Phormidium foveolarum.*and *Achanthes minutissima* with inorganic medium in the light (Sir-1), with inorganic medium in the dark (Sir-2), with organic medium in the light (Sir-3), with organic medium in the dark (Sir-4), together with chromatogram of alkane fraction typical for the control experiments (Sir-1C), Pr – pristine, Phyt – prutane, data taken from [33].

It has been shown in experiments, that *n*-alkanes are among the most biodegradable hydrocarbons and therefore they are easily broken down and preferentially depleted from soil samples [32]. Also it has been proven in simulation experiments of the biodegradation of two different samples of crude petroleum (paraffinic and naphthenic type) that microbial cultures which were isolated as dominant microorganisms from the surface of a wastewater canal of an oil refinery (most abundant species: *Phormidium foveolarum*, filamentous Cyanobacteria – blue-green algae and *Achanthes minutissima,* diatoms, algae) show a

strongly differentiated degradation behaviour with clear preference for the degradation of *n*-alkanes and isoprenoid aliphatic alkanes [33]. As can be seen in **Figure 1.3**, the largest degree of biodegradation was achieved in a medium containing the base nutrients $Ca(NO_3)_2.4H_2O$, $K_2HPO_4.7H_2O$, KCl, $FeCl_2$, K_2SO_4, pH≈8 exposed to light. Biodegradation activity is somewhat lower with the same medium in the dark. With a medium containing not only the nutrient broth, but also organic compounds: tryptone, yeast extract, glucose, at pH≈7), degradation occurs at a much lower rate, especially without light.

When crude oil or petroleum products are accidentally released to the environment, they are immediately subjected to a variety of weathering processes that lead to compositional changes and to the depletion of certain hydrocarbon compounds. Weathering processes include all previously mentioned physicochemical processes, such as dissolution, evaporation, photooxidation, polymerization, adsorptive interactions between hydrocarbons and the soil, and some biological factors. Furthermore, due to the fact that the degree of biodegradation is different for different types of petroleum hydrocarbons and varies depending on their nature, the weathering rate also depends on the type of petroleum contaminant. If we thus observe in the analysis of petroleum hydrocarbon contaminants changing patterns of hydrocarbons with time, this may be either due to the segregation of the oil according to the physical properties, or due to the action of bacteria and microorganisms. As these are only able to degrade certain classes of compounds, or at least they exhibit a strong preference for some over other compounds, this will lead to characteristic changes of the hydrocarbon pattern as observed by gas chromatography (**Figure 1.3**).

1.1.4. Possible toxic effects from exposure to petroleum hydrocarbons

As it was discussed earlier, crude oil and petroleum products are complex mixtures of groups of compounds. Many of the compounds are apparently benign, but many other are known to have toxic effects. Due to petroleum hydrocarbon toxicity, spilled hydrocarbons pose a threat that affects not only the sea and land, but also the lakes, rivers and groundwater, and can be harmful for animals and human health.

Much of what is known about the impacts of petroleum hydrocarbons comes from studies of catastrophic oil spills and chronic seeps. Large oil spills usually receive considerable public attention because of the obvious environmental damage, oil-coated shorelines, and dead or moribund wildlife, including, in particular, oiled seabirds and marine animals. The acute toxicity of petroleum hydrocarbons to marine organisms is dependent on the persistence and bioavailability of specific hydrocarbons. The exposure to them may alter an organism's chances for survival and reproduction in the environment, and the narcotic effects of hydrocarbons on nerve transmission are a major biological factor in determining the ecologic impact of any release. Marine birds and mammals may be especially vulnerable to oil spills. In addition to acute effects such as high mortality, chronic, low level exposure to hydrocarbons may affect reproductive performance and physiological impairment of seabirds and some marine mammals as well [11]. Petroleum contamination may also cause unfavorable impacts on nearby plants and animals.

Plants growing in contaminated soils or water may die or appear distressed. In turn natural seeps, leaking pipelines, production discharges release small amount of oil over long periods of time, resulting in chronic exposure of organisms to oil and oil chemical compounds. The lower molecular weight compounds are usually the more water soluble components of a product and hence attention has also been paid to the water soluble fractions of petroleum and related products. Concentrations in the environment are usually comparatively low and chronic effects are usually more significant [26]. The persistence of some compounds such as PAH in sediments, especially in urban areas is also an example of chronic pollution and toxicity.

Nowadays humans can be exposed to petroleum hydrocarbons through ingestion of contaminated drinking water and soil residues, inhalation of vapors and airborne soils, contact of contaminants with skin (dermal exposure) from many sources, including gasoline fumes at the pump, spilled crankcase oil on pavement, chemicals used at home or work, or certain pesticides that contain petroleum hydrocarbon components as solvents. Most petroleum hydrocarbon constituents will enter the bloodstream rapidly when inhaled or ingested. Incorporated petroleum hydrocarbons are widely distributed by the blood throughout the body and quickly are metabolized into less harmful compounds. Others may be degraded into more harmful chemicals. Even other compounds are distributed by the blood to other parts of the body and do not readily break down, but are accumulated instead in fat tissue. The resorption of petroleum compounds through dermal tissue is more slowly; that is why direct exposure of the skin to petroleum hydrocarbons is generally harmless when exposure is only occasional and of short duration.

Studies on animals have shown effects on the lungs, central nervous system, liver, kidney, developing foetus, and reproductive system from exposure to petroleum compounds, generally after breathing or swallowing the compounds. Health impacts of exposure to petroleum contamination may include lung irritation, headaches, dizziness, fatigue, diarrhea, cramps, and nervous system effects. Benzene and other chemicals found in petroleum products have been determined to be carcinogenic (cause cancer). More information regarding toxicity of petroleum chemicals is available, for example, from the Agency for Toxic Substances and Diseases Registry (ATSDR), an agency of the U.S. Department of Health and Human Services [34], or from European Chemicals Agency (ECHA) [35].

Oil products are complex mixtures of hundreds of chemicals, with each compound having its own toxicity characteristics. There are many difficulties associated with assessing the health effects of such complex mixtures with regard to hazardous waste site remediation. This means that the traditional approach of evaluating individual components is largely inappropriate. Toxicity information is in the best case available for the pure product; however, once a petroleum product is released to the environment, it changes its composition as a result of weathering. These compositional changes may be reflected in changes in the toxicity of the product.

One approach for assessing the toxicity of oil products is to use toxicity information from studies conducted on the whole product. A second approach is to identify and quantify all components and then consider their toxicities. This approach produces data which theoretically could be compared to the

known toxicity of each compound. The impracticality of this approach stems from its high analytical cost and the lack of toxicity data for many of the component chemicals found in hydrocarbon mixtures. A third approach is to consider a series of hydrocarbon fractions, determine appropriate tolerable concentrations and toxicity specific for those fractions. A number of groups have examined such an approach, but the most widely accepted and internationally used are the ones developed by the Total Petroleum Hydrocarbons Criteria Working Group (TPHCWG) and the Massachusetts Department of Environmental Protection (MA DEP) in the U.S., although they have been subject to adjustments in many cases. For example, in the U.K. the TPHCWG approach is modified and extended to consider heavier hydrocarbon fractions. It has been developed as part of the Environment Agency's [36] environment sciences programme and published in documents related to petroleum hydrocarbons [37].

The MA DEP introduced in 1994 the concept of petroleum hydrocarbon size-based fractions for use in evaluating the human health effects of exposures to complex mixtures of hydrocarbons [38,39], and provided oral toxicity values for each of the fractions. The toxicity value assigned for each fraction is used in dose response evaluations. Cancer risks or hazard amounts are subsequently summed across the fractions to get the total values. The TPHCWG has developed and published a series of five monographs [16,40,41,42] detailing the data on petroleum hydrocarbons and, in addition, has developed tolerable intakes for a series of total hydrocarbon fractions. The TPHCWG independently identified largely similar groupings of hydrocarbon fractions with somewhat different toxicity values in 1997. Of the 250 individual compounds identified in petroleum by the TPHCWG, toxicity data were available only for 95. Of these 95, the TPHCWG concluded that there were sufficient data to develop toxicity criteria for only 25.

As there are differences in toxicity between different hydrocarbon compounds, it is impossible to accurately predict toxic effects of contamination for which only total hydrocarbon data are available. Health assessors often select surrogate or reference compounds (or combination of compounds) to represent TPH so that toxicity and environmental fate can be evaluated. Correspondence date relating the toxicologically-derived hydrocarbon fractions and their toxicity values, to the analytically-defined reporting fractions (by MA DEP) are contained in **Table 1.7** for ingestion exposures and for inhalation exposures. Inhaled or ingested volatile hydrocarbons have both general and specific effects. The toxicity values are represented as a reference dose (RfD), what is the United States Environmental Protection Agency's (US EPA) maximum acceptable oral dose of a toxic substance. Significant efforts have been undertaken by MA DEP to describe an approach for the evaluation of human health risks from ingestion exposures to complex petroleum hydrocarbon mixtures. The methods offered by MA DEP for determination of air-phase (APH), volatile (VPH) and extractable (EPH) petroleum hydrocarbons [43,44,45] are designed to complement and support the toxicological approach. The ranges of quantified hydrocarbons within each method and their reporting limits are shown in the **Table 1.8**.

Table 1.7 Oral and inhalation toxicity values by MA DEP for petroleum hydrocarbon fractions and individual compounds present in petroleum products (NA- not applicable), data taken from [37,38].

Carbone Range	Compound	Toxicity Value, RfD Inhalation mg/m^3	Oral mg/kg/d	Critical effect
Aliphatic				
C5 - C8		0.2	0.04	Neurotoxicity
	n-Hexane	0.2	0.06	
C9 - C18		0.2	0.1	Neurotoxicity, hepatic and hematological
C19 - C32		NA	2	Liver granuloma
Aromatic				
C6 - C8	Use individual RfCs for compounds in this range			
BTEX	Benzene	NA	0.03	
	Toluene	0.4	0.2	
	Ethylbenzene	1.0	0.1	
	Styrene	1.0	0.2	
	Xylene (o-, p-, m-)	NA	2	
C9 - C18		0.05		Body weight reduction, hepatic, rental, and developmental effects
	Isopropylbenzene	0.4	0.1	
	Naphtalene	0.003	0.02	
	Acetanaphthene	NA	0.06	
	Biphenyl	NA	0.05	
	Fluorene	NA	0.04	
	Anthracene	NA	0.3	
	Fluoranthene	NA	0.04	
	Pyrene	NA	0.03	
C9 - C32			0.3	Neurotoxicity
C19 - C32		NA		

NA - not applicable

Table 1.8 The ranges of hydrocarbons quantified within the methods for determination of air-phase (APH), volatile (VPH) and extractable (EPH) petroleum hydrocarbons by MA DEP and their reporting limits, data taken from [38].

	APH		**VPH**		**EPH**		
	28-218 °C		36-220 °C		150-265 °C		
aliphatic	C5-C8	C9-C12	C5-C8	C9-C12	C9-C18	C19-C36	
aromatic		C9-C10		C9-C10		C11-C-22	PAH
				Reporting Limits (RLs)			
for the individual target analytes							
in air-phase	2-5 g/m^3						
in soil			0.05-0.25 mg/kg		20 mg/kg		0.2-1 mg/kg
in water			1-5 µg/L		100 µg/L		2-5 µg/L
for the collective hydrocarbon ranges							
in air-phase	10-12 g/m^3						
in soil			5-10 mg/kg		20 mg/kg		
in water			100-150 µg/L		100 µg/L		

The components of petroleum can be generally divided into broad chemical classes: alkanes, cycloalkanes, alkenes, and aromatics. A review of **Table 1.7** shows that a US EPA RfD is available for only one alkane, *n*-hexane. In general terms, alkanes have relatively low acute toxicity, but alkanes having carbon numbers in the range of C5–C12 have narcotic properties, particularly following inhalation exposure to high concentrations, because of their relatively high volatility and low solubility in water. Repeated exposure to high concentrations, for example, of *n*-hexane (RfD of 0.06 mg/kg/d) may lead to irreversible effects on the nervous system. Hexane is considered to be the most toxic compound in the C5-C8 aliphatic fraction. No RfDs are available for other alkanes, nor for any cycloalkane or alkene. Alkenes exhibit little toxicity other than weak anesthetic properties. Alkanes and cycloalkanes are treated similarly and have similar toxic effects.

Aromatic compounds with less than nine carbon atoms (such as benzene, toluene, ethylbenzene and xylenes - BTEX) are evaluated separately, because the toxicity values for each are well supported and these compounds have a wide range of toxicity. However, most of the smaller aromatic compounds have low toxicity, with the exception of benzene, which is a known human carcinogen (RfD of 0.029 mg/kg/d). Most petroleum hydrocarbon mixtures contain very low concentrations of PAHs. The major concern regarding PAHs is the potential carcinogenicity of some of these. Benzo(*a*)pyrene and benz(*a*)anthracene are classified as probable human carcinogens. Benzo(*a*)pyrene is normally considered to be the most potent carcinogenic PAH, but the carcinogenic potency of most PAHs is not well characterized. In case of spills of petroleum products affecting water, PAHs are not usually a specific concern, however, this concern becomes more specific if these compounds are released into the soil, due to a bioaccumulation of PAH in soil.

Different regulations and guidelines to protect public health are developed. These public health statements tell as well about petroleum hydrocarbons and the effects of exposure. The US Environmental Protection Agency (US EPA) [46] identifies the most serious hazardous waste sites in the U.S.A. EPA lists certain wastes containing petroleum hydrocarbons as hazardous. It regulates certain petroleum fractions, products and some individual petroleum compounds. General health and safety data are as well discussed by the Energy Institute [7], which is the main professional organization for the energy industry within the UK that promotes the safe, environmentally responsible and efficient supply and use of energy in all its forms and applications. The Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are other agencies that develop regulations for toxic substances in the U.S.A. The information provided by all of them is regularly updated as more information becomes available. The Dutch National Institute for Public Health and the Environment (RIVM) has been involved in a number of studies on risk assessment for petroleum hydrocarbons which were commissioned by the Dutch government and the European Commission [47]. Also the U.K. Environment Agency, mentioned before, is the leading public body protection and improving the environment in the United Kingdom, including protection petroleum contaminations.

1.2. Total petroleum hydrocarbons (TPH) and analytical methods for the determination of petroleum hydrocarbons in environmental media

Due to the compositional complexity of petroleum products, it is impossible to assess the extent of petroleum hydrocarbon contamination by directly measuring the concentration of each hydrocarbon contaminant. For this reason, at the present time no single analytical method is capable of providing comprehensive chemical information on petroleum contaminants. TPH is one parameter and definition that is currently widely used for expressing the total concentration of nonpolar petroleum hydrocarbons in soil, water or other investigated sample. In the U.S.A., for example, there are no federal regulations or guidelines for TPH in general. Many states have standards for controlling the concentrations of petroleum hydrocarbons or components of petroleum products. These are designed to protect the public from the possible harmful health effects of these chemicals. Analytical methods are specified as well, many of which are considered to be methods for TPH. These generate basic information that is a surrogate for contamination, such as a single TPH concentration. Such data are not suitable for risk assessment. However they are relatively quick and easy to obtain, and can offer a useful preliminary information.

The term TPH is widely used, but it is rarely well defined. In essence, TPH is defined by the analytical method – in other words, estimates of TPH concentration often vary depending on the analytical method used to measure it. So the ATSDR defines the TPH as a term used to describe a broad family of several hundred chemical compounds that originally come from crude oil. In this sense, TPH is really a mixture of chemicals. By the TPHCWG, the definition of TPH, also called “hydrocarbon index”, is referred sometimes to mineral oil, hydrocarbon oil, extractable hydrocarbon, oil and grease. As well as the TPHCWG says that the TPH measurement is the total concentration of the hydrocarbons extracted

and measured by a particular method and it depends on the analytical method used for determination. According to the MA DEP the TPH is also a loosely defined parameter which can be quantified using a number of different analyses and this parameter is an estimate of the total concentration of petroleum hydrocarbons in a sample. And again, depending on the analytical method used to quantify TPH, the TPH concentration may represent the entire range of petroleum hydrocarbons from C9 to C36 or the sum of concentrations of a number of single compounds (for instance benzene, toluene, ethylbenzene and xylenes) and groups of compounds (fractions e.g. primarily aliphatics C9-C18, C19-C36 and aromatics C11-C20). Great improvements in the definition and analysis of TPH were finally introduced by the International Organization for Standardization (ISO) [48] in 2000, when it published the standard method ISO 9377-2:2000 [49] for the quality control of water in which a method for the determination of the hydrocarbon oil index within the C10-C40 range in waters by means of gas chromatography is specified. The definition of "hydrocarbon oil index by GC-FID" was introduced, which defines the fraction of compounds extractable with a hydrocarbon solvent, boiling point between 36 ºC and 69 ºC, not adsorbed on Florisil and which may be chromatographed with retention times between those of *n*-decane ($C_{10}H_{22}$) and *n*-tetracontane ($C_{40}H_{82}$) (substances complying with this definition are long-chain or branched aliphatic, alicyclic, aromatic or alkylsubstituted aromatic hydrocarbons).

The TPHCWG and MA DEP evaluated the risk implications and arrived to the conclusion that TPH concentration data cannot be used for a quantitative estimation of the human health risk. The same concentration of TPH may represent very different compositions and very different risks to human health and the environment. Because the TPH parameter includes a number of compounds of differing toxicities and the health effects associated with exposure to particular concentrations of TPH cannot be determined. For example, two sites may have the same amount of TPH but constituents at one site may include carcinogenic compounds while these compounds may be absent at the other site. If TPH data indicate that there may be significant contamination of environmental media, than fractionated measurements and the separate determination of BTEX compounds and PAHs are necessary so that potential risk to human health can be quantitatively assessed [50]. The hydrocarbon index is thus a good indicator of the (magnitude of the) relative contamination of oil; however, it will not be suitable to give a true representation of the actual concentration of total petroleum hydrocarbons in the investigated sample. There are several reasons why TPH data do not provide the ideal information for investigated samples and do not establish target cleanup criteria. This is due to many factors including the complex nature of petroleum hydrocarbons, their interaction with the environment over time and the non-specificity of some of the methods used. The scope of the methods used for TPH determination varies greatly. There are few, if any, methods that are capable of quantifying all hydrocarbons without interference from non-hydrocarbons. All methods are subject to interferences from non-hydrocarbons, some to a greater extent than others.

There are numerous established analytical methods that are available for detecting, measuring, or monitoring of TPH and its metabolites. Analytical methods used for analysis of petroleum hydrocarbons

in environmental media should provide a sufficient degree of robustness. At the current time, however, the correctness and precision of results for the petroleum hydrocarbon determination strongly depends on the proper choice of method and measurement parameters whose correct selection is left to the judgment of the analyst. Besides methods that measure the TPH concentration, two other types of methods can be distinguished. These are methods that measure the concentration of a group or fraction of petroleum compounds and methods that measure individual petroleum constituent concentrations. For product identification the results of analyses of the petroleum groups or fractions can be useful, because they separate and quantify different categories of hydrocarbons. Individual constituent methods quantify concentrations of specific compounds that might be present in petroleum-contaminated samples, such as BTEX and PAHs, which can be used to evaluate human health risk.

There are several basic steps related to the separation of analytes of interest from a sample matrix prior to their measurement, such as extraction, concentration, and clean-up. These steps are common to the analytical processes for all methods, irrespective of the method type or the environmental matrix. Each of these steps together with the sampling, which is also important step in performing of petroleum analyses, affects the final result, and has a certain impact onto the measurement uncertainty [51,52].

Sample taking and sample handling has been recognized as probably the most significant factor that contributes random errors and uncertainties in the analysis of offshore oil in produced water. There are some general guidelines available through a number of studies that have been carried out on this subject. To separate the analytes from the matrix, extraction is performed using one of the many available extraction methods. Heating of the sample, purging with an inert gas can be used in the analysis of volatile compounds; solid phase extraction or extraction into a solvent is usually applied for water samples, the latter extraction method also being used for soil samples. For some types of solid samples the extraction efficiency depends on the extraction method and time. However, ultrasonication and extraction by shaking are equally used for this purpose. It was demonstrated by some studies, that extraction and clean-up are the most crucial steps in sample preparation procedures. According to the results the most critical factors affecting TPH recovery are extraction solvent and type of co-solvent, extraction time, adsorbent and its mass, and the TPH concentration [53]. The results of a study, where the occurrence of matrix effects in the gas chromatographic determination of petroleum hydrocarbons in soil was evaluated, indicate that solid phase extraction does not appear to be effective enough in removing interfering matrix components from the extract [54].

Most of the methods for the determination of TPH involve a clean-up step using Florisil (a particular form of magnesium silicate) and sodium sulphate (anhydrous) which essentially aims at removing the polar, non-petroleum hydrocarbons of biological origin and remaining traces of water. It appears that the found hydrocarbon concentration strongly depends on the used clean-up technique. The efficiency of the clean-up procedure for removing polar compounds is not limited to hetero-atomic substances like O-, N- or Cl-containing compounds. Also some hydrocarbons have a tendency to adsorb on Florisil, e.g. aromatic compounds with π-electrons or alkyl aromatics. The TPH recoveries after clean-

up procedure might depend on the composition of the oil investigated. Lower TPH recoveries may be expected for oils containing high concentrations of unsaturated hydrocarbons or PAHs. Also, lubricating oils often contain different amounts and types of (non-petrogenic) additives that may behave differently from the other compounds during the clean-up procedure [55].The results demonstrate also that the ratio of Florisil amount and extract volume are of importance for the recovery of the purified extracts [56].

The three most commonly used TPH testing methods include gas chromatography (GC) [49,57,58,59,60], infrared absorption (IR) [61,62] and gravimetric analysis [63,64,65]. Conventional TPH methods are summarised in **Table 1.9**.

Table 1.9 Summary of common TPH methods.

Analytical Method	Method Name	Matrix	Scope of method	Carbon Range	Aproximate Detection Limits	Advantages	Limitations	Reference
GC based	DIN ISO 9377-2:2000	water	solvent (hydrocarbon) extraction, clean-up using Florisil, evaporation, 1 µL injection; GC-FID	C10-C40	0.1 mg/L	detect broad range of hydrocarbons; provide information (e.g., a chromatogram) for identification	dose not quantify below C10; chlorinated compounds can be quantified as TPH	49
	OSPAR (2007)	water	*n*-pentane extraction, clean-up using Florisil, 50 µL injection; GC-FID	C7-C40 + TEX compounds	0.1 mg/L	does not need preconcentration step; detect broad range of hydrocarbons and polar hydrocarbons; provide information for identification	do not quantify below C7	57
	DIN ISO 16703:2005-12	soil	aceton/*n*-heptane extraction; clean-up using Florisil; evaporation; GC-FID	C10-C40	10 mg/kg	detect broad range of hydrocarbons; provide information (e.g., a chromatogram) for identification	dose not quantify below C10; chlorinated compounds can be quantified as TPH	58
	DIN EN 14039:2004	wastes	aceton/*n*-heptane extraction; clean-up using Florisil; evaporation; GC-FID	C10-C40	10 mg/kg			59

Table 1.9 Summary of common TPH methods (continued).

Analytical Method	Method Name	Matrix	Scope of method	Carbon Range	Aproximate Detection Limits	Advantages	Limitations	Reference
IR based	EPA 418.1 (1991/1992)	water, soil	freon extraction, silica gel treatment to remove polar compounds	most hydrocarbons with exception of volatile and very high hydrocarbons	1 mg/mL in water, 10mg/kg in soil	technique is simple, quick and inexpensive	freon is banned now; low sensitivity; lack of specificity; prone to interference; provides quantitation only	62
	ASTM D7678 - 11 (2011)	water, wastewater	solvent (cyclic aliphatic hydrocarbon) extraction, clean-up using Florisil, IR absorption in the region of 1370-1380 cm-1 (7.25 – 7.30 microns)	most hydrocarbons with volatile	0.5 mg/mL	technique is simple, very quick; a more complete fraction of extracted petroleum hydrocarbon is accessible		61

Table 1.9 Summary of common TPH methods (continued).

Analytical Method	Method Name	Matrix	Scope of method	Carbon Range	Aproximate Detection Limits	Advantages	Limitations	Reference
Gravimetry	EPA 413.1 (1979) ASTM D4281-95(2005)e1	most appropriate for wastewater, sludge, sediment	freon extraction, solvent evaporation	anything that is extractable (with exception of volatiles which are lost)	5 mg/mL in water, 50mg/kg in soil	technique is simple, quick and inexpensive	freon is banned now; low sensitivity; lack of specificity not suitable for low boiling fractions; prone to interference (organic acids, phenols and other polar hydrocarbons); provides quantitation only	63,65
	EPA 1664 (1999)	most appropriate for water and wastewater	*n*-hexane extraction; silica gel treatment to remove polar compounds, solvent evaporation	anything that is extractable (with exception of volatiles which are lost)	5 mg/mL	technique is simple, quick and inexpensive	low sensitivity; lack of specificity not suitable for low boiling fractions; prone to interference; provides quantitation only	64

Methods based on solvent extraction followed by quantitative infrared absorption measurement (at frequency 2930 cm^{-1}, which corresponds to the stretching vibration of aliphatic CH_2 groups), have been widely used in the past for TPH measurement because they are simple, quick and inexpensive. However, the use of these methods is discontinued, since the sale and use of freons (required for the extraction of hydrocarbons from the sample) no longer is allowed, and freons are generally phased out world-wide due to their ozone-layer destructing potential. Recently a new IR-based method was introduced, based on Freon free extraction. This method defines oil and grease in water and wastewater as the fraction that is extractable with a cyclic aliphatic hydrocarbon (for example cyclohexane) and measured by IR absorption in the narrow spectral region of 1370-1380 cm^{-1} (which corresponds to the excitation frequency of the symmetrical deformation vibration of CH_3 groups) using mid-IR quantum cascade lasers (QCLs) [61]. The method also considers the volatile fraction of petroleum hydrocarbons which is lost by gravimetric methods that require solvent evaporation prior to weighing, as well as by solventless IR methods that require drying of the employed solid phase material prior to measurement. Similarly, a more complete fraction of extracted petroleum hydrocarbon is accessible by this method as compared to GC methods that use a time window for quantification, as petroleum hydrocarbons eluting outside these windows are also quantified. On the other hand, IR-based methods do hardly provide any information on the chemical composition of the oil, or the presence or absence of other relevant compounds (aromatics, PAHs). In contrast, they would even detect compounds that are not typically considered as TPH, such as surfactants which also may absorb IR radiation due to the presence of CH-bonds. However, this statement is only partially true, since it depends mainly on the clean-up whether the IR method determines also other compounds than the TPH.

Gravimetric-based methods are also simple, quick, and inexpensive; they measure anything that is extractable by a solvent, not removed during solvent evaporation, and capable of being weighed. Consequently, they do not offer any selectivity or information on the type of oil detected. Gravimetric-based methods may be useful for oily sludges and wastewaters at high(er) concentrations, but are not suitable for measurement of light hydrocarbons (less then C15) which will be lost by evaporation below 70-85 ºC.

GC-based methods are currently the preferred laboratory methods for TPH measurement because they detect a broad range of hydrocarbons, they provide both sensitivity and selectivity, and they can be used for TPH identification as well as quantification. The potential of GC for producing information on the product-specific hydrocarbon pattern has been for long recognized by researchers in the field of petroleum hydrocarbon analysis [66,67,68].

Currently, there are several standard methodologies based on GC for different types of samples (water, soil, wastes). ISO has published the standard ISO 9377-2:2000 for the quality control of water and specifies a method for the determination of the hydrocarbon oil index within the C10-C40 range in waters by means of gas chromatography. The method is suitable for surface water, waste water and water from sewage treatment plants and allows the determination of the hydrocarbon oil index in concentrations

above 0.1 mg/l. Due to systematic differences which became evident between the results from the DIN ISO method and the IR based method, the GC-based method was subsequently modified [69,70]. As a result, the modified version of DIN ISO 9377-2:2000 – the OSPAR (Oslo-Paris commission) reference method [57] was published in 2005 and taken into force as a reference method in the field of petroleum production in January 2007. The OSPAR reference method is applicable for the determination of dispersed oil content in produced water and other types of waste water discharged from gas, condensate and oil platforms. It also allows the determination of the dispersed mineral oil content in concentrations above 0.1 mg/l, and includes the determination of certain hydrocarbons within the C7-C10 range with the TEX compounds (toluene, ethylbenzene and *o*-/*p*-/*m*-xylene) being reported separately.

GC-based methods are based on the extraction of water samples with a nonpolar (hydrocarbon) solvent, the removal of polar substances by clean-up with Florisil and capillary gas chromatography measurements using a nonpolar column and a flame ionization detector (FID), cumulating the total peak area of compounds eluted between *n*-decane ($C_{10}H_{22}$) and *n*-tetracontane ($C_{40}H_{82}$) for the DIN ISO 9377-2:2000 standard method and for the DIN ISO 16703:2005-12 [58] standard method for soil samples. The OSPAR method was modified in order to include the determination of certain hydrocarbons with boiling point between 98ºC and 174ºC (that is, from *n*-heptane to *n*-decane), with the TEX (toluene, ethylbenzene and *o*-/*p*-/*m*-xylene) compounds being determined separately by integration and subtraction of their peak areas from the total integrated area. The GC-based methods usually cannot quantitatively detect compounds with lower boiling point than *n*-heptane because these compounds are highly volatile and are interfered by the solvent peak. Furthermore, the EPA method 8240 [60] exists which is used to determine volatile organic compounds in a variety of waste matrices by gas chromatography/mass spectrometry (GC/MS). It can be used to quantitate most volatile organic compounds that have boiling points below 200 ºC and that are insoluble or slightly soluble in water. The estimated quantitation limit of the EPA 8240 method for an individual compound is approximately 5 µg/kg (wet weight) for soil/sediment samples, 0.5 mg/kg (wet weight) for wastes and 5 µg/L for ground water.

GC based methods are suitable for surface water, waste water and other types of waste water discharged from gas, concentrate and oil platforms and allow the determination of hydrocarbon oil concentration above 0.1 mg/L. To reach the required detection limit, the method according to DIN ISO 9377-2:2000 foresees preconcentration of the extracts by solvent evaporation which bears the risk of losing the more volatile constituents of the sample. In contrast to this, the OSPAR method does not allow for any external apparatus for preconcentration, for which reason the GC must be equipped with an injection system that allows the injection of a volume of up to 100 µL of the extract. This is most easily realised with programmed-temperature vaporiser large volume injectors (PTV-LVI). This technique can reduce the loss of volatile analytes, increase sensitivity and is a viable, fast and automated alternative to an external preconcentration procedure [71,72,73].

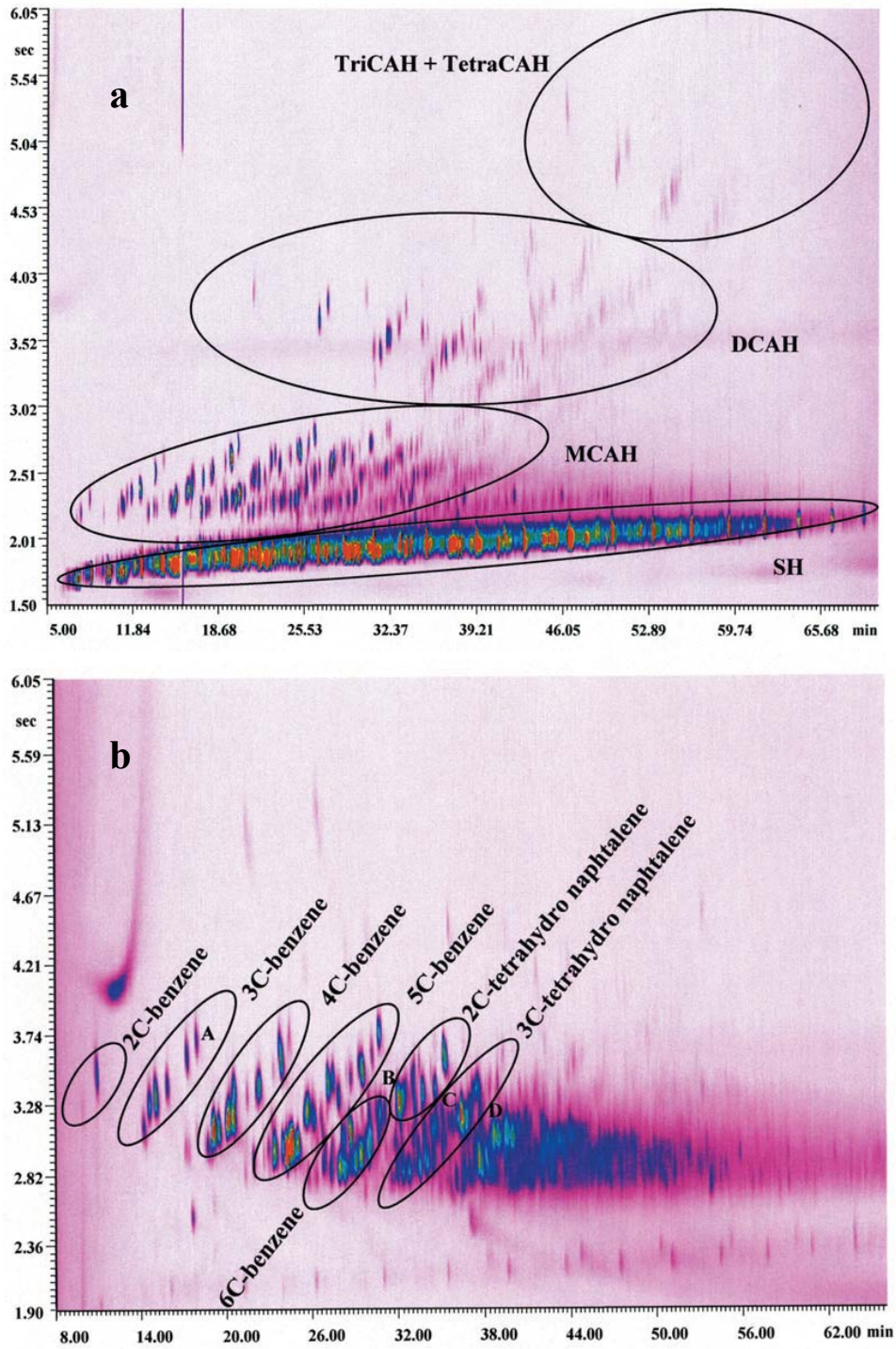


Figure 1.4 a: TIC GC × GC-qMS (quadrupole-MS) chromatogram of diesel oil. SH, saturated hydrocarbons; MCAH, monocyclic aromatics; DCAH, dicyclic aromatics; TriCAH, tricyclic aromatics; TetraCAH, tetracyclic aromatics. **b**: TIC LC-GC × GC-qMS chromatogram of the monocyclic aromatic fraction of diesel oil, A: Indane, B: 1,2,3,4-Tetrahydro-2,7-dimethyl naphthalene, C: 1-Cyclohexyl 3-methyl benzene, D: 1,2,3,4-Tetrahydro-2,5,8-trimethyl naphthalene data taken from [77].

Petroleum products easily contain thousand of different compounds. Classical capillary GC cannot resolve such mixtures to the level of individual compounds. A powerful analytical tool for separation of complex mixtures, such as petroleum hydrocarbons, is comprehensive two-dimensional gas chromatography (GC×GC or 2D-GC) [74,75,76]. The use of 2D-gas chromatography with MS detection

(GC×GC/MS) is expected to not only allow the separation of the various constituents of complex TPH samples, but also their identification based on MS detection (**Figure 1.4b)**. It is known that a certain class of chemical compounds (a series of 'homologues') is forming a very distinct, clearly identifiable pattern in the two-dimensional space of the GC×GC separation. The diesel total ion (TIC) GC×GC/MS chromatogram, illustrated in **Figure 1.4a**, is characterized by very typical group-type patterns: saturated hydrocarbons, which present low second dimension retention times, are followed by monocyclic and dicyclic aromatics; tri- and tetracyclic aromatics are the most retained on the secondary polar column [77]. Moreover, partial overlapping between chemical groups occurs, the monocyclic aromatics are situated in a rather narrow band and the tri- and tetracyclic aromatics are hardly visible in the 2-D chromatogram. The analytical potential of such two dimensional system is great.

1.3. Large volume injection (LVI) technique in gas chromatography (GC)

When trace sample components have to be determined at very low concentration levels, LVI-GC is an attractive approach to achieve these goals. It is a good alternative to an external evaporative pre-concentration step in the extraction procedures of complex samples. The procedure for pre-concentration is time-consuming and sometimes difficult to automate. There is a risk that volatile components are lost in the evaporation step, unstable components can decompose, especially if the elevated temperatures are used for evaporation. The LVI technique has been investigated for the analysis of a great variety of samples as a replacement of an external pre-concentration step [78,79] and proved to be a very accurate technique. Also, LVI can be helpful in the on-line combination of the sample preparation step, i.e. solid-phase extraction and chromatographic analysis (SPE-GC-FID) [80,81].LVI can also be used as an interface for the on-line connection of liquid chromatography (LC) with GC [82,83,84].Two-dimensional LC-GC systems have become popular lately, in order to reach efficient sample clean-up and/or a group-type separation of the analytes in complex samples.

In the past the main obstacle to LVI has been the difficulty in removing the large amount of solvent from the system in an efficient, simple and rugged manner so that chromatographic performance is not compromised. The classical split/splitless injection only allows introducing the limited a sample volume of 1-2 μL onto capillary columns in most cases (depending on the solvent and used system the volume can be increased up to 5-10 μL). There were various techniques (some of them built in-house) that have been developed for this purpose but they often require specialized hardware and considerable skill on the part of the operator. Many authors have worked on developing, characterising and investigating these techniques [80], in particular Grob and co-workers [85,86,87,88]. In principle, the LVI techniques can be separated into three groups:

- The on-column technique [89,90]
- Loop-type injector (mostly as interface for coupling LC with GC) [91]
- Programmed-temperature vaporiser (PTV) injector [80]

Large volume injection requires that the solvent is vaporised as it is being injected. On-column injection addresses this problem by using a retention gap of few meters of uncoated deactivated capillary, which provides room for the large volume of solvent injected to condense (since it is injected into the column *below* the normal boiling point of the solvent) and to expand. Loop-type injection is based on an interface that connects a sample loop with a capillary column (retention gap) using a multi-port valve. It is mainly used for LC-GC coupling. The technique is based on the concept that the carrier gas pushes the sample from the loop into the GC capillary against its own vapor pressure, generated by a column temperature slightly exceeding the solvent boiling point at the carrier gas inlet pressure, where the solvent is evaporated.

Programmed-temperature vaporizing injection is the most widely used technique for LVI. Using the PTV, solvent removal becomes a fast, easy and reproducible process. It is based on the so-called solvent vent or solvent elimination technique. With this technique the solvent is evaporated in the liner of the injector and the solvent vapor is discharged via the split exit of the injector. This is possible but requires extreme care in setting up liner temperatures, gas flows and injection speed (special autosampler may be required). This technique and other important questions related to its optimisation will be described in detail in *section 1.4*. The principles of the on-column approach and loop-type injection for LVI will be briefly discussed in following.

Several other novel injection techniques for LVI have been reported with the intention to yield *or*: lead to robust, easy and flexible LVI approaches. They include direct sample introduction (DSI) [92,93], splitless overflow, at-column [94], and ‘‘through oven transfer adsorption desorption’’ (TOTAD) [95,96]. A comprehensive review of these techniques and of the fundamentals of LVI was given by Hoh and Mastovska [71].

1.3.1. On-column LVI

Large-volume on-column injection techniques were introduced by Schomburg *et al*. in 1977 [97], denoted as direct sampling, and further described by Grob [98]. The basic principle of on-column large volume injection is very straightforward. A key component of the on-column LVI technique is a retention gap (a piece of a deactivated, uncoated capillary column), which is attached to the front of the analytical column to retain the injected liquid. The training guide for LVI of ATAS GL Sciences Company [99] has been used to describe it here.

A retention gap is installed before the analytical column. The large volume sample is than introduced directly into this retention gap using an on-column injector. During introduction of the sample the temperature of the column oven is low, some 15ºC *below* the normal boiling point of the solvent. This means that, as in the cool on-column injection of a standard volume, the sample is introduced in the liquid state. Discrimination or degradation is hence absent because the sample is directly introduced from the syringe into the column system without an intermediate evaporation step. It is evident that the retention

gap, in order to accommodate the entire sample volume, should be sufficiently long. As a rough rule of the thumb: every microliter of sample results in an increase of the zone length of the wetted zone of roughly 20 centimetres. This means that for a 100 μL injection a retention gap of at least 20 meters is required. As the solvent plug may move along the retention gap, a safety margin of some 5 meters has to be added to this length. The use of such a long retention gap not only makes the column system expensive, it also renders evaporation very slow. Due to the long retention gap the column system has a serious flow resistance. This means that the gas flow rate through the system is low and, hence, evaporation of the solvent is slow. A second disadvantage of the on-column method for large volume injection is that in the simple set-up described above the entire volume of solvent has to be discharged from the system via the detector. Many of the more sensitive and/or selective GC detectors such as the nitrogen phosphorous detector, the flame photometric detector, electron capture detector and the mass spectrometer, however, do not tolerate the introduction of large volumes of solvent. To overcome this disadvantage, systems for on-column large volume injection are very often equipped with a so-called "solvent vapor exit (SVE)". This is an additional gas exit installed between the retention gap and the analytical column. A schematic representation of a system for on-column large volume injection in capillary GC is given in **Figure 1.5**.

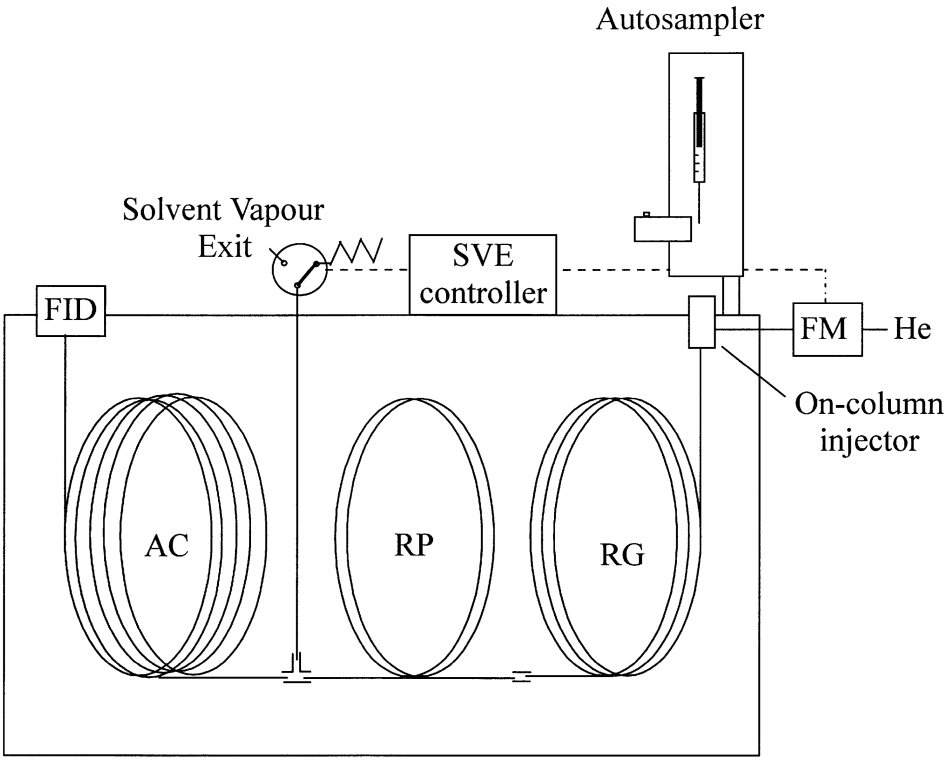


Figure 1.5 Set up of the on-column large volume injection-GC system. Abbreviations: AC, analytical column; RP, retaining precolumn; RG, retention gap; FM, flow meter; He, helium; SVE, solvent vapor exit [89].

The SVE is opened during solvent evaporation and is closed when solvent evaporation has reached completion. An advantage of the use of an SVE is that solvent vapors no longer have to be discharged via the detector. In this way the detector is protected from large volumes of solvent vapor.

Moreover, if an SVE is used, the back pressure provided by the column system is significantly reduced, which results in a significantly higher column flow at a given inlet pressure. Evaporation of the sample solvent is hence faster. Apart from these advantages the use of an early solvent vapor exit also has several disadvantages; these include more expensive instrumentation and the greater complexity of the system.

Using the SVE, highly volatile analytes can be easily lost by concurrent evaporation with the solvent. The closure time of the SVE is very critical. This fully concurrent solvent evaporation prevents the solvent trapping effect, resulting in loss of highly volatiles [87,71]. To reduce the loss of highly volatiles, several methods were suggested. One method employs co-solvent trapping [100], i.e. addition of a small portion of a solvent with higher boiling point, which is effective in some applications (i.e. loop-type sample introduction). Another way to overcome this problem is the installation of a so-called "retaining precolumn" (approximately 2 m) between the retention gap and the SVE [101]. This column, in contrast to the retention gap, does contain stationary phase, it provides some separation between the wide solvent peak and the volatile components. In this approach, determining the closure time of the SVE is less critical, still a certain minimum difference in boiling point between the solvent and the first components of interest is required. Quantitative recovery of *n*-C7 in a large volume injection using hexane as the solvent is impossible. For a really reliable method a boiling point difference of at least some 35°C to 40°C is required.

Despite the evident advantages of on-column sample introduction one would, for practical reasons, usually try to avoid the use of this technique, even in analyses in which only small volumes are injected. Only if all alternative injection techniques fail, one would resort to on-column injection. This is because on-column injection is less rugged, more difficult to automate and because column contamination by high molecular weight impurities occurs more readily than in (hot) split and splitless or PTV injection. In principle the same remarks also hold for on-column large volume injection. From the theoretical point of view, on-column large volume injection is superior to the other methods of large volume injection. For practical reasons, however, one would again often try to avoid the use of the on-column interface in large volume injection. Contamination of the retention gap readily occurs in the on-column injection of large volumes. This results in losses of adsorptive and/or unstable components and makes it necessary to replace the retention gap and to re-optimise the time of closing the SVE. Moreover, the various column connectors incorporated in a system for on-column large volume injection form each a potential source of leakage [99].

1.3.2. Loop-type injection of large volume

The loop-type interface was introduced for fully concurrent solvent evaporation in 1986 [91] and was the interface installed on the first commercial on-line HPLC–GC instrument. It was the most widely used interface for more than a decade, **Figure 1.6**.

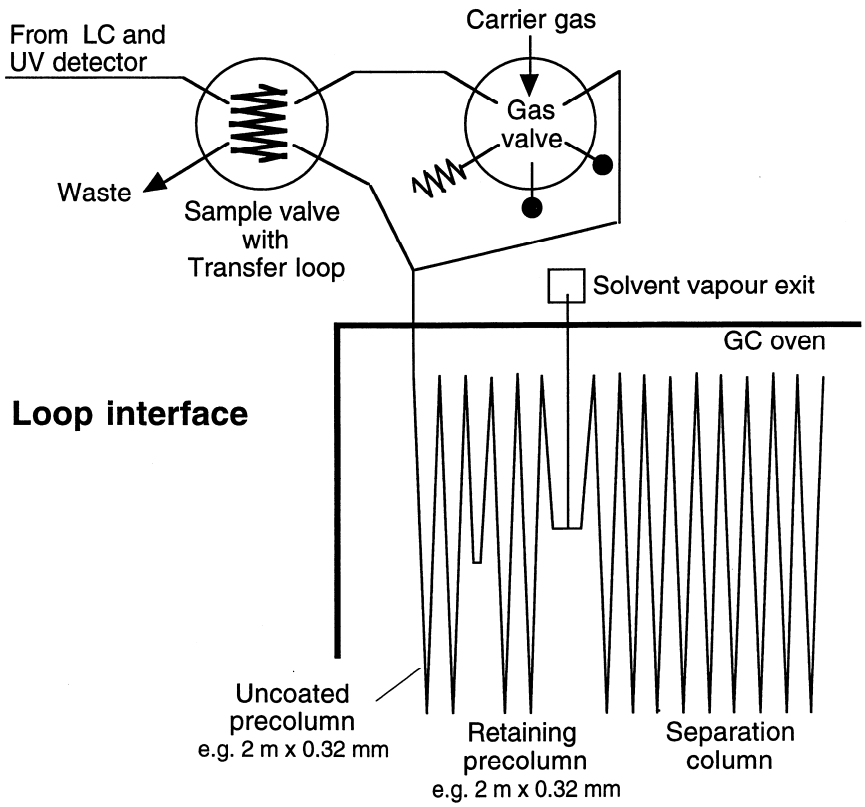


Figure 1.6 Loop type interface for LC–GC transfer involving concurrent eluent evaporation [102].

The loop-type interface consists of a 6- or 10-port valve equipped with a loop, in which the sample is loaded, e.g. after HPLC separation. By switching the valve the sample is pushed by the carrier gas into the retention gap (into the GC) at a temperature equal or *above* the eluent boiling point, where the solvent is evaporated. The solvent evaporates concurrently during its introduction and the transfer occurs at a rate corresponding to the evaporation speed through the SVE. In fact, the carrier gas pushes the eluent against its own vapor pressure, which has to exceed the column inlet pressure and stopping the eluent to flow into the GC column, which due to its smaller i.d. and greater length offers a significant flow resistance [91], **Figure 1.7**. In this way the flow rate is automatically adjusted to the evaporation rate. Basically, only the oven temperature during transfer needs to be optimised. While the solvent evaporates, an increase in pressure can be observed, and a subsequent pressure drop indicates that the solvent has evaporated. If a vapor exit is used, it is closed some 10-30 seconds later to ensure that all solvent vapors have left the column system. A 2–3 m retention gap and a short piece of a retaining precolumn (1–2 m) are usually placed before the SVE (similarly to on-column LVI), as shown in **Figure 1.6**. A simple detector (e.g. a thermal conductivity detector) often is integrated in the SVE to monitor the elution of the solvent vapor. When analyzing high boiling analytes the retaining pre-column is not necessary. A restrictor can be mounted on the vapor exit valve to purge the solvent vapor exit and thus prevent remaining solvents interfering with the subsequent analysis by backflow. Once the injection is finished and the solvent has been evaporated, the SVE is closed and a normal GC separation is performed [83,103].

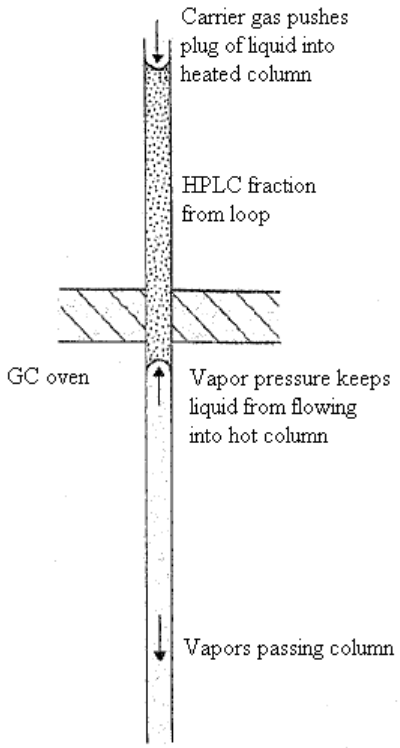


Figure 1.7 The concurrent solvent evaporation using the loop-type interface [91].

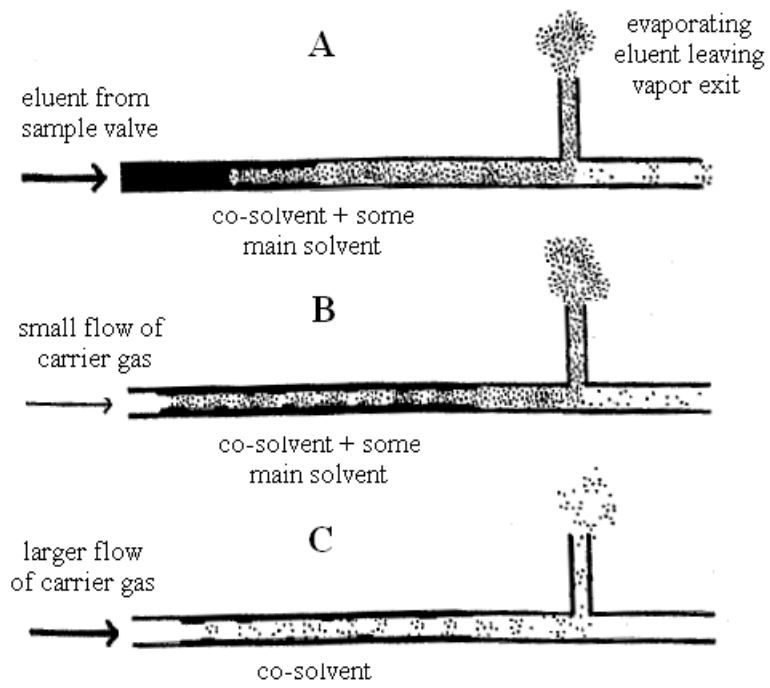


Figure 1.8 Transfer by concurrent solvent evaporation with co-solvent trapping [106].

A larger amount of solvent can be transferred (by using a relatively short retention gap), but loss of volatile compounds occurs. In fact volatiles co-evaporate with the solvent through the SVE or, if venting is delayed, the most volatile analytes reach the detector before the end of solvent evaporation. The first sharp peaks are eluted 60–120°C above the transfer temperature. To partially reduce such a drawback, the so called "co-solvent trapping technique" can be employed [104,105]. It is achieved by adding a small amount of a higher boiling co-solvent to the main eluent. The co-solvent left behind as a liquid forms a film as in the retention gap technique, enabling the exploitation of the solvent trapping effect for the volatile compounds. The transfer in this case can be shortly described as follows [106] (**Figure 1.8**): (A) The solvent plug completely blocks the carrier gas; only solvent vapor flow through the uncoated precolumn, most of it leaving through the vapor exit; (B) The plug of liquid has disappeared; the thick layer of co-solvent and main solvent on the capillary wall exhibits a vapor pressure that allows a small flow-rate of carrier gas to pass; (C) Only co-solvent is left in the uncoated precolumn, with a low vapour pressure that allows a large carrier gas flow-rate to pass. At this point, the vapor exit must be closed. To simplify the optimisation, the co-solvent is usually chosen such that it forms an azeotropic mixture with the main solvent and the mixture evaporates in known ratio of the components virtually independent of the pressure.

The advantage of the loop-type interface is that the operation is simple. The transfer is almost completely self-regulated; the only parameter to be adjusted is the oven temperature, and only a short

uncoated precolumn is needed. Large sample volumes up to several millilitres are easily transferred. At least in theory, the method is suitable for aqueous eluents as well. In practice, however, complete evaporation of polar solvents and solvent mixtures turns out to be very difficult. The loop-type interface with concurrent solvent evaporation technique is only suitable for relatively non-volatile analytes, however.

1.4. Programmed-temperature vaporiser (PTV) for large volume injection (LVI)

1.4.1. The principle of PTV injection

A PTV system was presented by Vogt *et al.* in 1979 [107]. He described the introduction of large sample volumes (up to 250 µl) into capillary CG columns at a controlled rate in the trace analysis of high-boiling compounds (the system was tested on *n*-alkane (C18-C32) sample). The temperature of the liner filled with glass wool was chosen below the boiling point of the solvent and the solvent was continuously evaporated and vented through the split line. Later, based on this idea, the PTV inlet was developed by Poy *et al.* [108], who first realized that the technique of programmed temperature sample introduction had a number of advantages over conventional split/splitless and on-column injection. For example, discrimination due to differences in boiling point can be minimized by introducing the sample in the liquid state onto a cold liner, and subsequently raising the temperature of the liner to the normal temperature of a conventional hot inlet. Poy proposed the name "programmed temperature vaporiser" injector, which is now a widely accepted term in chromatographic nomenclature.

A schematic drawing of a PTV inlet is given in **Figure 1.9**. As can be seen from this figure, the PTV consists of the same elements as a classical split/splitless inlet, but it is constructed to have only low thermal mass and is equipped with an efficient heating and cooling system. Heating is accomplished using a special wrapped heating wire configuration that provides linear, homogeneous heating of the inlet body at a user selectable rate. Heating is controlled using a sophisticated electronic controller. Cooling is realized by means of liquid nitrogen, CO_2 or Peltier cooling. As with the split/splitless inlet, the heart of the system is the liner. The carrier gas is connected through the sampling head and enters the liner at the top. The split flow leaves the liner at the bottom. There is a solenoid valve in the split line that allows switching from split to splitless operation.

Operational steps of the PTV technique are the following:

1. The sample solution is injected (in the concurrent evaporation mode) into the liner at a controlled rate at a temperature below the boiling point of the solvent.
2. During injection the split vent is opened, the solvent vapor is purged from the liner and leaves through the open split vent. Less volatile compounds will remain within the liner.

3. When all, or most of the solvent has evaporated, the vent is closed and the temperature of the liner is raised to vaporize the analyte residues. These are then transferred, without splitting, to the chromatographic column where chromatographic separation takes place.
4. The split vent may be reopened after a further delay to assist in the removal of less volatile analytes from the liner after each injection.

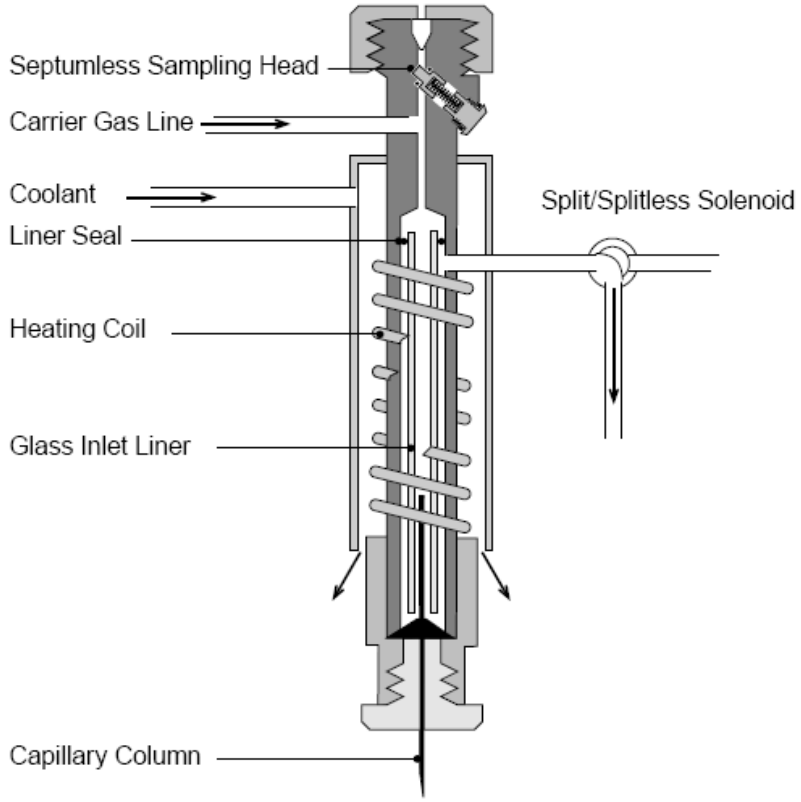


Figure 1.9 Shematic representation of PTV inlet (GERSTEL-CIS) [109].

According to the training guide for LVI of ATAS GL Sciences Company [99], when the sample is injected into a straight, empty liner, typically a fine spray of liquid is formed which reaches the liner wall and spreads out as a film of liquid. Because the split line is open, a high flow of gas passes across the liquid film, so evaporation of the solvent occurs first, as this has the lowest boiling point. Other components also start to evaporate eventually, but only after all of the solvent has evaporated. Just before this happens, the split exit should be closed. The injector is heated at this time, so all of the solutes are rapidly transferred to the column.

A number of parameters can be controlled for PTV. For a better understanding, the reader is referred to **Figure 1.10, (**redrawn from [110]), which shows the sequence of events for an injection with the PTV injector.

The events are organized according to different phases:

1. Pre-injection: During this phase (*Prep run time*) the sampler is preparing for injection, the GC and injector temperatures are at their initial values, and the split valve is open. The column head pressure is at normal operating pressure until just before the injection.

2. Sample injection + venting: The sample is injected into the liner, and the split valve remains open during injection. The duration of this phase is determined by the sample volume and injection speed (e.g. 50 µL @ 5µL/sec. would take 10 sec). The column head pressure is at ambient pressure, this prevents flow of analytes and solvent onto the column. Adjustment of vent pressure and vent flow allow a faster maximum injection speed. After the sample is injected into the inlet and the needle is withdrawn, the GC run starts (*Start run*).
3. Post injection + venting: depending on the particular application the inlet can stay at its initial temperature for a defined time (*Init Time*) and in the solvent vent mode for defined time as well (*vent end time*). In some cases this is needed to complete the solvent elimination.
4. Sample transfer and analysis: The sample is transferred onto the column in "splitless" mode by rapidly heating the inlet and analysed by mean of GC.
5. Inlet purging: The phase of splitless mode is defined by *Purge time*, the time when the spit vent of the inlet is reopened in order to purge it from sample remains in liner.

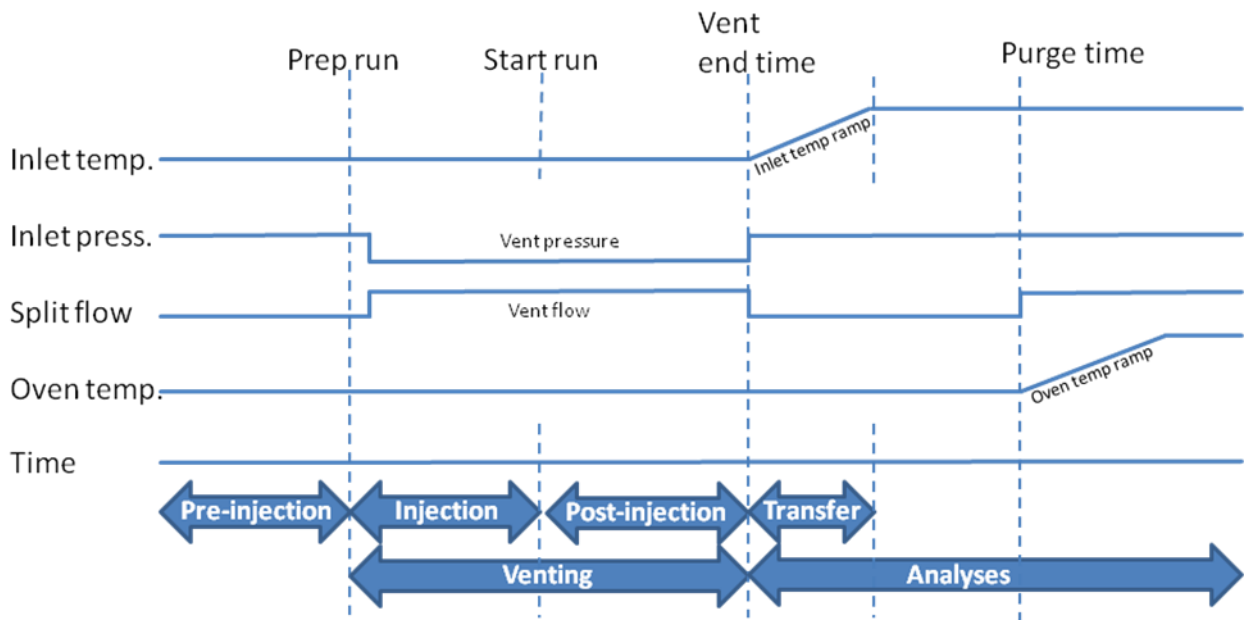


Figure 1.10 Time chart for control parameters for solvent vent mode PTV-LVI.

By using the PTV solvent vent injection the volume injected into the liner can be far above a volume of about 1 µL, which is usual in the case of hot sample injection. LVI by means of PTV with solvent elimination can be described by means of three approaches that are based on each other and will be described in the next section.

1.4.2. Programmed temperature vaporizer-large volume injection (PTV-LVI)

A critical issue in LVI is the huge solvent vapor volume resulting from the expansion of the large volume of solvent injected. To overcome this problem, large volume injection using PTV with solvent elimination can be applied. This can be realised in three different approaches: rapid or at-once injection, multiple, and speed-controlled injection.

At-once injection refers to one rapid injection of sample as a liquid within 1-2 sec into the cold inlet (at a temperature below the solvent's boiling point) and subsequent evaporation of the solvent in a controlled way. Upon injection it is important that the liquid sample is retained in the liner because otherwise part of the sample will be vented – in the liquid state- via the split exit and/or flood the analytical column. This would lead to loss of analytes and distorted peak shapes, respectively. Mol *et.al.* [111,112] described in detail the principles and the optimisation of this technique. They suggested several guidelines for the maximum volume injection volume depending on the selection of the liner size and packing material, **Table 1.10**.

Table 1.10 Guidelines for the selection of liner and injection mode in large volume PTV injection when hexane is used as a solvent [112].

	Analyte		
	Very labile/adsorptive	Other	
Sample volume	>5	<100-120	>100-150
Liner I.D. (mm)	1	4	1-4
Packing	Empty	e.g., Supelcoport, Chromosorb-750 coated with Dexsil	
Injection mode	Repetitive (n x 5µL) or speed controlled	At-once	Repetitive (n × V_{max}) or speed controlled
Volatility application range	$\geq C_{13}$	$\geq C_9$	$\geq C_{13}$, C_{10-30}[a]

[a] When packing material is Tenax

Liners with small diameters, accordingly with smaller volumes, result in faster transfer, so analytes leave the liner faster and at lower temperature, which is ideal for the injection of small volumes (1-2 µL). However, when injecting a larger sample volume, for example 20 µL, into a liner with an inner diameter of 1.2 mm, only a small amount of liquid can be accommodated. In this case a smaller sample volume needs to be injected repeatedly or the entire sample volume is introduced in a speed controlled manner. Using 3.4 mm liner, sample volume of up to 150 µL could be injected at-once [113], but the optimisation of the solvent vent time is required in this case. This time is needed to evaporate and eliminate the solvent via the split exit prior to transfer of the analytes to the analytical column.

In order to increase the capacity of the liner, and thus to enhance retention of the liquid sample, packing material in the liner can be used. Maximum load-ability is achieved when the polarities of the packing material and the solvent match. Packing material should be sufficiently inert, however, some interaction with the analytes can be advantageous, especially for volatile analytes that otherwise might

easily be lost together with the solvent during the solvent evaporation. The use of packed liners is often advantageous not only for at-once LVI, but for LVI in general.

The process of at-once injection into the cold injector can be repeated several times. This is the **multiple** injection case. With repetitive injection fractions of the sample smaller then the maximum volume that the liner can accommodate are rapidly injected. The time between two injections is equal to the solvent elimination time. After injection and solvent elimination of the last fraction, all analytes accumulated in the liner are transferred to the analytical column in the splitless mode by increasing the temperature of PTV. The optimisation of this LVI technique is straightforward as for the at-once mode [112].

The repeated introduction of sample portions in multiple injections can be replaced by a continuous process, so a large volume of sample is introduced into the liner at a constant and controlled rate. This is the case of **speed-controlled PTV injection**, which allows the injection of volumes up to 1000 μL by simultaneous injection and solvent evaporation. This requires special autosamplers, allowing to control the speed of the injection. The maximum allowable speed of large sample volume introduction in this case is determined by the solvent elimination rate. Atheoretical model was proposed by Staniewski and Rijks [114] to predict an optimum combination of the speed of sample introduction, the initial liner temperature and the purge gas flow rate.

The saturated vapor volume (V_g) at a given temperature (T_{inlet}), which corresponds to a defined liquid volume (V_l) can be calculated according to the following equation:

$$V_g = \frac{V_l \rho R T_{inlet}}{M P_j} \qquad (1.1)$$

where:

ρ – density of solvent [g/mL]

M – molecular weight of solvent [g/mol]

R – gas constant [0.08312 l*bar/K*mol]

T_{inlet} – initial temperature of inlet [K]

P_j – partial pressure of solvent [bar]

In deriving this equation it is assumed that the solvent vapor exhibits ideal gas behaviour. During the introduction of LVI the speed of sample introduction into the liner of the injector should not exceed the solvent elimination rate. In the steady stage the mass flow of the liquid solvent introduced into the liner equals the mass flow of the corresponding solvent vapor at the exit of the liner, i.e., the amount of liquid solvent in the liner remains constant. Assuming isothermal evaporation conditions and further assuming that the gas leaving the liner is saturated with solvent vapor, the maximum injection speed which equals the solvent elimination rate can be calculated as follows:

$$v = \frac{M \cdot P_s}{\rho \cdot R \cdot T_{inlet}} \cdot \frac{P_a}{P_{inlet}} \cdot v_{sf} \qquad (1.2)$$

where:

M – molecular weight of solvent [g/mol]

P_s – vapor pressure of solvent at initial inlet temp. [bar]

ρ – density of solvent [g/mL]

R – gas constant [0.08312 l*bar/K*mol]

T_{inlet} – initial temperature of inlet [K]

P_a – ambient pressure [usually 1.013 bar]

P_{inlet} – inlet pressure [gauge+ambient pressure, bar]

v_{sf} – split flow [mL/min]

According to the **Equation 1.2** the inlet pressure and the, vapor pressure of solvent at initial inlet temperature are important parameters in order to provide a correct solvent evaporation. The equation further shows that by reducing the column head pressure, increasing the injection temperature (which increases vapor pressure of solvent) and increasing the purge gas flow rate the solvent elimination rate can be increased. The success of the separation of the solvent from the most volatile solutes in the injector depends also on solvent physico-chemical properties, evaporation temperature, solvent evaporation time and the split flow-rate. Adaptation of the conditions for the separation of solvent and analytes in the inlet in PTV solvent split injection proved a complex and many-faceted problem, the solution of which requires careful optimisation.

When optimising speed controlled PTV poor recoveries can be found for all components (both volatile and non-volatile) if the injection rate exceeds the evaporation rate, because the liquid sample is lost *via* the split exit. Before starting the optimisation it is thus necessary to estimate the maximum solvent evaporation rate, which is the same as the injection rate, since both processes must be in equilibrium. The systematic approach of Staniewski and Rijks [114] described before can be used for calculating the evaporation rate of different solvents.

1.4.3. Parameters to be optimised for PTV-LVI

Large volume sample introduction using PTV is an attractive technique. Three injection modes (at-once injection, multiple, and speed-controlled injection) can be applied depending on the sample volume, volatility and thermostability of the analytes. During the method development for PTV-LVI it is often observed that instead of reaching optimum conditions easly, one goes from one type of error (loss of volatiles) immediately to the other type of error (losses of both volatiles and non-volatiles). Thus the method must be optimised and this optimisation must be done with respect to both the efficiency ofsolvent elimination and component recovery. The preferred injection mode and liner configuration are determined by the volatility of analytes and the sample volume.

There are a wide number of published studies published on the optimisation of PTV-LVI parameters [115,116,117,118,119,120], but in spite of this there are no general guidelines in the optimisation procedure and the practical adjustments that are required. For this reason, each PTV system needs to be optimised individually with respect to the purpose of analysis. Optimisation of the PTV-LVI method involves the adjustment of many parameters while all of these parameters are mutually dependent. To obtain good accuracy of analytical data, careful optimisation of all parameters has to be carried out. Based on the literature research and our own experience in this area the PTV-LVI parameters to be optimised and recommendations for this purpose are summarised in the **Table 1.11** and will be discussed in details in the experimental part (*Chapter 3*).

Table 1.11 Guidelines for parameter optimisation for PTV-LVI.

PTV-LVI parameters	**Recommendations**
Maximum injection volume	▪ Speed-controlled injection.: any volume, usually > 100 µL ▪ Rapid/at-once injection and Multiple injection: < V_{max} that the liner can accommodate (to prevent flooding of the liner the maximum sample volume has to be determined first)
Liner packing bed	▪ Speed-controlled injection: small amount, bed height of 10 to 15 mm ▪ At-once and multiple injection: amount of packing proportional to maximum injection volume
Liner packing material	▪ Should be capable of retaining a large volume of solvent (in case of at-once and multiple injection) ▪ Should be sufficiently inert, the components should not exhibit too strong an adsorption on the packing material ▪ Should have a sufficient thermal stability and should give low blank
GC oven initial temperature	Can be equal or some 10 ºC above the solvent vent temperature, this prevents possible recondensation of solvent vapors in the column
PTV initial temperature	▪ Generally at least 30 ºC below the boiling point of the solvent ▪ or some 250 ºC below the boiling point of the analyte (analytes are retained in the liner during solvent elimination only if their vapor pressure is sufficiently low)
PTV heating program rate	Usually quite rapid (as rapid as possible by the instrumentation), but depends on the nature, properties, thermal stability of the analytes and an their interaction with packing material
PTV final temperature	Some 25 ºC above the elution temperature of the last component ensures quantitative transfer of the solutes
Solvent elimination time (Vent Time)	Split exit should be closed shortly before evaporation of the solvent has been completed
Split flow	Usually 100-250 mL/min, the higher the split flow rate, the faster is evaporation. In turn too high split flow (> 400 mL/min) causes stronger cooling effect of the liner (temperature drop ~40 ºC), cooling effect is less pronounced at lower (< 0 ºC) PTV temperatures
Splitless (transfer) time	30-60 sec after PTV reaches its final temperature
Inlet pressure during transfer	Can be advantageous to increase the inlet pressure during the splitless transfer step in order to reduce the residence time of analytes in the (hot) liner

The choice of the liner and packing material has enormous importance. The packing not only serves to retain the sample liquid, it also helps to prevent transfer of high-boiling matrix constituents to the analytical column. However, packing materials can cause negative effects, such as degradation of analytes, strong and even irreversible retention of analytes due to their strong affinity toward the packing material, carry over, and limitation in the heating temperature. Packing materials have different nature based on their composition (glass wool, Carbofrit, organic polymers, such as Tenax, polydimethylsiloxane (PDMS) and phenylmethylsilicone chemically bonded silica (PMSS)), the choice of which depends on each particular analysis case.

After selection of the liner and packing material and at the beginning of the optimisation procedure it is recommended to perform be a 1 μL splitless injection of the standard mixture. This is the reference chromatogram. Then a large volume injection is carried out. A large volume of a sample diluted in proportion to the increase in injection volume (*e.g.* 125 μL of a 125 times diluted standard) is injected. This is done at the tested/chosen PTV conditions. A comparison of the large volume injection and the reference chromatogram will indicate whether (and to which extent) components are lost.

Miñones Vázquez *et al.* [72], in 2001, using a PTV injection mode were looking for suitable conditions to increase sensitivity in the trace analysis of aliphatic hydrocarbons (C14-C36). Apart from the PTV-LVI mentioned before and found in the literature, affecting the chromatographic signal, others were studied whose influence has not been considered until then. They include length of the microsyringe needle, adsorbent distribution in the liner and injection volume on analyte discrimination.

Since these studies were performed with mixtures of aliphatic hydrocarbons, which also are in the focus of our research, a short summery of it is given below as an example of optimisation.

Two different microsyringe needle lengths were used in this study, 5 and 7 cm. Chromatographic experiments were better when the 7 cm needle was used, because the sample liquid is released in the centre of the glass wool packing. Packing the liner with glass wool appears to be an efficient means of increasing the contact area. The distribution of the packing material in the liner showed to be an important parameter. The highest-molecular-mass compounds were discriminated when the irregular distribution was used. A higher and more equal signal was observed for all the compounds (C14-C36) when the packing was homogeneously placed. The rate of injector heating showed an effect on the response of compounds. When the 6 ºC/min ramp was used, a big discrimination in the signal of the high-molecular-mass analytes was observed. The best and most homogeneous signal was obtained when the 200 ºC/min ramp was use. The best split time (time when the split valve is opened) was when the valve was open for times between 0.1 and 0.3 min. When the split valve remained open for long period of time, there was discrimination of the more volatile analytes.

Using PVI LVI, the authors selected conditions for the trace analyses of aliphatic hydrocarbons in the range C14-C36 and proved the best performance of the analysis. The proposed best programmed-temperature split/splitless technique consists in injecting 50 μL of the sample by discharging a microsyringe with 7 cm needle quickly (1 sec) into a liner of 2 mm I.D. homogeneously packed with

glass wool. During injection the split vent was open for an initial split time below 0.3 min in order to vent the solvent with a split flow-rate of 40 ml/min. Then, the split vent was closed and the injector was heated at a high rate and remains at its final temperature for 11 min. The proposed gas chromatographic sampling technique increases the sensitivity in trace analysis of aliphatic alkanes, and good recoveries and satisfactory RSD values can be obtained when it is used in the analysis.

1.5. Comprehensive two-dimensional gas chromatography (GC×GC)

1.5.1. Introduction

Gas chromatography (GC) is a powerful separation technique that is suitable for determining (semi-) volatile compounds and also less volatile analytes if these are first transformed into volatile derivatives. During the more than 50 years of GC history, many examples have illustrated the potential of GC for accurate identification and quantification of individual analytes in many types of different real-life mixtures. Nowadays, it is the preferred separation approach in a variety of application areas. However, the improved detection capabilities provided by different detectors have also shown that, in many cases, aroma, food, petrochemical and environmental samples are much more complex than was assumed a decade ago [121]. Real-world samples are often characterized by a number of chemical groups and, therefore, by random peak distribution that require high-power separation.

The overall separation and resolving power of a single GC column can be described in terms of its peak capacity, which is the number of peaks that can be separated from one another up to a predefined resolution [122]. In conventional one-dimensional GC (1D-GC), values of peak capacity approaching 1000 are very difficult to attain, if not impossible. The most common approach to improve peak capacity is to use a long column, but, due to the square-root dependence of resolution (peak capacity as well) on effective plates, this option has a limited effect on resolving power. In an 11 h GC run, a 400 m column with more than 1.3 million effective plates, representing the upper limit of efficiency that is available to the chromatographer using commercial instrumentation, was far from sufficient for the complete separation of a sample of only medium complexity (gasoline) [123]. A more effective way to enhance separation power and peak capacity is through multidimensional separation. Conventional multidimensional gas chromatography is a heart-cut technique in which a single fraction (or a few specific fractions at the most) of the primary column effluent are transferred to a secondary column, with different characteristics, for further separation. The theoretical peak capacity of such a system is the sum of peak capacities of the first and the second dimensions, the latter multiplied by the number of heart-cuts [124], **Figure 1.11**. This method proved to be useful only in target analysis, where a limited number of heart-cuts need to be analyzed in the second dimension. If the entire sample requires analysis in two

different dimensions, then comprehensive two-dimensional gas chromatography (GC×GC) must be considered.

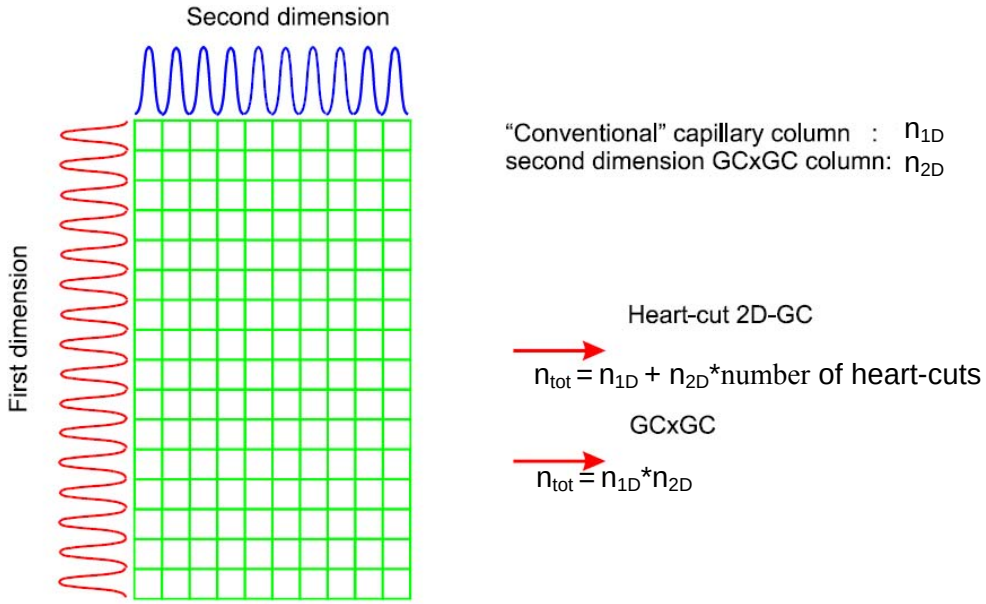


Figure 1.11 The increase in peak capacity of GC×GC [125].

GC×GC has been developed to meet an increasing need for complex sample analysis and to address limitations such as peak capacity, dynamic range, and restricted specificity of 1D-GC, that is, to improve the global efficiency of the separation. GC×GC has been defined as a chromatographic technique in which a sample is subjected to two different separation processes coupled on-line [126]. GC×GC is based upon separations utilizing two chromatographic columns of different selectivity, connected in series through an interface known as the modulator. The main role of the modulator is to trap and/or sample the primary column effluent and periodically inject it into the second column. The more different the separation mechanisms applied, the more orthogonal is the system and the higher is its separation power [126]. Separation systems are considered orthogonal if the two separation mechanisms are independent of each other, so that the distribution of retention factors in one dimension is not correlated with the distribution of retention factors in the other dimension [127]. In GC×GC, the theoretical peak capacity is the product of the peak capacity of the two dimensions, **Figure 1.11**, and it is the number of peaks that can be potentially located in two dimensional space, with the specific resolution value.

The potential ability to provide a substantially larger peak capacity is not the only advantage of GC×GC over 1D-GC [128]. GC×GC systems offer several features that cannot be matched by 1D-GC. Thus, GC×GC offers higher speed in terms of number of peaks resolved per unit of time and better separation power due to increased resolving power. GC×GC can expose the *structural composition* of a sample in a way that 1D-GC cannot. Structured chromatograms are typically generated when compounds belonging to homologous series are present in the sample. GC×GC also has a unique *class selectivity* feature that makes it possible to remove a majority of peaks of no interest from the second dimension

(2D) separation. And finally thanks to the focusing step during modulation very narrow solute bands are generated, so the sensitivity of GC×GC is increased [129].

GC×GC separation is increasingly important for the separation of complex samples. It provides the basis for a better understanding of them. The unsuspected complex nature of many real world samples has been elucidated through the enhanced resolving power of these techniques.

In this chapter the principles and most commonly used instrumentation of GC×GC together with optimisation and quantitative aspect of this technique will be shortly described.

1.5.2. Principles of GC×GC separations

The first step in understanding comprehensive multidimensional separations is to grasp the concept of a separation "dimension", and what makes a separation "comprehensive". A separation dimension can be defined as a technique that relies on a single "bulk parameter" to separate compounds. In GC the boiling point and partition coefficient (which depends on the nature of the stationary phase, the nature of analytes and instrumental parameters) are bulk parameters. If multiple compounds have the same value for this parameter, they will not be separated using this particular separation dimension. However if a different stationary phase is chosen for the separation, then the parameter that governs the separation changes, and the separation becomes possible in this second (OR: further) dimension. When dealing with mixtures of compounds, it is often impossible to separate all of them using any single separation dimensions. This has driven the development of techniques that rely on multiple dimensions to perform separation of such multidimensional samples [130].

Accordingly, the hypothetical sample in **Figure 1.12** [126], containing compounds differing in shape, colour and size, has a dimensionality of three. If such a sample contains a reasonable amount of compounds, then there is no chance of separating all its constituents in a 1D-system. Either the separation is performed according to size, but then the mixture cannot be separated according to colour nor shape, or the separation is performed according to colour, but then size and shape will not contribute to the separation. Or the separation is performed according to shape, and size and colour will interfere. The only possibility of separating all of the constituents is in using an orthogonal 2D-separation system (in the case of GC × GC, this implies that the two columns involved should retain compounds based on different retention mechanisms). And if there is a match between the dimensionality of the sample and of the separation system, we can use the total separation space that is available very efficiently to accommodate separated analytes and structures will appear in the colour plot as is depicted in **Figure 1.12**.

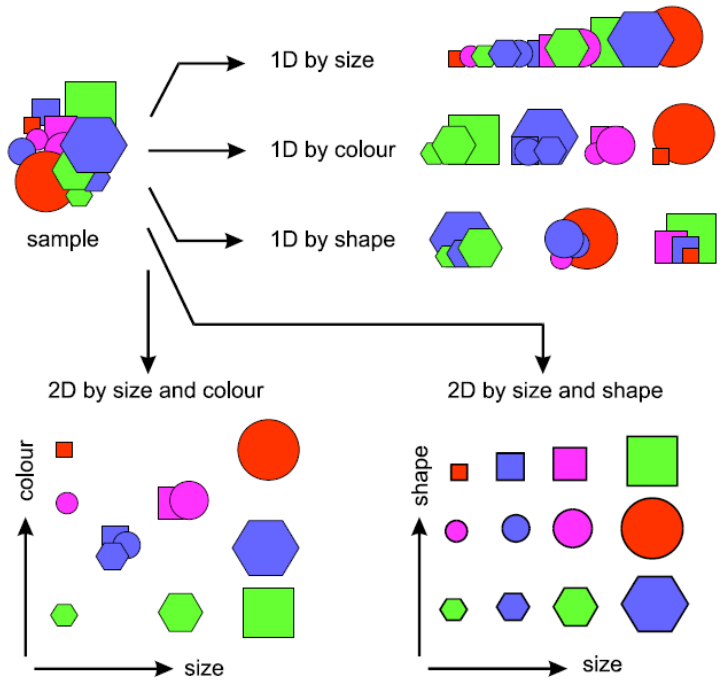


Figure 1.12 Match between separator and sample dimensionality in GC×GC [126].

For analytical samples, in principle, all kinds of stationary phases can be used in the first dimension of a GC×GC system, but generally non-polar phases are used (based on analyte volatility only). Because of the fast (and, thus, isothermal second-dimension separation, for analytes of equal volatility, there will be no boiling-point contribution in the dimension, so only the specific interaction with the stationary phase will govern the retention, and the separation will indeed be orthogonal [131]. For the second dimension, a variety of phases can be selected as well, depending on the desire analyte-stationary phase interactions. Graphically this can be expressed within a Cartesian plane, with perpendicular axes, one axis being volatility and the other axis polarity. If the retention times of a solute on the two columns are independent, it can be represented as in **Figure 1.13**. But the most important consequence of the orthogonality is that the eluting components will be arranged according to their chemical structures on the separation plane. In other words, the contour plot (which is a projection of the intensity to the plane of the graph) will exhibit a great deal of order in bands and clusters of members of the same chemical family, so the separation will be structured [125]. The reverse type column combinations are also popular, that is, those having a medium-/polar 1D column and a nonpolar 2D column. This is especially helpful when highly polar or ionogenic analytes are mostly present in the analysed mixture.

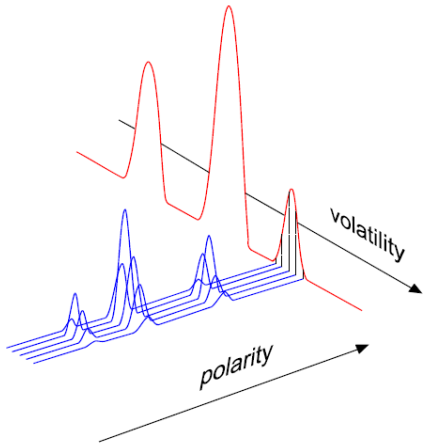


Figure 1.13 The orthogonality of GC×GC [125].

As mentioned above, there are two types of multidimensional gas chromatography: heart-cut GC and comprehensive multidimensional GC. In heart-cut GC, two separation columns are used, but only a small portion of the material exiting the first column is introduced to the second column. In other words, only a small region of the first dimension is subjected to both separation dimensions (see **Figure 1.14A**). Techniques have been developed that allow for multiple heartcuts to be taken from a single chromatogram, but allowing less time for the cut fraction to be separated (**Figure 1.14B**). As can be seen in **Figure 1.14C** in the limiting case when the number of heartcuts gets high enough and the time for the heartcut separation gets short enough, one accomplishes a comprehensive multidimensional GC separation. Material exiting the first dimension is sampled periodically in such a way that the separation in the first dimension is preserved and all of the compounds in the sample are subjected to both separation dimensions. GC×GC is in effect a separation where very many sequential heart-cuts are taken [130].

To define when a two-dimensional separation can be called comprehensive three conditions were established. The first and second conditions distinguish between heart-cut two-dimensional separation and comprehensive two-dimensional separation. Therefore, a two-dimensional separation can be called comprehensive if [132]:

1. Every part of the sample is subjected to two different separations;
2. Equal percentage (either 100% or lower) of all sample components pass through both columns and eventually reach the detector;
3. The separation (resolution) obtained in the first dimension is essentially maintained.

The most important aspect of preserving resolution in the first dimension is the number of second-dimension chromatograms (number of the heart-cuts) recorded across the width of a first-dimension peak, with three or four being a minimum number.

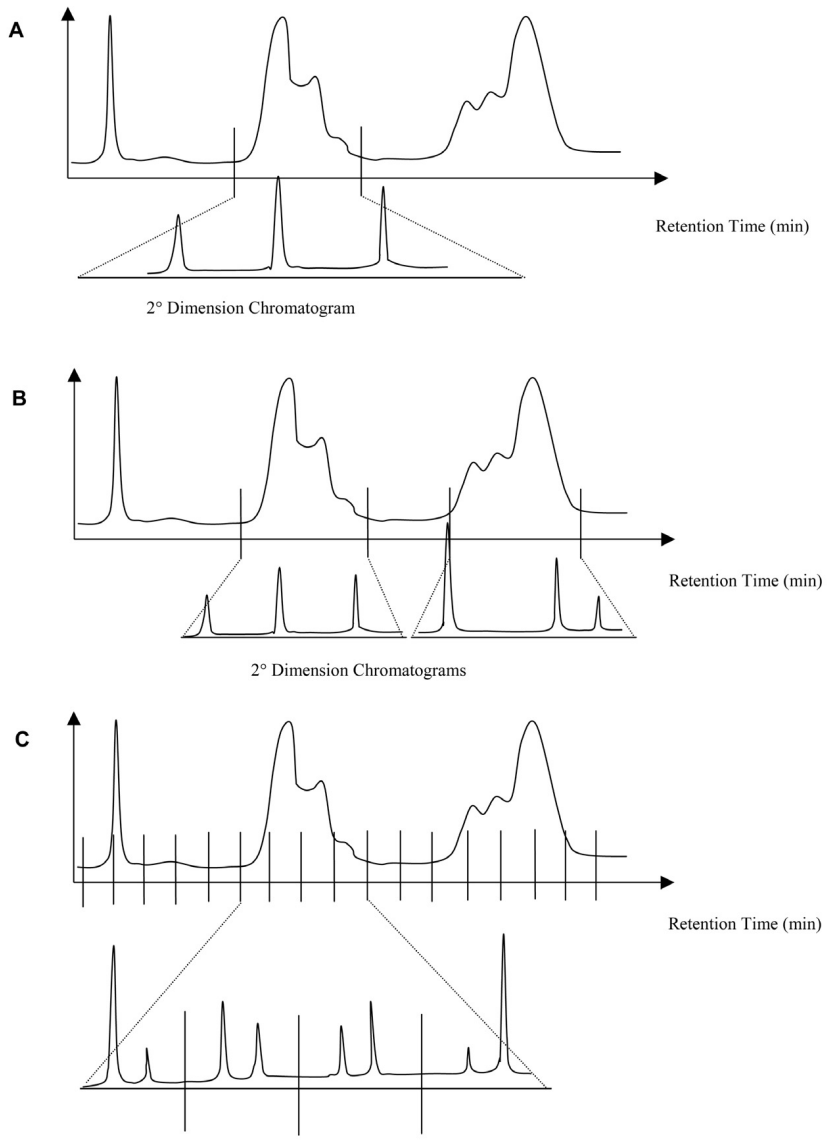


Figure 1.14 The concept of multidimensional GC. (A) single heart-cut GC analysis, where a large portion of the effluent from the primary column with coelutions is diverted to the second dimension column and separated over an extended period of time. (B) dual heart-cut GC analysis, where two regions with coelutions are diverted to the second dimension column, but with less time to perform each separation. (C) comprehensive two-dimensional GC analysis occurs when the size of the sequential heart-cuts is very short, as are the second dimension chromatograms [130].

1.5.3. Instrumentation for GC×GC

The hardware setup of a GC×GC is quite simple and can be built on any commercially available GC instrument. The typical setup for GC×GC analysis is shown in **Figure 1.15**. In GC×GC the separation process is carried out on two columns of different selectivity connected in series with a so-called modulator located in between as an interface. The two columns used in GC×GC systems can be placed in one oven, or a second optional oven can be used for the second column. The use of two different ovens for each column (dimension) provides more flexibility in method development and allows

better control over secondary column separation. The modulator collects small fractions of the solute stream eluting from the first column and introduces them to the second column, so the entire sample is subjected to reanalysis and no fraction go unexplored. Since the separation mechanism differs in the two columns, compounds coeluting in the first column, usually of conventional dimensions (e.g., 25-30 m × 0.25 mm ID × 0.25 µm), are likely to be separated in the second column. The second-dimension separation of each fraction has to be completed before the next fraction is subjected to reanalysis, so the second column has to be short, commonly a short micro-bore capillary segment (e.g., 1-2 m x 0.10 mm ID x 0.25 µm). Such a setup makes it possible to have a 2 to 8 sec analysis time for each of the three to four heart-cuts (or modulations) across a first-dimension peak with a typical baseline width of 5 to 30 sec. I order to have three to four modulations per peak the peak must be wide, so the temperature rate of GC×GC should be slower than that of 1D-GC (some 1 to 3 °C/min) [121,129]. The primary columns are typically coated with nonpolar stationary phases (although other options are increasingly being explored); some common choices include 100% poly(dimethylsiloxane) or 95 : 5 methyl/phenyl polysiloxane. The stationary phase chosen for the second-dimension column should offer a different separation mechanism than that in the primary column; hence, it should be distinctly different from the latter. The secondary columns are usually coated with polar stationary phases. Typically, the stationary phases are 50:50 phenyl/methyl polysiloxane or "wax" [poly(ethylene glycol)]-based; sometimes, though, good results can be achieved with liquid crystal, fluorinated, chiral phases and ionic liquid columns [133,134,135].

Thus, the only piece of hardware required to turn a 1D-GC system into a GC×GC system is a modulator mounted inside (or outside) gas chromatographs. However, the detectors used for GC×GC must be characterized by short rise time and small internal volumes to be compatible with the fast separation occurring in the second column.

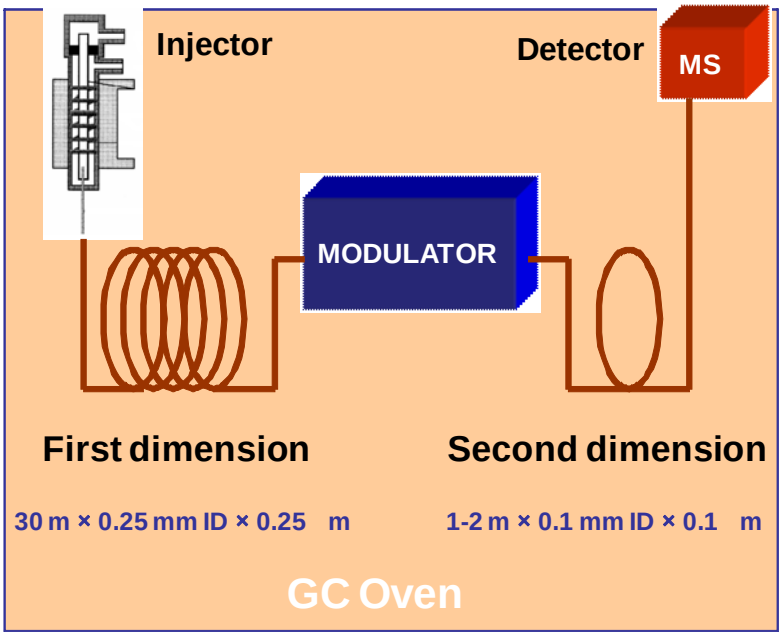


Figure 1.15 Schematic of the basic setup for GC×GC.

1.5.3.1. Modulator

The modulator is arguably the most important component, heart of any GC×GC system. Direct serial connection of two different columns without a modulator results in a one-dimensional (1D) separation because analytes separated on the first column are not prevented from coelution at the exit of the second column. Their elution order might even be reversed. The modulator allows the flow of analytes from the first column to the second column to be controlled, which changes the nature of the separation fundamentally. Following the same separation in the first column the modulator traps and focuses the first band, and then injects it into the second column while collecting the following band. This following band is injected into the second column and is then separated into two bands on the second column, while the next band is collected by the modulator. This sequence of events assures that separation achieved in the first-dimension column is preserved and that additional separation in the second column is possible. To sum up, the role of the modulator is to trap, refocus, and inject the primary column heart cuts sequentially into the second dimension. The time taken to complete a single cycle of events is called the modulation period.

Two main classes of modulators are used: thermal modulators and valve-based or flow modulators [136]. Both these types use, although in a different way a compression in space of the accumulated fraction. This has to be done in order to make the fraction suitable in acting as a narrow injection pulse for the fast second dimension column. Bandwidths of the injected pulses are, dependent on the type of modulator, in the order of <10 to 200 msec. A major result of this space compression and the use of a fast second column is the fact that peaks elute from this column as very narrow peaks, which have, in view of the conservation of mass, enhanced signal intensities. A graphical example of the enhanced peak amplitudes is given in **Figure 1.16** [137].

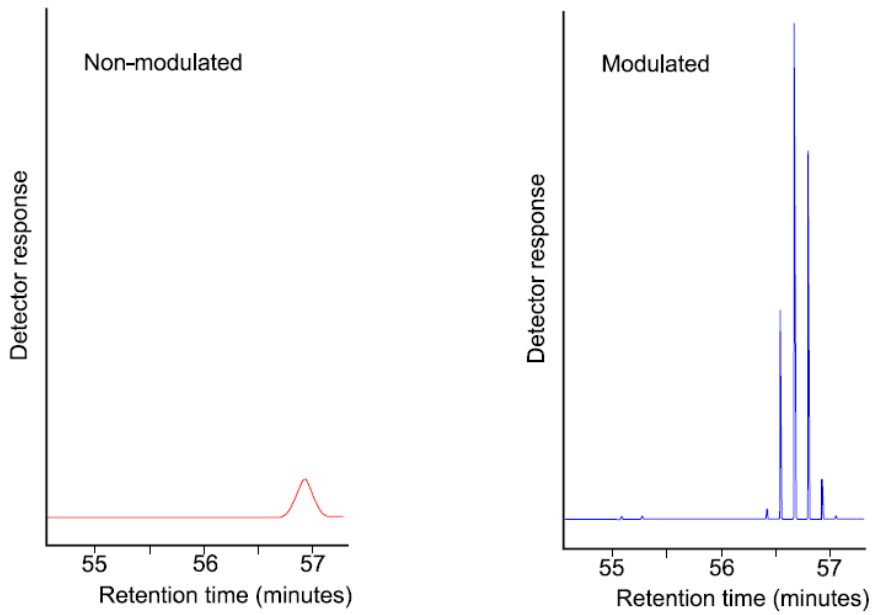


Figure 1.16 Non-modulated peak in 1D-GC and modulated peak in GC×GC [137].

Thermal modulators currently are the most frequently used and in turn can be broken down into two categories: heating modulators (those whose principle involves a temperature increase) and cryogenic modulators.

In heating modulators a short section of a column is rapidly and reproducibly heated, so the substances retained on the stationary phase of this modulation section are pulsed into the second column. The rotating heating modulator, known as the sweeper, introduced by Phillips in 1999 [138], **Figure 1.17**, was the first commercially available modulator. Desorption of the trapped material and its reinjection into the secondary column were accomplished by moving a slotted heater along the trapping capillary, heating it locally to a temperature higher than the oven temperature by about 100°C. This modulator worked satisfactorily in many different applications. Several other heating modulators were introduces as well, however, they have significant disadvantages: it was practically impossible to collect volatile compounds with the heating trap; limited choice of stationary phases (since the final oven temperature had to be 100°C lower then upper working temperature of the stationary phase); and difficult optimisation. These modulators did not find large use.

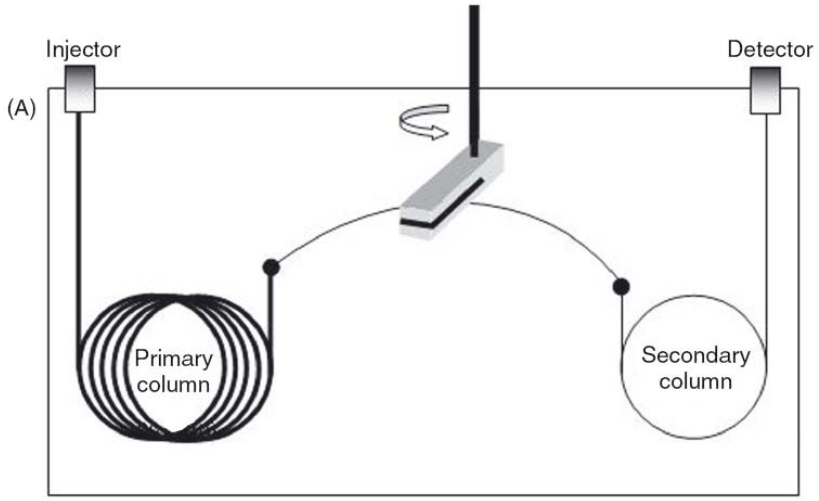


Figure 1.17 Diagram of the rotating sweeper heating modulator [130].

Cryogenic modulators, other type of thermal modulators, use cooling rather than heating to create the required pulsing effect. The first cryogenic modulator, the longitudinally modulated cryogenic system (LMCS), was developed by Kinghorn and Marriott in 1999 [139]. It is based on the use of cryogenic trap that can be moved longitudinally along the column to focus analytes in the beginning of the second column. A schematic diagram of the LMCS is presented in **Figure 1.18**.

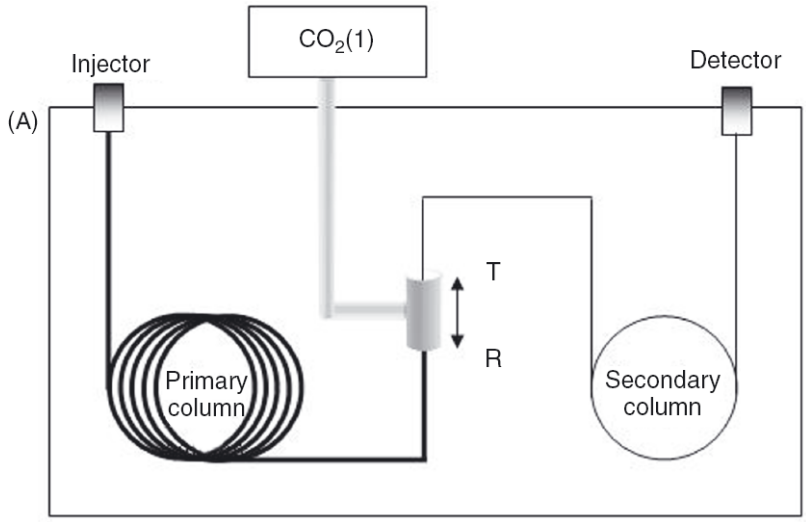


Figure 1.18 Schematic of the LMCS modulator. R: focusing position, T: releasing position [130].

As the effluent exits the primary column, analytes are cold-trapped in a small region of the column marked "R". The cold trap is then moved to a region of the column downstream of the trapping position ("T"). This results in the cold spot being rapidly heated back to the oven temperature, which causes the analytes trapped in the stationary phase to continue their separation. As the desorbed band moves along the column carried by the carrier gas, it is re-trapped in the downstream segment where the cryotrap is positioned ("T"). In this way, analyte breakthrough was prevented. The cryotrap is then moved back to its initial position ("R"), releasing the analyte band into the second column, while a new portion of the primary column effluent is trapped in the upstream position.

This modulator was the first reliable cryogenic modulator for routine use. However, there were some limitations to this approach. The use of liquid CO_2 as the cryogenic agent allows the trap to be cooled to only about −50°C. This temperature is not sufficiently low to trap highly volatile analytes (up to approximately *n*-C6). And the use of a moving shuttle at the fragile GC column could potentially yield to operational problems over time.

Later several different non-moving cryogenic modulators were introduced, such as dual-stage quad-jet (two cold CO_2 and two hot air jets), a dual-stage dual-jet (two cold CO_2 jets for trapping the analytes, which were then remobilized by the heat from the oven air) and a single cryojet capable of dual-stage modulation [136].

The most successful modulator version, a dual-jet dual-stage modulation with the use of a delay loop, was developed and introduced by Ledford and co-workers in 2002 [140]. This "loop-modulator" uses cold gas cooled with liquid nitrogen and hot gas heated by a heater, **Figure 1.19**. The loop-modulator is a length of column looped through the paths of intersecting hot and cold gas jets. Every few seconds, the hot jet fires, thereby heating the cold spots and releasing trapped analytes, **Figure 1.19 and 1.20**. Two cold spots are used because two cryogenic focusing steps produce very sharp injections onto the second column and it prevents analyte breakthrough in the moment, when the hot jet is open.

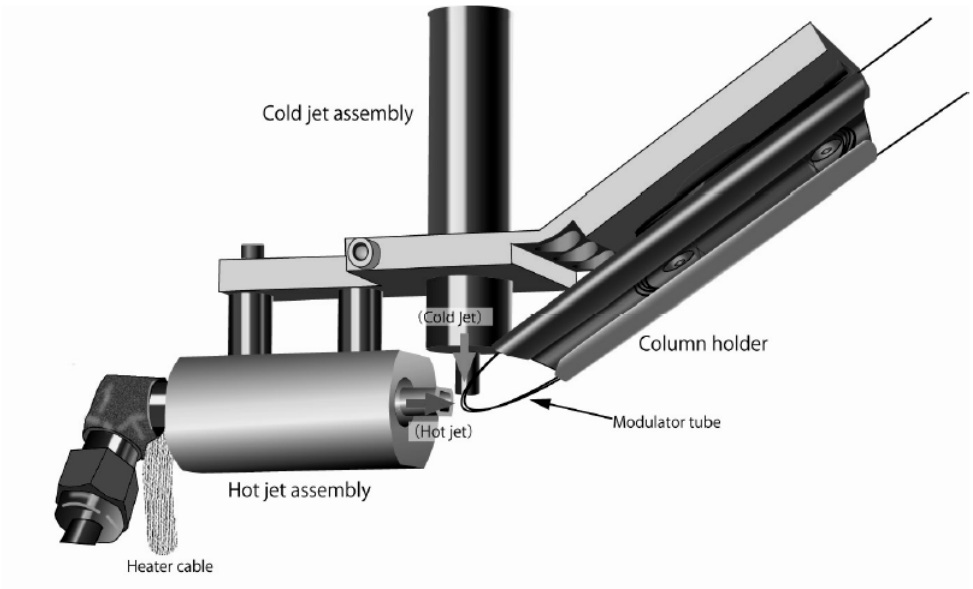


Figure 1.19 Dual-jet (hot and cold) dual-stage loop-type (ZOEX) modulator [141].

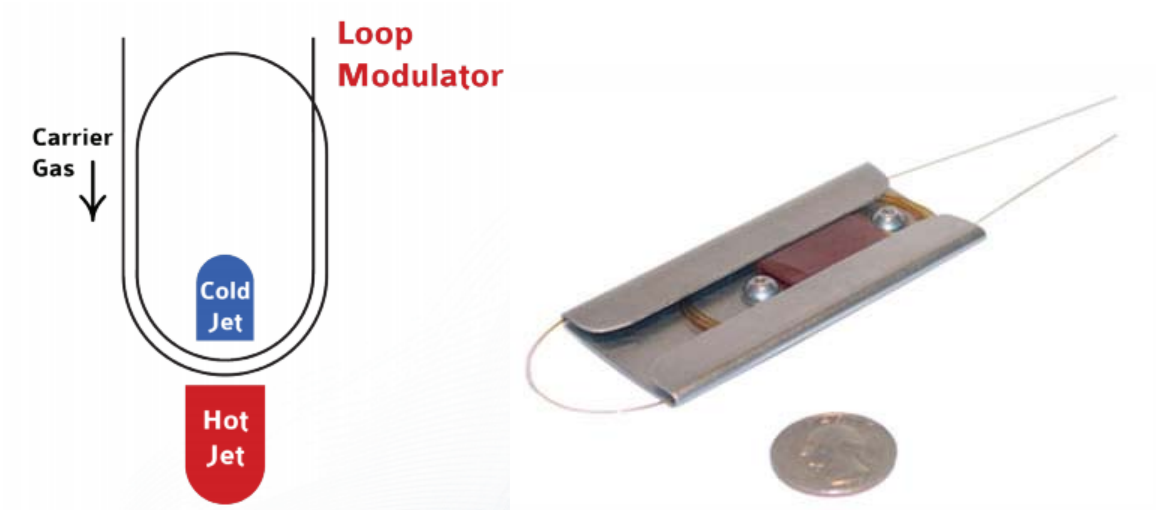


Figure 1.20 Right: Design of the loop modulator; Left: Column (loop) holder [142].

The **Figure 1.21** describes the dual-stage modulation processes in the loop modulator. Substances eluting from the first column are trapped by the cryogenic jet (sampling) and then released into the delay loop by a brief firing of the hot jet (release). By the time substances travel through the delay loop, the cold jet is active again, and they are trapped a second time by the same cryogenic jet (sampling) but at the end of the loop. The next firing of the hot jet released a sharp chemical pulse in the second dimension while admitting the next pulse in the delay loop.

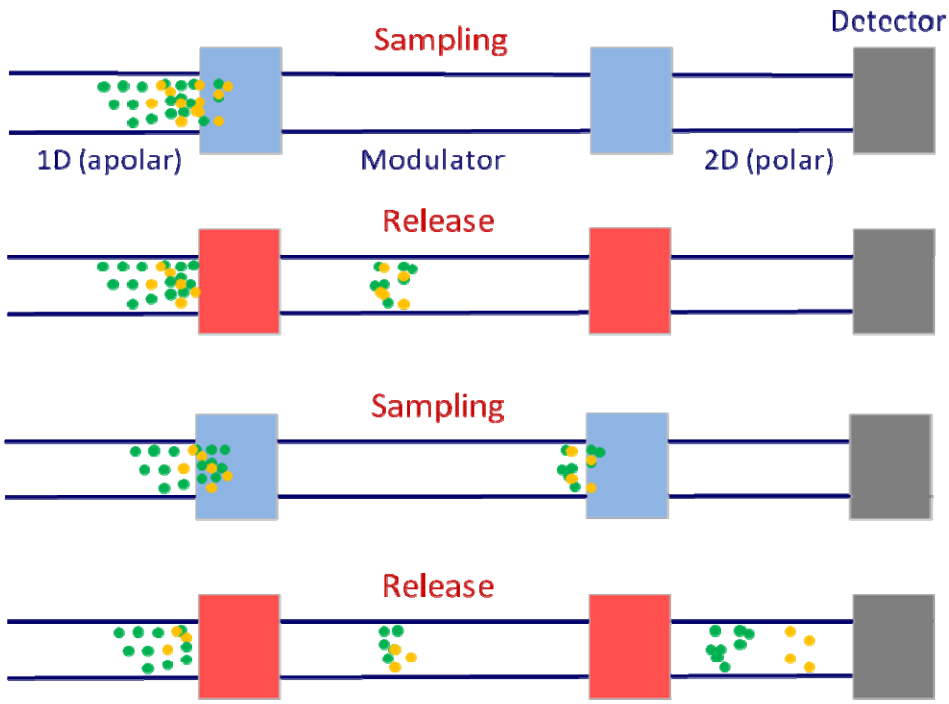


Figure 1.21 Dual-stage modulation processes in the loop modulator with one cold jet and one hot jet (from personal communication with L. Mondello, P. Tranchida).

This modulator, available commercially from ZOEX Corporation, represents one of the simplest dual-stage cryogenic designs. It achieves good modulation of compounds with a wide range of boiling points through the thermal absorption and desorption of samples, but it requires fine adjustment of the length of the loop and the velocity of the carrier gas.

Valve-based comprehensive GC×GC was also actively developed in the past decade. Valve-based modulation is a simple way to generate GC×GC separations without using cryogenic fluids, thus reducing significantly operating costs. A further practical advantage of valve-based modulators is their low construction costs. More than ten new valve-based modulators were introduced up to now. Diaphragm valves fitted with sample loops are the most common low duty cycle modulators, whereas fluidic modulators that employ differential flow conditions are the most common high duty cycle modulators [143]. In this type of modulators, effluent from the first column is sampled in the loop by the action of the usually six port valve during a very brief period before being sent to the second column. As an example, one such valve-based device, proposed by Tranchida *et al.* in 2010, offers a flexible loop-type flow modulator [144], with the possibility to optimise flows, using a waste branch bridging the interface and a needle valve.

Figure 1.22 shows this the seven-port flow modulator device. The interface comprises a metallic disc (2.5 cm diameter, 7 mm thickness), and internal rectangular channels (250 µm width/75 µm depth), connecting ports 1-2-3 and 4-5-6/7. A two-way electrovalve is located outside the GC oven and is connected to an advanced pressure control (APC) unit. Two metallic branches connect the valve to the interface in positions 2 and 5. The primary and secondary columns are linked to positions 1 and 6, respectively. A 40 µL stainless-steel loop (20 cm × 0.71 mm OD × 0.51 mm ID) bridges positions 3 and

4; the size of the loop is chosen considering the modulation period, first-column flow and second-column dimensions. It is noteworthy that the flow exiting the loop is divided between the channels linked to ports 6 and 7. The flow rate in the 2D column is – depending on the actual interface – very high, and can sometimes even reach 40 ml/min. This is not compatible with MS detection, or, requires a corresponding post-column split which would reduce sensitivity according to the split ratio. Flow splitting depends on needle-valve regulation and remains unaltered during the analysis because the valve-restriction parts are located within the GC oven [136].

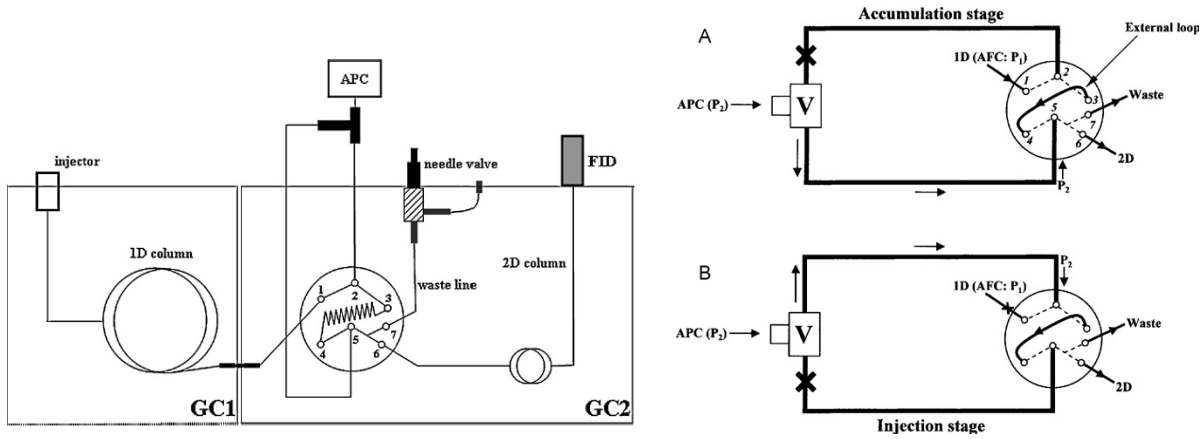


Figure 1.22 Left: Scheme of the flow modulator GC×GC set-up; Right: seven-port flow modulator device in the accumulation (A) [144] and injection (B) modes. Abbreviations: V: two-way solenoid valve; APC: Advanced pressure controller [136].

1.5.3.2. Detector

The detector is another important GC×GC system component. The very fast separation in the second dimension results in peak with widths of, typically, 100-600 msec. Peak width depends on the type of modulator used, on gas flow and the dimensions of the second column. The narrow peaks require a fast detector, providing a small internal volume, a short detector rise time and a high data acquisition rate to ensure a proper reconstruction of the second dimensional chromatogram. It is generally accepted that at least 10 points per peak should be available to correctly define a peak [145].

For narrow peaks a very fast acquisition rate of 50-100 Hz is required. The most commonly employed detector in GC×GC has thus been the flame ionization detector (FID) which is capable of very high acquisition rates of 50-250 Hz. The FID is a universal detector with response proportional to the compound's carbon content and is suitable for most quantitative applications. All early applications were in the field of petrochemical analyses and the FID was the detector of choice. Other detectors, such as micro-electron capture detector (μECD – with a data acquisition range of 50 Hz), flame-photometric detector (FPD with higher detector gas flow), atomic emission (AED) – 10 Hz, nitrogen- and sulfur-chemiluminescence detectors (NCD and SCD – 100 Hz,) are compatible with GC×GC and have been reported for GC×GC analyses as well [146].

Detection by MS is often preferred for compound identification, due its high information content. The coupling of MS to a GC×GC system has been highly desirable since the birth of comprehensive two-dimensional gas chromatography. At that time used quadrupole MS (qMS) instruments offered only a very low acquisition speed (ca. 2.4 scan/sec). Under such conditions, less than 3 data points per peak could be obtained, which was not enough to correctly define a narrow chromatographic peak. A time-of-flight (ToF)-MS was the ideal solution to satisfy the requirements of GC×GC narrow peaks. Normally this type of MS provides high mass resolution (5-10 ppm), but have only a moderate scan speed (ca. 10 Hz). TOF-MS is either used in the high resolution mode at which, due to the coaddition of many individual scans the cycle time is still relatively high, or you can operate it in low resolution mode where a smaller number of scans is coadded, resulting in data acquisition frequencies of up to 200 Hz with modern instruments. So ToF-MS is vogue today for hyphenation with the GC×GC technique, however it is an expensive one [147].

With the new generation of rapid scanning qMS instruments (such as the Shimadzu QP2010 Ultra qMS which is the fastest scanning quadrupole instrument currently on the market) the before mentioned limitations of older qMS instruments could be overcome. It thus became possible to obtain an average of more than 15 data points per peak, through the application of a 50 Hz acquisition rate and a 40-341 m/z mass range. The availability of such a very rapid qMS represents an important step towards the expansion of quantification methods in GC×GC/MS [148,149]. The advantage is that it is a much less expensive instrument, compared with the well-established TOF-MS instrumentation.

1.5.3.3. Visualization

In a typical experiment of comprehensive 2D chromatography, the massive amounts of data acquired in both dimensions create large data files, which need to be processed in order to become useful. One of the biggest challenges for comprehensive multidimensional separations is handling these complex data. The entire GC×GC process requires data elaboration and visualization though transformation of the raw chromatographic data into its two- or three-dimensional representation, **Figure 1.23**. The raw signal is the continuous record of the large series of short second-dimension chromatograms with high data acquisition rate. From the early stages of development of comprehensive 2D-GC, data processing tools have been established (*see more in Section 3.5*).

Figure 1.23A shows a simulated output from a (single channel) detector. Dedicated software packages stack second-dimension chromatograms side by side and, considering the modulation time, derive the first and second dimension retention times for each peak. Since the modulation time is known, then the application of the slicing methodology is straightforward, and the resulting **Figure 1.23B** shows the same data set but now under a form of a 2D chromatogram. After data processing, it may be necessary to smooth the chromatogram by interpolation of data points between 1D chromatograms mainly along the

first dimension in order to visualize and obtain a 2D chromatogram, as shown in **Figure 1.23C** and **D**. [150].

For representation of a 2D data matrix either the contour plot, the contour plot or the 3D plot are the types of graphs that easily allow visualizing and understanding the distribution of peaks in a chromatogram. As shown in **Figure 1.23C**, such a graph does not provide quantitative information but it is an excellent visualization tool which becomes the first essential step for further processing of the data obtained. The use of a colour coded scale for peak intensity allows an immediate qualitative assessment of the different peaks and respective heights. **Figure 1.23D** uses the same data as in **Figure 1.23C** for simulating a typical 2D chromatogram, and the 3D plot provides already quantitative information, thus becoming the best approach to observe, analyse, and comment the visual features of 3D peaks shape and form. When dealing with higher order data dimensions, it is not possible to produce conventional contour and 3D plots, since a 2D chromatographic system coupled to a multichannel detector will produce a three dimensional (3D) data array with every single data point not only representing intensity, but an entire spectrum [150].

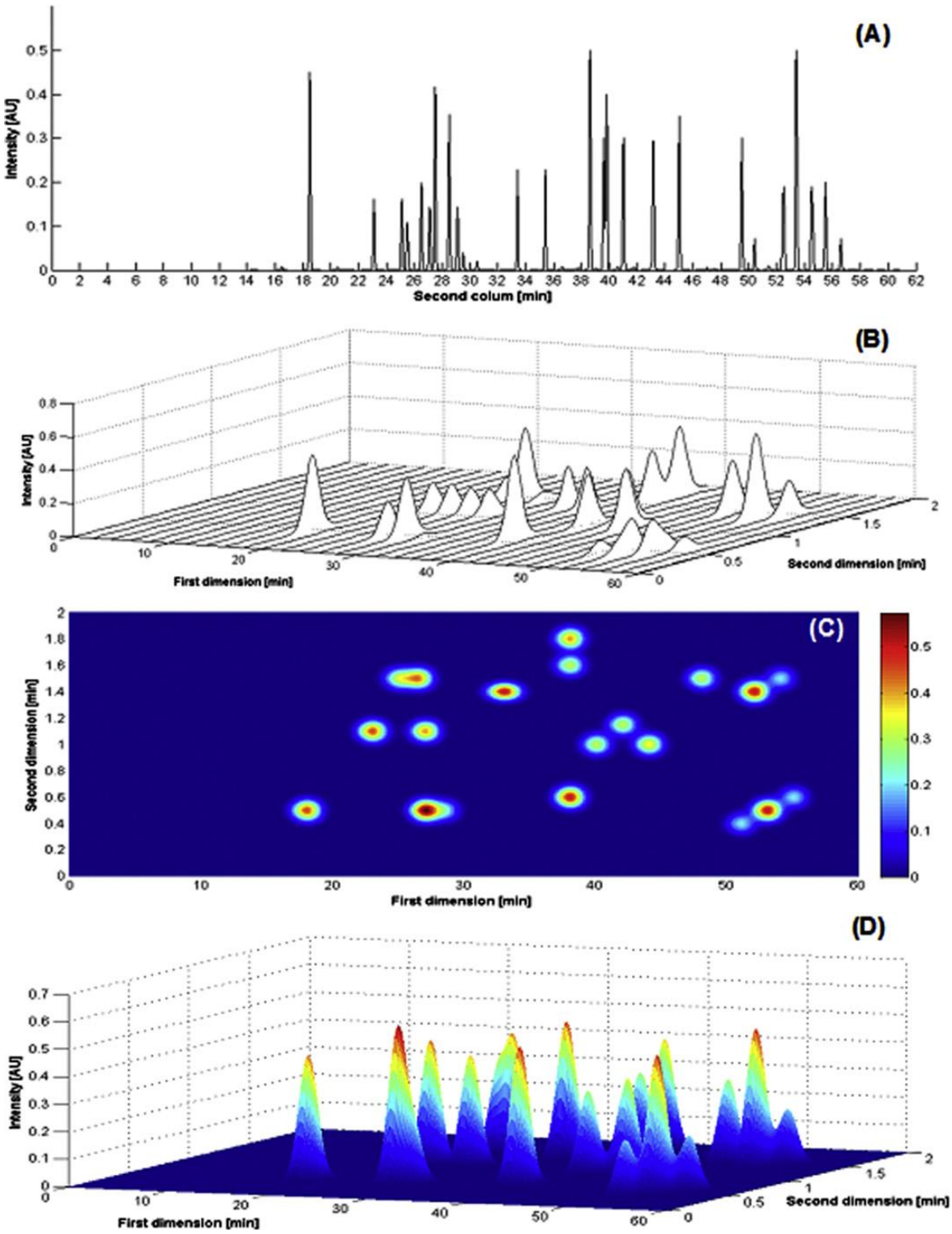


Figure 1.23 Representation of a simulated data set for comprehensive 2D liquid chromatography: (A) raw data from the detector positioned at the end of the second column; (B) layout of the sliced 1D chromatograms defined by the modulation time; (C) contour plot of a 2D chromatogram after smoothing; and, (D) 3D plot of a 2D chromatogram after smoothing [150].

1.5.4. GC×GC optimisation and troubleshooting

GC×GC method optimisation is far more complicated that in 1D-GC. Solid knowledge of the basic chromatographic theory and general practical experience in the GC field are of great help for effective GC×GC optimisation. Method optimisation in GC×GC is mainly devoted to maximizing separation power and sensitivity. The main operational conditions that must be optimised are the modulation parameters, stationary phase chemistry, column dimensions, carrier gas flow, temperature programs and detector settings [151].

Before optimising the conditions of the instrumentation it should be emphasized that modifications of some of the settings of GC×GC will also influence others. It is quite obvious that GC×GC method optimisation is not straightforward. **Figure 1.24** illustrates the complex interplay between different parameters in GC×GC separation [152]. Individual parameters are indicated by ovals, with instrumental parameters that are directly controllable by the chromatographer shown in blue. Green arrows point to parameters whose values increase as the input parameter value increases; red arrows indicate opposite influences (the value of the parameter decreases as the value of the input parameter increases), red/green dashed arrows indicate uncertain influences. The relationships shown in the diagram are valid when only one instrumental parameter is changed at a time, with the rest held constant. For example, increasing the oven temperature programming rate causes the elution temperature of an analyte to increase (as illustrated by the green arrow), while the first dimension peak width and the analysis time decrease (as illustrated by the red arrows). Increasing the inlet pressure has the opposite effect on the elution temperature (as illustrated by the red arrow), while the analysis time decreases as well (red arrow). Other relationships can be studied in a similar way.

Based on my personal experience and on literature studies the most important recommendations and guidance for optimisation of GC×GC parameters are summerized and presented in the **Table 1.12**. It is also discussed below how to cope with problems occurring during the optimisation procedure ("troubleshooting").

The modulator is the most important part of the GC×GC system. The major role of it is to trap, refocus, and re-inject the effluents from the 1D column. Modulation parameters must be tuned in relation to each specific application and type of modulator. Heat-based modulators (e.g. the rotating sweeper heating modulator) are not considered here since they are rarely used in present time. Method optimisation using the flow modulator is even more challenging then for thermal modulators. The flows must be tuned carefully for 1D and 2D.

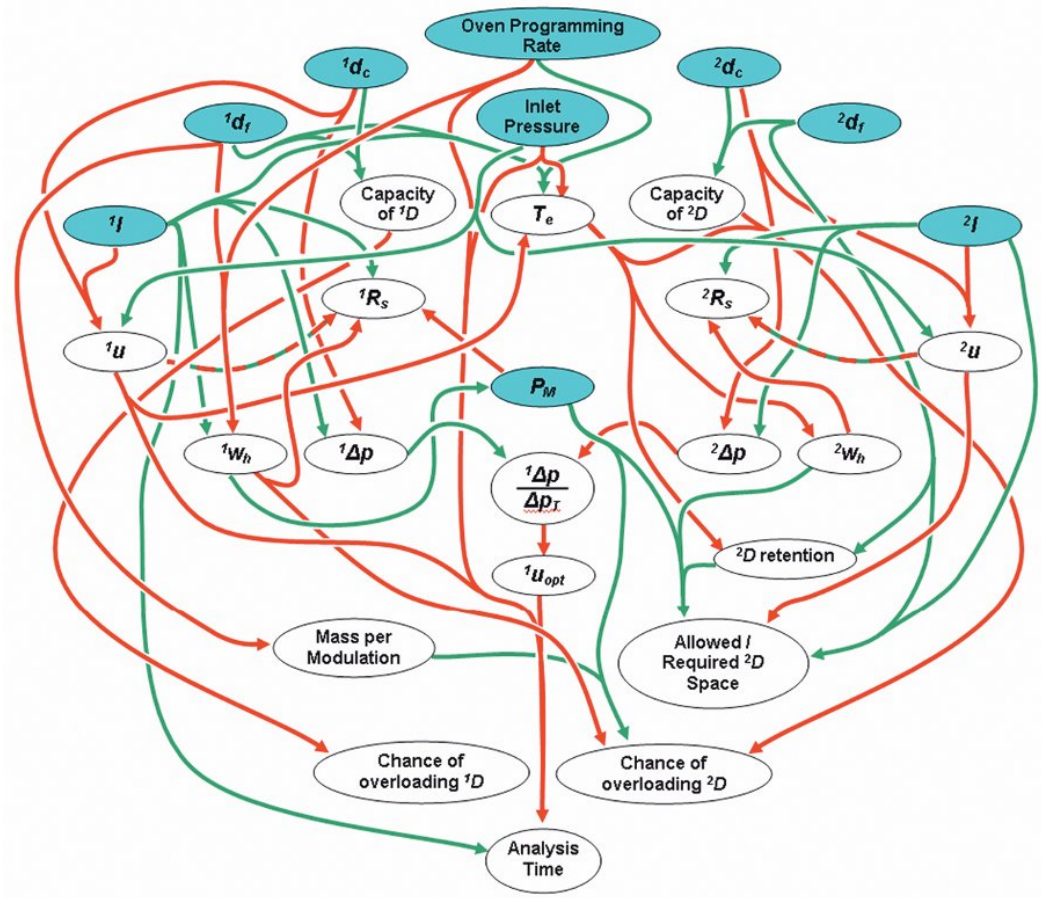


Figure 1.24 Interplay of parameters in GC×GC separations. Green arrows point to parameters whose values increase as the input parameter value increases; red arrows indicate opposite influences. Symbols: ^1d_c: ^{1}D column diameter, ^2d_c: ^{2}D column diameter, ^1d_f: ^{1}D column film thickness, ^2d_f: ^{2}D column film thickness, ^{1}l: ^{1}D column length, ^{2}l: ^{2}D column length, Te: elution temperature, ^{1}u: ^{1}D column linear velocity, ^{2}u: ^{2}D column linear velocity, ^1w_h: ^{1}D peak width, ^2w_h: ^{2}D peak width, $^1\Delta$p: pressure drop in the ^{1}D, $^2\Delta$p: pressure drop in the ^{2}D, $^1\Delta p/\Delta pT$: pressure drop in the ^{1}D compared to the total pressure drop, ^1R_S: ^{1}D resolution, ^2R_S: ^{2}D resolution, $^1u_{opt}$: ^{1}D column optimal linear velocity [151].

Table 1.12 Recommendations/guidance for optimisation of GC×GC parameters.

Parameters to be optimised	***Recommendations and guidance***
1. Modulation parameters	
Modulation period/frequency	- Must be sufficiently short (2-8 sec, depending on application), at least 2.5-3 modulations per peak eluting from 1D column; at the same time also not too short in order to avoid wrap-around. - *Flow modulators* are characterized by the same requirements, but optimisation is more complicated. Flow in the 1D column and volume of collection loop define the modulation time. The flow from collection loop must be very fast: 10-20 mL/min in 100-200 msec.
Modulation temperature (for cryogenic modulators)	- Must be low enough in order to trap/focus the effluents from 1D column. Desorption temperature must be high enough to generate sharp and symmetric injection bands in the 2D column. - *LMCS*: cooled by liquid CO_2. The trapping temperature is below that of the GC oven, usually ~100°C lower than the GC elution temperature; reinjection of the trapped effluent accomplished at oven temperature.

Table 1.12 Recommendations/guidance for optimisation of GC×GC parameters (continued)

Parameters to be optimised	***Recommendations and guidance***
Modulation temperature (for cryogenic modulators)(continued)	- *Dual-stage loop-type modulator*: N_2 gas cooled by liquid N_2 is used for the cold jet and allowing compounds from C4 to C40 to be properly modulated, but the N_2 gas flow is important (~10-15 L/min). If it is too high, then trapping temperature is too low, overcooling of the effluent from the 1D column occurs (specially for low volatile compounds), so the desorption and reinjection of the trapped effluent is not efficient. If the flow is too low, then trapping temperature is too high and breakthrough happens. The temperature of the hot jet (~40°C above the elution temperature) and hot jet duration (300-600 msec) must be high enough for efficient desorption and reinjection of trapped analytes.
2. Column combination	
Stationary phase chemistry	- Must offer the maximum use of the two-dimensional separation space, must offer effective separation in both dimensions and provide a structured/ordered separation - Separation must be orthogonal. Typically non-polar column in 1D (provides separation according to vapor pressure of analytes) and more polar in 2D (separation according to their polarity). Reverse order is also possible, when 1D column is more polar and 2D column less polar. - Ionic liquids (ILs) stationary phase can be tailored to have different degree of polarity, so can offer unique selectivity toward non-polar solutes.
Column dimensions	- 1D column is generally long, typically 15-30 m × 0.25 mm i.d., film thickness of 0.2-1 µm - Separation in 2D column must be fast (must be shorter then modulation period), typically columns of 0.5-2m length × 0.1-0.25 mm i.d. with film thickness of 0.1-0.25 µm are used - In order not to overload 2D column with focused 1D band, the concentration of the injected analyte must be controlled.
Carrier gas linear velocity	- Must be a compromise in terms of linear velocities in two dimensions: In 1D usually near the optimum ~20-35 cm/sec In 2D far above the optimum ~ 160-330 cm/sec - An approach to reduce the flow in the 2D is the use of a flow splitter prior to or after the modulator.
3. Temperature programming	
	- To produce relatively broad peaks in the 1D of 15-30 sec width, in order to have enough modulations per peak, generally, the temperature programming rate is slow, e.g. 1-5 °C/min. - If one GC oven used for both column, too slow heating rate can course wrap-around. - If two GC ovens are used, the same temperature programming rate is used, but with a 10-30 °C offset of the 2D column.
4. Detection parameters	
	- Detector should be able to detect peaks of 100-800 msec at least with 10 data points across the peak - Data collection rate and acquisition speed should be high enough

Figure 1.25 shows our experimental verification of the effect of modulation period on the GC×GC separation of 3 fatty acid methyl esters (FAME: C18:1, C18:2, C18:3) and on the number of modulations per peak. The separation was done using the DB-5 × BPX-50 column combination under similar chromatographic conditions, but using different modulations periods of 4 and 8 sec. A dual-stage loop-type modulator was used with the hot jet pulse of 600 msec. Although this example is not very critical in terms of separation, it still can be seen that the shorter modulation period of 4 sec (**Figure 1.25A**) is favorable over the longer one of 8 sec (**Figure 1.25B**), since the separation between the three peaks is better. In contrast, the peaks are more closely spaced in the second case. Moreover, a modulation period of 4 sec gives 3 modulated peaks for each compound, while within a modulation period of 8 sec – only 2 modulations are obtained which is not satisfactory.

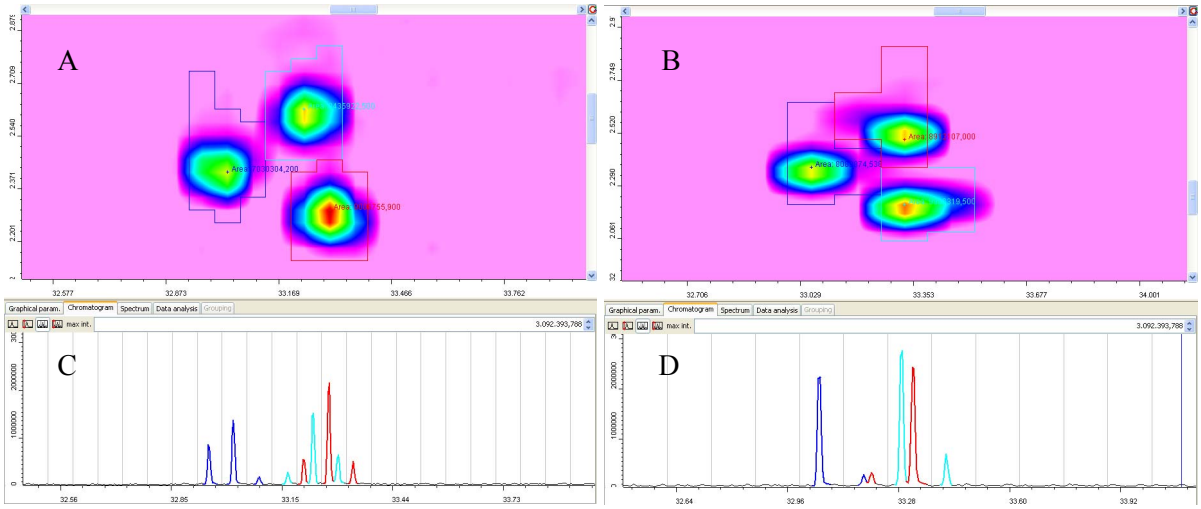


Figure 1.25 The effect of the modulation period on the peak separation and number of modulations per peak. A: modulation period of 4 sec, B: modulation period of 8 sec, C: raw data chromatogram with modulation period of 4 sec, D: raw data chromatogram with modulation period of 8 sec; peaks belonging to the same compound have the same colour.

If the modulation period is too short the so called wrap-around can occur. This is the case when the 2D retention of the analytes becomes longer then the modulation period, which causes some or all of the analytes not to elute in their own, but rather in a subsequent 2D chromatogram. This effect can change the representation of the data and make their interpretation difficult. **Figure 1.26** represents an example of a partial wrap-around, occurring for some of the late eluting peaks.

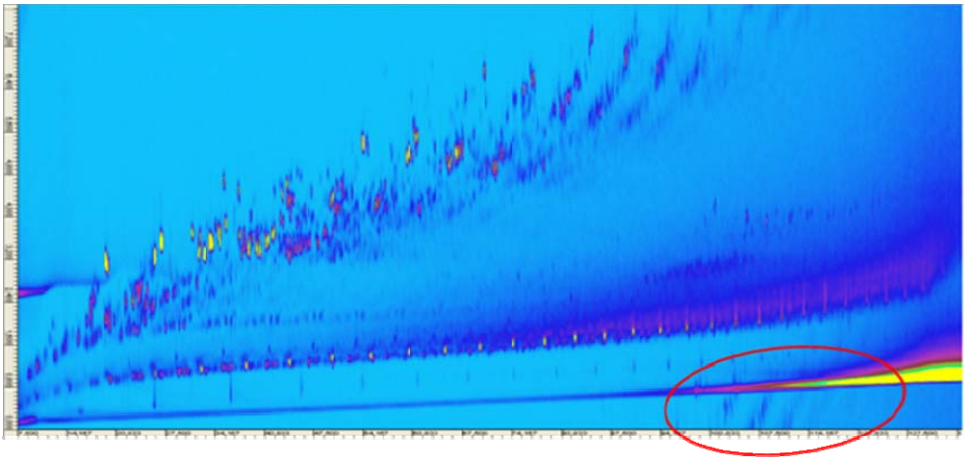


Figure 1.26 The effect of wrap-around. Column set of a column (DB-5MS, 30 m × 0.25 mm i.d × 0.25 µm) in the first dimension and of a column (BPX-50, 1.2 m × 0.1 mm i.d × 0.1 µm.) in the second dimension, modulation time 8 sec, time windows of x-axis: 7.5-128 min, y-axis: 0-8 sec.

The thermal modulator must generate sharp and symmetrical injection bands and introduce them into the 2D column. Trapping and desorption temperature control is important to get effective modulation. In case of dual-stage loop-type modulator these are the temperature of cold and hot jets. The temperature of the cold jet in this type of modulator depends on the cold N_2 gas flow (cooled by liquid N_2). Typically this flow is 10-15 L/min. If the flow is too high, accordingly the jet's temperature will be too low. It this case the effluent from the 1D column can be overcooled and the remobilization of the analytes will be far more difficult and will need a longer time, thus not being completely accomplished within the same modulation. This will lead to tailing in the first chromatographic dimension, or carry-over from one to the next 2D-chromatogram, **Figure 1.27**. The overcooling effect can also happened if the modulation time is too long and a heat pulse is too short at the same time. This effect is more pronounced for compounds with higher boiling points (>C20).

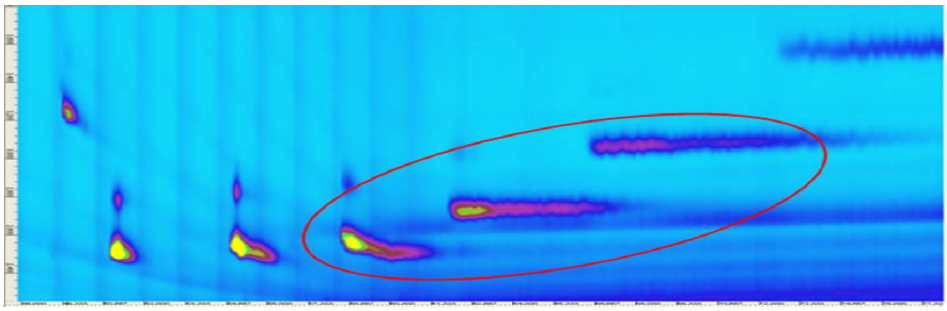


Figure 1.27 The effect of overcooling. Ccolumn set of a column (DB-5MS, 30 m × 0.25 mm i.d × 0.25 µm) in the first dimension and of a column (BPX-50, 1.2 m × 0.1 mm i.d. × 0.25 µm) in the second dimension, modulation time 8 sec, time windows of x-axis: 49-77 min, y-axis: 2.2-3.8 sec.

In order to provide good remobilization of the analytes trapped in the modulation loop, the release (= reinjection) temperature must be high enough. In case of LMCS, this temperature cannot be controlled independently and is equal to the GC oven temperature at the moment of elution. In the loop-type modulator the temperature of the hot jet is normally 40°C above the elution temperature and the hot jet

pulse duration must be long enough (300-600 msec) for efficient desorption and reinjection of trapped analytes. Another problem, breakthrough (**Figure 1.28**), can be observed if the trapping temperature of the modulator is not low enough. Usually this happens with highly volatile analytes. Breakthrough can be also observed if the modulator is overloaded (the concentration of the effluent is too high), or the flow rate in the delay loop is too high.

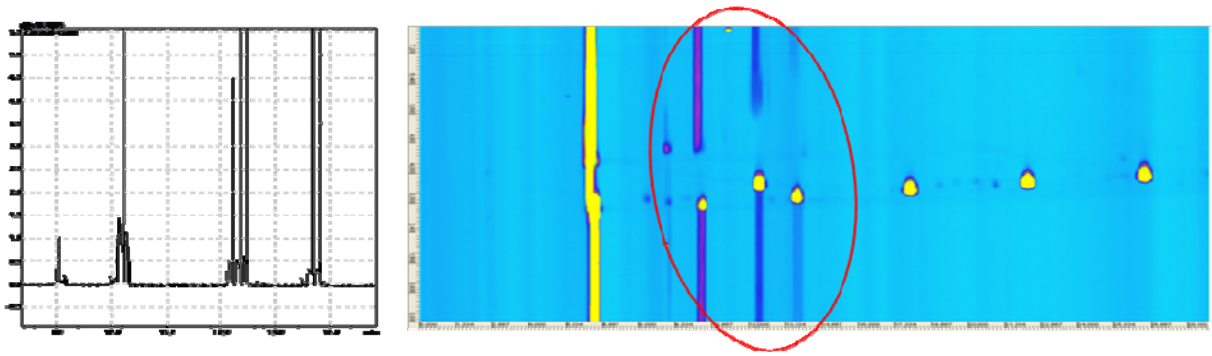


Figure 1.28 The effect of breakthrough. Left: raw GC×GC chromatogram, Right: GC×GC chromatogram. Ccolumn set of a column (DB-5MS, 30 m × 0.25 mm i.d. × 0.25 µm) in the first dimension and of a column (BPX-50, 1.2 m × 0.1 mm i.d. × 0.2 µm) in the second dimension, modulation time 8 sec, time windows of x-axis: 0-28 min, y-axis: 0-8 sec.

The choice of columns in GC×GC separation is of enormous importance. The optimisation of the column set includes finding the suitable stationary phase chemistry and column dimensions (length, diameter and film thickens) for both dimensions in GC×GC separation. The most important aim of an optimised GC×GC separation is to maximise the use of the 2D separation space and to achieve an effective separation, which is done by having an orthogonal separation. To obtain such an orthogonal separation for particular analyses requires a proper stationary phase combination. For recommendations and guidance for the choice of stationary phases and column dimensions see **Table 1.12**. In theory, short, narrow bore columns with thin films of stationary phase should always provide the most efficient separation. In GC×GC, however, problems arise when the 2D column becomes overloaded, which occurs quite frequently with thermal modulators due to the zone compression during trapping [151]. The concentration effect of the focused 1D band may lead to overloading of the 2D column even when the 1D column is not overloaded, so the concentration of the injected analyte should be controlled.

As it is mentioned in the **Table 1.12**, the most employed column set uses a conventional 25-30 m × 0.25 mm ID capillary column in the first dimension and a narrow-bore 0.5-2 m × 0.1 mm ID column in the second dimension. Both columns are connected in series, and as both have quite different internal diameters, linear velocity in the two columns will be different. Using such a configuration, the full potential of GC×GC is hardly ever realized due to performing the separation under conditions which are non-optimum for either of the two dimensions. In **Figure 1.29** the van Deemter plots for both columns of a 2D-column set (= a 30 m × 0.32 mm column for the first dimension and a 2D column of 1.5 m × 0.18 mm) are presented as function of the inlet pressure. As can be seen, by choosing the flow such that the second column is at its optimum (where the height of a theoretical plate is the smallest, so the number of

theoretical plats is the highest, which occurs at a linear velocity of 80 cm/sec corresponding to a column head pressure of ~100 kPa), the average linear velocity of the carrier gas in the first column is only 10 cm/sec, which is far from the optimum flow rate for this column. And on the contrary, by choosing the optimum flow rate for the first column, that is 18 cm/sec, the gas flow in the second dimension is 160 cm/sec, what again is far from the optimum flow rate for this column. It becomes clear that there must be always a compromise in terms of linear velocities in two dimensions: in 1D this is usually near to optimum, that is, ~20-35 cm/sec, in 2D far above the optimum, at ~ 160-330 cm/sec. Among the several options to better match the very different flow requirements of the first and second dimension separation, which all bring different limitations, like reduction of the head pressure, using a longer 2D column or a column with larger internal diameter, there is an alternative option, proposed by Tranchida [153].This author proposes the use of a flow splitter prior to or after the modulator. This system enables independent adjustment of the carrier gas liner velocity in both dimensions which also has the advantage that column overload becomes less likely in the second dimension.

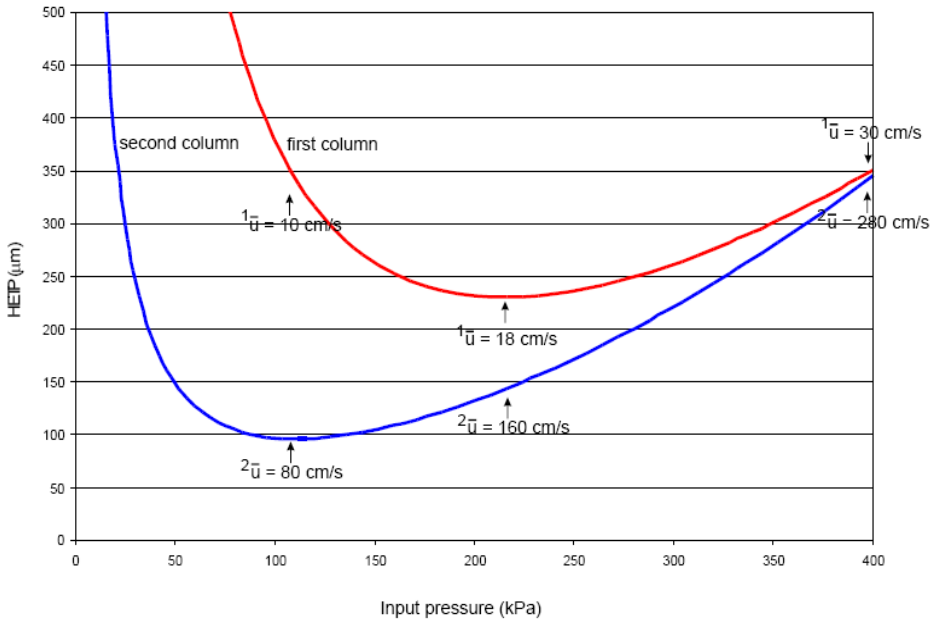


Figure 1.29 HETP vs. inlet pressure curves for the column set of a column (30 m ×0.32 mm i.d) in the first dimension and of a column (1.5 m × 0.18 mm i.d.) in the second dimension [125].

Another important parameter in optimisation of GC×GC is a temperature programming. Changing temperature programming of the first column will not only influence the elution temperature, but also the peak widths of eluting compounds. This will affect the number of modulations that can be made across a first-dimension peak. Generally, the temperature programming rate in GC×GC should be rather slow, i.e. only 1–5 ºC/min, to produce relatively broad peaks in the 1D thus allowing the required 2.5–3 modulations of each peak. However, excessively slow temperature rates can adversely affect sensitivity and/or cause wrap-around. When two independent ovens are used, the 2D column can be programmed at the same rate, but with 20–30 ºC offset from the 1D rate [151]. This difference in temperature may influence the retention and improve separation in the second column.

1.5.5. Quantitative aspects in GC×GC

Comprehensive two-dimensional gas chromatography has the potential to provide both quantitative and qualitative analysis. There are fundamental differences in the way 1D-GC and GC×GC results are evaluated for quantification. Since 1D-GC produces a single measured peak in the chromatogram, there is a single retention time, and associated with this a single peak response. GC×GC produces a series of modulated peaks at the detector. Thus, the peak metrics of retention, area and height for one component are now not simple single values for one peak, but rather are derived from the multiple peak distribution generated by the modulation process [154].

In quantitative GC×GC analysis, peak areas or volumes can be used in quantitation. The determination of peak areas is not as straight forward as in one-dimensional GC, because in GC×GC a single compound is divided into several fractions, the belonging of them to this single compound has to be recognized and the areas of the individual fractions have to be summed up to obtain the total area for the target compound. This can be done either automatically or manually by drawing summation boxes. The automatic integration requires a sophisticated algorithm which is able to recognize which peaks should be included in the summation and then to perform this calculation.

The conventional (manual) integration and summation of selected modulation peaks, and integration of the peak volumes in the 3D chromatogram, can be used in some cases for quantitative evaluation of GC×GC results [155,156]. This is possible and works with satisfactory precision mostly for all target analyses, but in case of samples with complex matrix it is time consuming and hardly realised. Different "multiway techniques'' (when data can be arranged in matrices) [155], "in-house" integration packages and chemometric methods [157] were reported in order to improve and facilitate the quantitation process. Some of them failed due to lack of sophisticated integration algorithms to cope with less-ideal situations.

Due to the large amount of data that are produced by GC×GC and the time required to analyze and quantitate, many researchers have turned to chemometrics to simplify data analysis. Chemometrics uses mathematical techniques to extract useful information from datasets. Chemometric techniques, such as principle component analysis (PCA) and component discriminate analysis (PCDA), have been used to find similarities and differences between samples. Chemometrics techniques that simultaneously deconvolute and quantitate have been reported in GC×GC such as the generalized rank annihilation method (GRAM) and parallel factor analysis (PARAFAC). Chemometric calibration techniques have also been applied to GC×GC, such as principal component regression (PCR), partial least squares (PLS), and multilinear partial least squares (NPLS) [158].

Chemometrics is the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods, and spans a variety of methods to be applied in these studies, e.g. parameter optimisation, peak detection, alignment of retention time shift, and other aspects of signal processing [159].

The interesting structure of comprehensive multidimensional separation datasets is their high-dimensionality, with retention time in the first dimension (^1t_R) as abscissa and retention time in the second dimension (^2t_R) as ordinate axes (or vice versa), with the z-axis being either a single response variable or a multivariate (spectral) response, e.g. as with MS. Further, changes in a mixture's composition can be employed as the fourth axis to enhance recovery of 'buried' information. The high dimensionality of the comprehensive multidimensional separation data structure is represented in **Figure 1.30**. This makes method development a task for chemometrics and provides new opportunities for and challenges to conventional mathematical tools in the chromatography experiment [159].

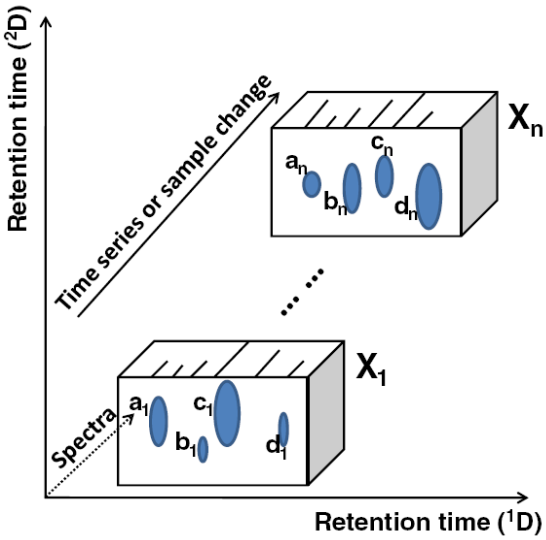


Figure 1.30 Data structure of comprehensive multidimensional separations with the highest dimension attained being 4. Each box, from X_1 to X_n, represents a tensor data with two dimensions of retention time (^1t_R and ^2t_R), and spectra as the third dimension. a_1, a_n, b_1, b_n, c_1, c_n, d_1, d_n show the representative image peaks in the two-dimensional separation space. ^{1}D first dimension, ^{2}D second dimension [159].

Classic 1D software systems can be used to acquire GC×GC data streams. To treat the datasets acquired from comprehensive two-dimensional separation a number of commercial software packages have been developed specifically: ChromaTOF optimised for the Pegasus 4D system (Leco, USA), HyperChrom (Thermo Fisher Scientific, USA), Transform (ITT Visual Information Solutions, USA), GC Image (GC Image, Lincoln, USA), ChromSquare (Chromaleont/Shimadzu). These systems present data in the now familiar 2D format, and process such data taking into consideration interpretation of the 2D data structure. These systems include a collection of chemometric methods for data processing. They can be employed for data acquisition, comparison and visualization, peak detection and discrimination, deconvolution, quantification, classification, calibration, mass-spectral similarity matching, result reporting, and other functions [159]. Integration of peaks for quantitation is also realized by mean of these softwares.

There are two main algorithms for the quantification of peaks in comprehensive multidimensional chromatography: the two-step algorithm and the watershed algorithm. In the following, I would like to discuss the two commercially available software packages for comprehensive two-dimensional

chromatography that have been used in this work and that are built on the base of the two mentioned algorithms. These two algorithms have been compared recently, and while both provide excellent quantitative results, the watershed algorithm was found to detect resolved peaks with a greater accuracy than the two-step method when a correction for second dimension retention time shifts was performed for both algorithms However, it is important to note that neither algorithm can guarantee successful peak detection under all conditions and as peak width and noise increase both methods begin to detect peaks less accurately [160,161,162].

GC Image software [163], developed at the University of Nebraska-Lincoln, is one of several available solutions for visualizing, analyzing, and reporting on data from GC×GC and comprehensive two-dimensional liquid chromatography (LC×LC) based on the watershed algorithm. The watershed algorithm takes the retention plane and detects peak apexes on the image. Then, it grows the peaks around these apexes by adding pixels with lower values, essentially growing each peak from the top down in three dimensions until either the baseline is reached or data points start to climb, as would happen when reaching a partially coeluting neighboring peak. Since visualization is primarily a qualitative technique, this software treats the data space resulting from the 2D chromatograms as image. The separated analytes are associated in groups of datapoints, pixels, so they can be detected and accurately quantified. GC Image sums pixel intensity corresponding to a single analyte, thus providing quantitative information while also utilizing the second-order data structure. In image processing, each such group of pixels is called a blob and the operation of segmenting pixels into blobs is called blob detection. GC Image implements blob detection. The blob detection algorithm uses a greed dilation that successively attaches the largest-valued unassigned pixel to a neighboring blob or forms its own blob if no neighboring peak has been established [164].

GC Image maintains computed attributes and metadata about each blob [165]. The computed attributes include: 1) Peak location – the coordinates of the peak apex in each dimension and retention times of the peak; 2) Peak Area – the number of pixels in the blob, i.e., its size in number of datapoints irrespective of its values; 3) Peak Volume – three-dimensional peak volumes are calculated by means of imaging, the sum of the values of the pixels in the blob, i.e., the total of its values or total response; 4) Peak value – the largest value or apex of the pixels in the blob. GC Image graphically highlights detected blobs in the Viewport of the Image Viewer, e.g., as shown in **Figure 1.31**, and presents metadata and computed attributes for all blobs in an interactive table. The Peak Volume values are used for peak quantification, so quantitative analysis is performed from the analysis of the blob volumes.

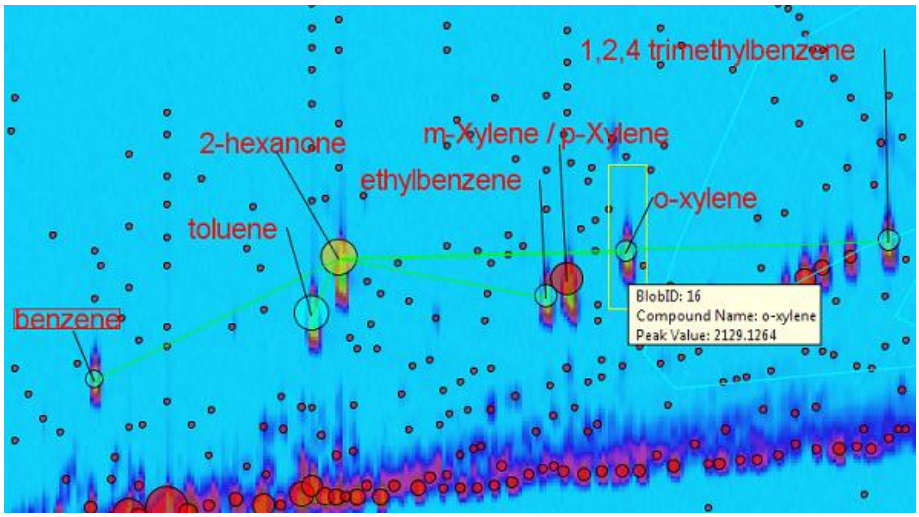


Figure 1.31 Graphical highlights show the unflagged blobs (circles in red), included blobs (circles in light blue), internal standards (circles in yellow), and associations between included blobs and internal standards (lines in green) [163].

The second software that was used in the frame of this work is the ChromSquare [166] workstation software, developed from analysts for analysts in the laboratory of Prof. Luigi Mondello at the University of Messina/Italy, based on the two-step algorithm. The two-step algorithm detects sub-peaks in each second-dimension chromatogram individually and then combines those which are deemed to be due to a single compound based on criteria such as retention times in both dimensions, mass spectral match quality between peaks (if a MS detector was used), and unimodality. ChromSquare provides all means for quantitative and qualitative analysis of data from comprehensive liquid chromatography (LC) or GC, with and without MS detection. It enabled both the conversion of the instrumental data into two- and three-dimensional chromatograms and direct peak integration.

The integration procedure in this software is based purely on chromatographic properties and differs from peak integration in GC Image software, where 2D peaks are integrated as images or considering peak volumes. The quantification process in ChromSquare software is done by automatic integration of each 2D peak. One of the greatest difficulties encountered if a conventional integration procedure is done relies on the proper determination of peaks that belong to the same compound. To this aim, the software contains the algorithms that enable the simultaneous identification and integration of analyte bands relative to the same solute. This fundamental data-handling process is achieved by considering detection data points during each modulation period. Obviously, both the modulation frequency and the detector sampling rate were involved. Hence, second-dimension peaks which eluted in the same data-point window, in consecutive modulation periods, are correctly identified by the program. Moreover, the reconstructed logical peak shape of peak groups produced by the same compound, is accounted for: for example, a compound eluting in the same second-dimension datapoint window as a previous one, without following the expected logical peak pattern, would be considered as a different compound [167].

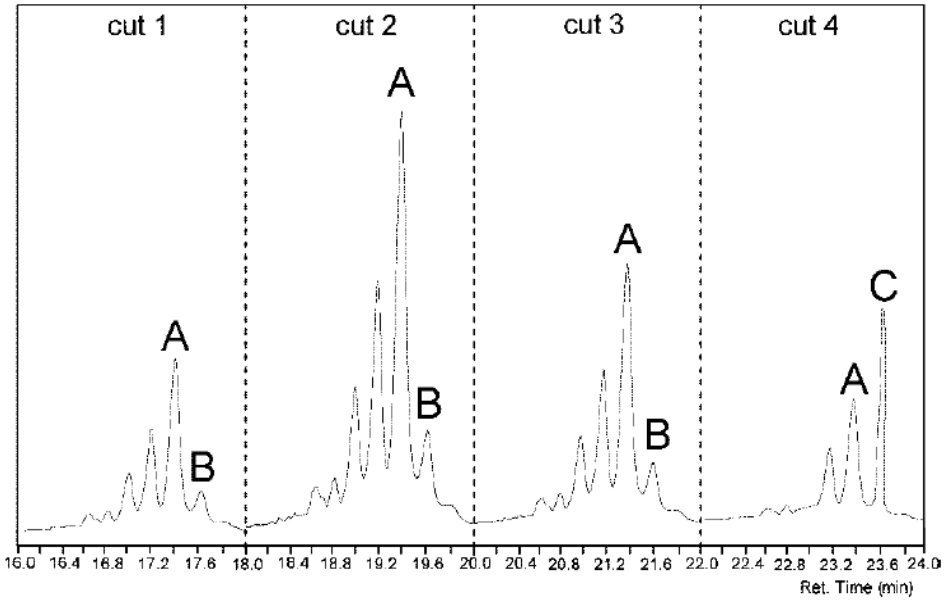


Figure 1.32 Four untransformed second-dimension chromatograms related to an LC×LC application. The peaks marked as A, B, and C represent different compounds [167].

The next step, once each second-dimension peak is assigned to a single compound, is the quantification process: all peak areas relative to each specific component group are measured and subjected to summation; thus, the total area for every 2D peak present in the 2D plot is derived. Besides this automatic integration function, the operator can select specific target components for quantitation.

When the detection and integration of peaks can be done by one of the above-described software, the subsequent quantitative analysis is done according to established schemes. Internal or external calibration can be used for target analyses and group type quantitative analysis [168,169,170]. It was also shown that the normal (one-dimensional) GC calibration can successfully be used to quantify compounds analysed by GC×GC [171].

Using multidimensional approaches, analytes can be quantified at levels substantially lower than those possible by one-dimensional techniques due to the larger signal that substantially improves detection limits of GC×GC over 1D GC. While a theoretical treatment of the detection limit and limit of quantification remain to be performed, it has been shown that the conventional definition of the limit of quantification does not apply to comprehensive multidimensional separation data. It has been shown that the modulation process introduces a new source of error to the measurement [172]: errors due to the summation of multiple peaks (smaller sub-peaks have potentially significant errors), errors due to phase shifting (would be most significant with small analyte peaks and modulation period is short). Harynuk *et al*. [173], presented the results of a study into the limits of quantification and detection (LOQ and LOD) in separations GC×GC using and the "two-step" integration algorithm as an example. Simulation of chromatographic data permits precise control of relevant parameters of peak geometry and modulation phase. Results are expressed in terms of the dimensionless parameter of signal-to-noise ratio of the base peak (S/N_{BP}) making them transferable to any result where quantification is performed using a two-step algorithm. Based on these results, the LOD is found to depend upon the modulation ratio used for the

experiment and vary between a S/N_{BP} of 10−17, while the LOQ depends on both the modulation ratio and the phase of the modulation for the peak and ranges from a S/N_{BP} of 10 to 50, depending on the circumstances.

1.6. GC×GC for petrochemicals

Petrochemical samples are perhaps the most complex samples known to analytical chemists. The number of compounds in petrochemical samples increases with the boiling point, and the complexity of their composition increases with increasing molecular weight of the compounds. Most analytical methods for oil characterization rely on gas chromatography, where by using a non-polar column a volatility-based distribution is obtained. No single 1D GC operation can come up with a full separation of such huge number of compounds. It is even unlikely that any peak in such 1D GC represents one single component, **Figure 1.33**. For this reason multidimensional separations are successfully used for this purpose. GC×GC has a huge potential for investigating complex matrixes such as petroleum products owing to its resolution power.

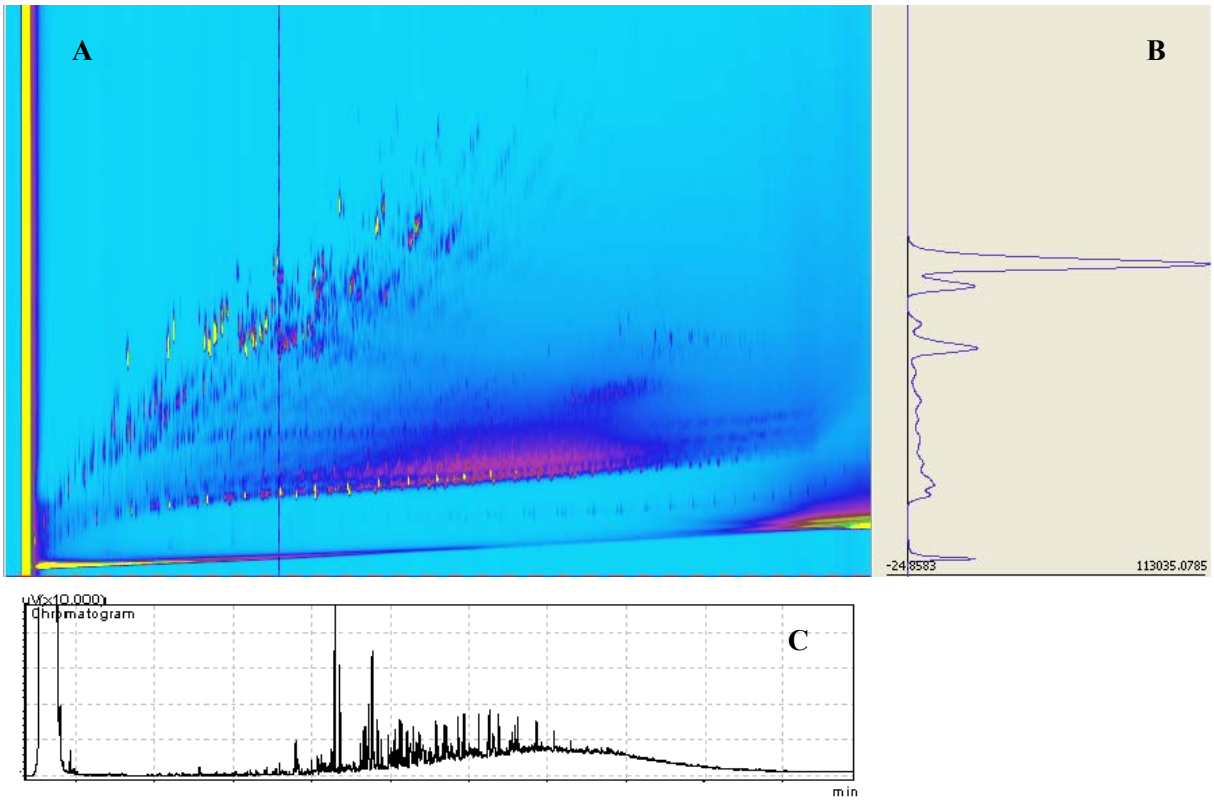


Figure 1.33 A: Contour plot of a GC×GC separation of light heating oil (column set of a column DB-5MS, 30 m × 0.25 mm i.d. × 0.25 µm in the first dimension and of a column BPX-50, 1.2 m × 0.1 mm i.d. × 0.1 µm in the second dimension, modulation time 8 sec, time windows of x-axis: 0-140 min, y-axis: 0-8 sec) and B: a single second dimension chromatogram from the same separation. The second dimension chromatogram was obtained at the first dimension retention time of 44.5 min. C: 1D-GC chromatogram of the light heating oil (DB-5MS, 30 m × 0.25 mm i.d. × 0.25 µm).

GC×GC has originally been developed for the comprehensive characterisation of petrochemical samples. Comprehensive multidimensional separation coupled to a FID or MS provides the general characterization of petroleum fractions showing the unique separations of classes of structurally related compounds. The well-known polarity versus volatility separation achieved using first a conventional non-polar column connected to a fast semi-polar column in the second dimension leads to structured chromatograms where hydrocarbons are arranged according to their chemical compound class and to the number of carbon atoms. Although the flame ionisation detector (FID) is the most appropriate detector for routine and quantitative analysis of hydrocarbons, hyphenation with a mass spectrometer or specific detectors – such as the atomic emission detector (AED) or sulphur chemiluminescence detector (SCD) – is recommended for identification or speciation [174]. The use of MS detection, instead of the commonly applied FID, provides only limited information for the characterization of high-boiling fractions of the petroleum hydrocarbons. The mass spectra of the many structurally related compounds tend to be closely similar and molecular ions are frequently missing [175]. However, MS detection still is the main tool for target and speciation analysis.

GC×GC is of great usefulness for the entire spectrum of petroleum analyses, from the profiling of potential oilfields during exploration, to monitoring product streams during refinery operations, to assessing the presence of petroleum and its combustion products in the environment. Since GC×GC GC×GC was introduced a reasonable number of reviews for its petrochemical applications have been published [75,158,175, 176]. Petroleum analyses can be classified as follows: group-type analyses (including fingerprinting techniques) and the analysis of one or more target compounds in a petroleum sample. The majority of petrochemical analyses involves a group-type separation rather than a target analysis, and will be discussed in more details in the next section.

A unique outcome of GC×GC are the well-structured chromatograms where representatives of different chemical classes occupy different locations within the two-dimensional (2D) separation space. The presence of an ordered structure in the GC×GC plot allows the classification and identification of chemical compounds through a visual inspection of the contour plot.

Typical classes or groups of petrochemicals identified by GC×GC include:

- Acyclic alkanes (paraffins), linear and branched alkanes (*iso*-alkanes);
- Cyclic alkanes (naphthalenes), containing either one (mono) or more (poly) saturated or naphthenic five- or six-membered rings – which may have one or more (branched) paraffins attached to the ring;
- Unsaturated alkanes or alkenes (olefins), either linear, branched or cyclic – in general crude oils;
- Aromatics, which can be subdivided into mono-, di-, tri- and poly-aromatics; these may have one or more paraffins or naphthalenes attached to the ring system.

The combination of a first non-polar column with a second semi-polar column is very often used for hydrocarbon analysis. BPX-50 (50% phenyl polysilphenylene-siloxane column) appeared to be one of the most promising stationary phases of intermediate polarity for use as the second column in the

separation of naphthenes from paraffins, and aromatics from saturate hydrocarbons [177], **Figure 1.34 A**. This combination leads to orthogonal separation, based on two independent chemical properties – volatility and polarity – and is referred to as normal phase GC×GC (nP-GC×GC). The phenyl groups in the siloxane backbone possibly show extra affinity to the ring structures of the naphthenes and aromatics.

To improve the separation between saturates and aromatics and, consequently, integration results, a reversed polarity set of columns, i.e. a first long polar column (BPX-50) connected to a second short nonpolar column (DB-1: dimethylpolysiloxane) can be applied [178]. The reversed phase GC×GC (rP-GC×GC) separation is presented in the **Figure 1.34 B**. As a first observation, the separation space was extended compared to nP-GC×GC separations meaning that the peak capacity was better adjusted to the analytical problem.

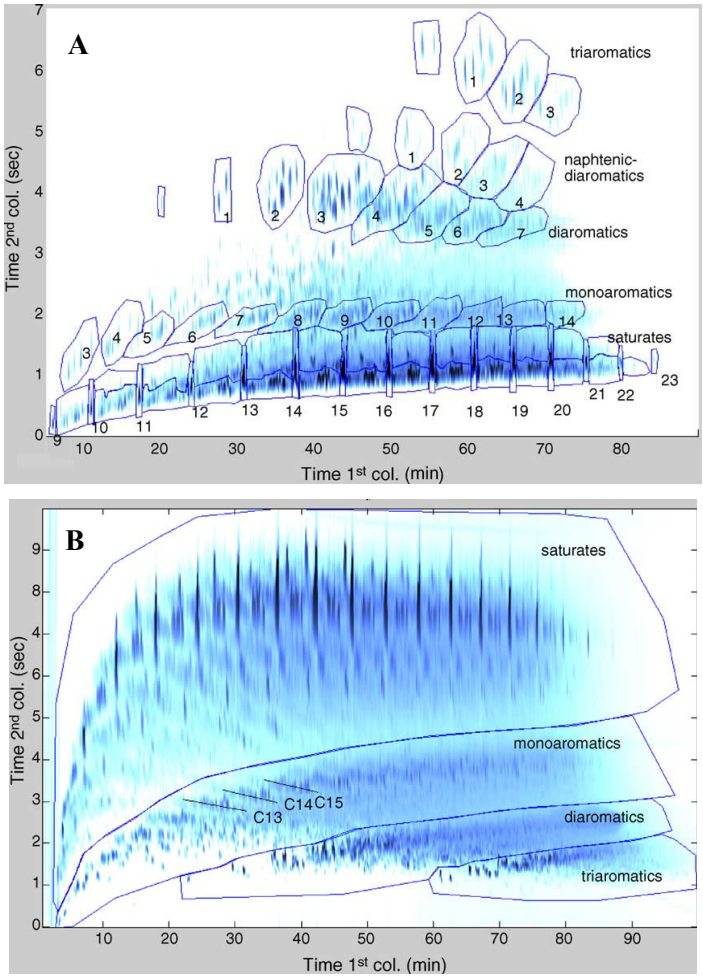


Figure 1.34 A: nP-GC×GC detailed group type separation of a diesel: elution zones of saturates, aromatics, diaromatics and triaromatics are plotted according to the number of carbon atoms. The number inside each zone indicates the number of carbon atoms of the alkyl chain or alkyl groups.
B: rP-GC×GC group type separation of a diesel. The pattern describes elution zones of saturates, aromatics, diaromatics and triaromatics in a reversed order compared to nP-GC×GC. Bands of C13, C14 and C15 alkylbenzenes are represented with straight lines [174].

The retention of a compound is inversely proportional to its vapor pressure and to its activity coefficient toward the stationary phase at infinite dilution. In a non-polar/polar system, two analytes having the same vapor pressure would coelute after the first separation but may be separated in the second

dimension on the basis of the difference in their activity coefficient. In a polar/non-polar system, the first dimension separation is governed by both volatility and molecular specific interactions. In this case, the orthogonality between the two separation dimensions may be somewhat reduced, because volatility is involved in both separation mechanisms, however, it clearly is a comprehensive two-dimensional separation system: and analytes coeluted after the first separation may also be separated in the second dimension if their vapor pressures are different, also meaning that they have different polarities. This situation typically occurs for the separation between saturates and aromatics. In the first dimension, aromatics are coeluted with saturates of lower volatility. As the separation in the second dimension only depends on volatility, the aromatic compound has a lower retention time than the saturate compound introduced at the same time in the second column. This process is similar for the separation between mono-, di-, and triaromatics. In the second dimension of rP-GC×GC, compounds elute from the more to the less polar. The strength of GC×GC still relies on the possibility of resolving in a second dimension of separation two compounds that were coeluted after a first separation dimension, owing to a difference in a specific chemical property [174].

The structure of GC×GC chromatograms allows getting more detailed information on the sample composition than that provided by a simple group-type separation, **Figure 1.35**. Indeed, isomers are grouped in the same location of the chromatogram, and can be recognized as the roof-tile effect, where each tile represents a certain class of structurally related compounds, having the given number of carbon atoms but in different structural arrangement. The roof-tile effect becomes evident as the different tiles are piled up one after another, and it helps identifying different classes of compounds [75,177].

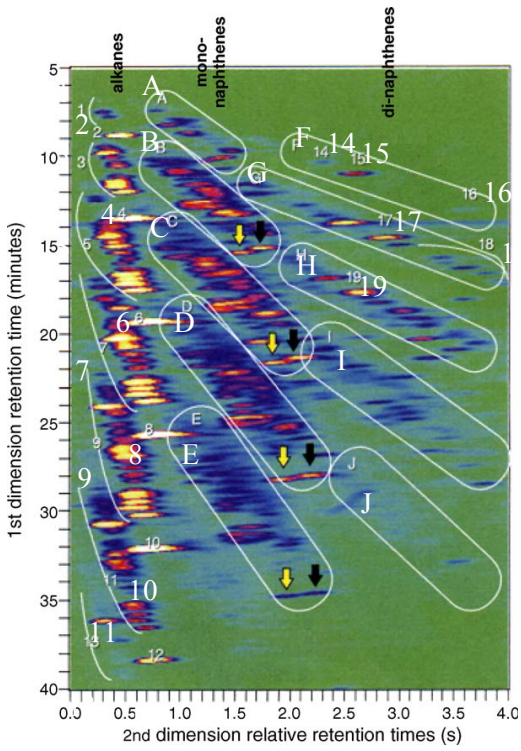


Figure 1.35 Contour plot of a nP-GC×GC separation of a non-aromatic solvent.
1. branched C10, 2. n-C10,
3. branched C11, 4. n-C11,
5. branched C12, 6. n-C12,
7. branched C13, 8. n-C13,
9. branched C14, 10. n-C14,
11. branched C15, 12. n-C15,
13. branched C16, 14. unknown,
15. trans-decalin, 16. cis-decalin,
17. trans-methyl-decalins,
18. cis-methyl-decalins,
19. C2-decalins,
A – E: mono-naphthenes C10 through C14, in which the yellow arrows indicate the n-alkylcyclopentanes and the black arrows the n-alkylcyclohexanes, F – J: dinaphthenes C10 through C14 [177].

As an example of the group type separation, different condensate oil samples are analysed by GC×GC in the work of Huitong *et al.* [179]. Condensate oil, with high light hydrocarbon content, provides abundant geochemical information. Because of strong volatility of condensate oil, the normal analysis methods cannot meet the analytical requirement. Light hydrocarbon components are separated completely without the influence of co-eluting peaks, and the fingerprint information of the light fraction such as propane remains intact. Sixty-seven compounds are detected in the range of nC3-nC8 by using GC×GC, 13 more than the compounds recognized in the conventional GC. These compounds are divided into 12 compound classes by GC×GC according to the structure in the range between C3 and C31, **Figure 1.36**.

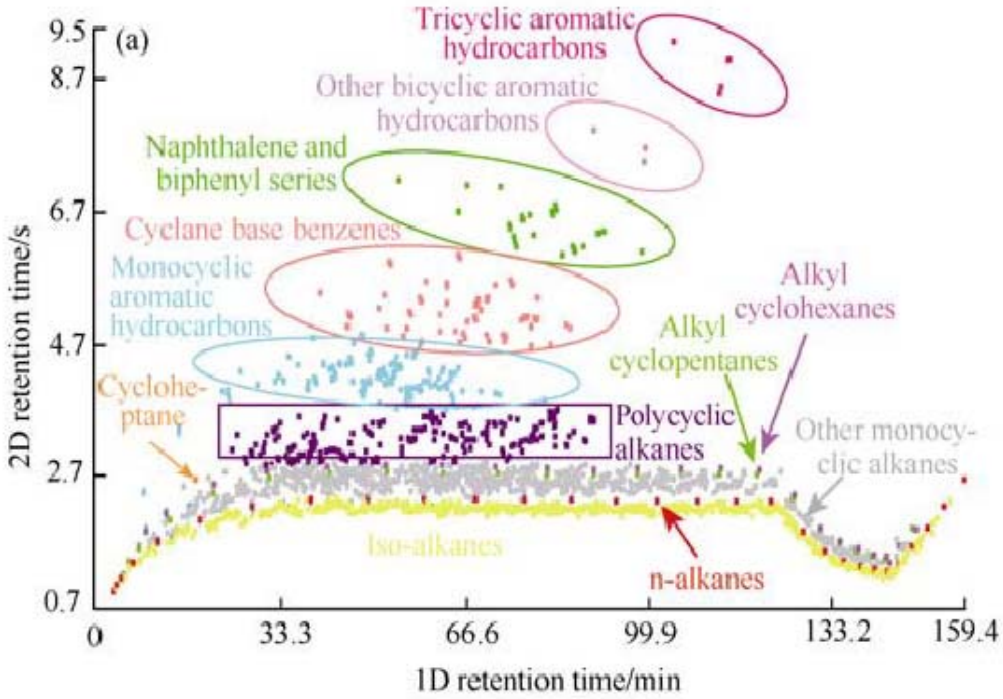


Figure 1.36 GC×GC color contour chromatogram of condensate oil with 12 compound classes [179]. Conditions: 1D - Petro column (50 m × 0.2 mm × 0.5µm), 2D - DB-17HT column (3 m × 0.1 mm × 0.1µm), 1D oven - 35 ºC (0.2 min) → 230 ºC at 1.5 ºC/min (0.2 min) →300 ºC at 4 ºC/min (15 min), 2D oven – offset of 10 ºC, modulation period 10 sec, hot pulse 2.5 sec.

The interest to heavier cuts of crude oil, such as extra heavy gas oils and vacuum gas oils is increasing recently, due to about declining natural oil reserves. These samples are much more complex than those from lighter oil sources, what makes the analyses of them more challenging. Furthermore, heavy fractions tend to include a higher proportion of nitrogen-, sulfur-, and oxygen-containing species than the lighter fractions; compounds which cause problems both with refinery operations and in finished products, making for interesting analytical targets.

In the search for alternative fuel sources, the products of Fischer–Tropsch (FT) reactions which convert materials such as coal and natural gas into clean, high value fuels have been extensively investigated by the use of multidimensional separations. These fuels are generally simpler than conventional fuels, and do not have any N- or S-containing compounds present. However, they do contain

large quantities of oxygenated products. Characterization of FT fuels for the optimisation of catalysts and reaction conditions require group-type separations of the products which contain a variety of linear and branched alkanes, and alkenes, with numerous oxygenated species such as alcohols, ketones, aldehydes, and carboxylic acids [179].

In the area of GC×GC analysis of petroleum and fuels, the most important technological advance in the last few years has probably been the development of the ionic liquid columns. They are known to have unique physicochemical properties. Since ionic liquids can interact with molecules via a range of physicochemical processes, they can act like polar solvents for polar molecules, and like nonpolar solvents for nonpolar molecules. This "dual nature" of ionic liquids makes them desirable materials for GC stationary phases. In addition to having retention mechanisms that are very different from conventional siloxane-based phases, some also have high temperature stabilities (290–300ºC), a feature crucial for high-temperature analysis of petroleum samples. In particular, the imidazolium-based ILs are known to possess wide liquid ranges and good GC column efficiencies [180,181].

Ionic liquid stationary phases are suitable for group separation analyses of aromatics from aliphatics, and FAMEs from aliphatics. In addition, they offer high phase stability in conjunction with extremely high polarity [182]. In this way ionic liquid stationary phases offer interesting possibilities for the second dimension in GC×GC. The high selectivity of comprehensive GC, combined with MS identification, made it possible to detect and identify more compounds which were co-eluting in one dimension GC. Fatty acids with different double bonds and carbon numbers are group-type separated on the 2D space, if SLB-IL 82 was the second column [183]. A reversed mode using IL59 provided an innovative sulfur group type separation, especially for naphthenoaromatic S-compounds family [184,185].

There are a lot of other group-type applications of GC×GC for different petroleum samples, e.g., biomarkers in crude oil, fatty acid methyl esters (FAME) in biofuel, environmental monitoring of petroleum products and others, see **Table 1.13**.

Table 1.13 Group-type applications of GC×GC to petroleum samples.

Group-type	Instrumentation	Sample type
PIONA (Parafins, Isoparaffins, Olefins, Naphthenes, Aromatics)	GC×GC	Different petroleum products (for their characterization), e.g. heavy and vacuum oils
BTEX (benzene, toluene, ethyl benzene and xylenes)	GC×GC LC-GC×GC	Gasoline
Aromatic content by ring number	GC×GC	Crude oil
Aliphatics and aromatics	GC×GC SFC-GC×GC LC- GC×GC	Middle distillates, diesel
N-, S-containing compounds	GC×GC/MS GC×GC-NPD, NCD GC×GC-SCD GC×GC-ToFMS	e.g. Heavy and vacuum gas oils, different fuels (monitoring in terms of environmental impacts when fuel is consumed and as catalysis in the intermediate refining steps)
O-containing compounds	GC×GC GC×GC/MS	Products of Fischer–Tropsch (FT) reactions
Fatty acid methyl esters (FAME)	GC×GC GC×GC/MS	Biofuel
Group-types analyses	GC×GC/MS Ag ion-HPLC-GC× GC SPE-GC×GC	Environmental weathering and biodegradation products of petroleum samples and biofuel
Biomarkers (pristine, phytane, alkylated aromatics, S-containing compounds, steranes, triterpanes, hopane)	GC×GC	Crude oil

SFC – supercritical fluid chromatography; LC – liquid chromatography; NPD – nitrogen phosphorus detector; NCD – nitrogen chemiluminescence detector; ToFMS – time-of-fligh masspectrometer; Ag-ionHPLC – silver ion HPLC; SPE – solid phase extraction

Comprehensive coupling of liquid chromatography (LC) to GC×GC has strongly gained popularity recently. In LC-GC×GC analyses of petroleum samples, the LC separation is usually an offline fractionation step to separate aliphatics from aromatics before the multidimensional GC separation. Normal phase or silver ion LC (Ag ion-HPLC) is usually implemented in this set-up [186,187].

A good example of the detailed group-type characterization by LC-GC×GC of hydrocarbons present in motor oils is presented in the **Figure 1.38**. With conventional GC, motor oil is not well separated due to its complexity, resulting in a ‘‘hump” in the chromatogram: the unresolved complex mixture (UCM). With HPLC-GC×GC, motor oil hydrocarbons can be quantitatively separated into four major groups: alkanes, cycloalkanes, alkenes and aromatics.

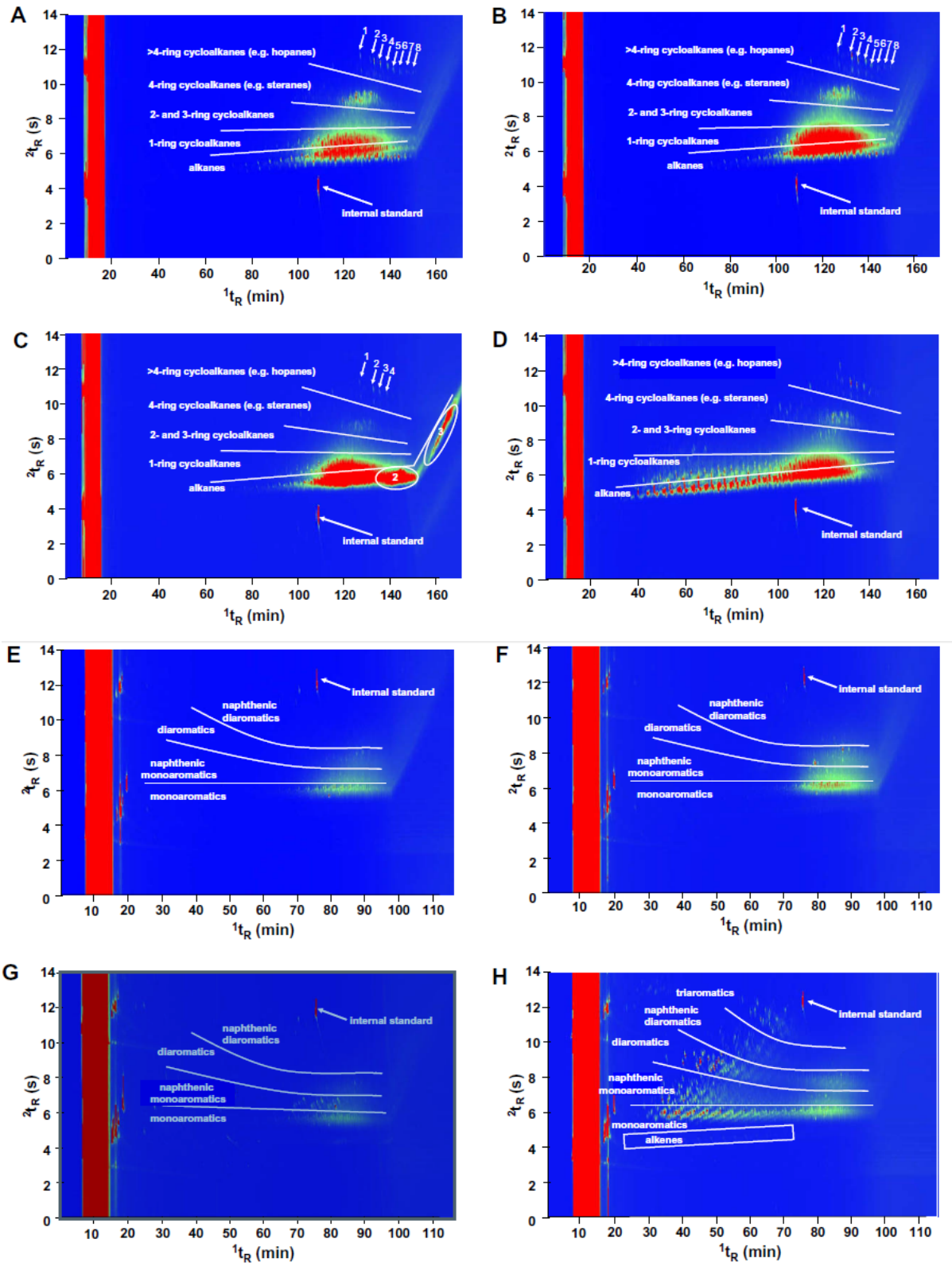


Figure 1.38 HPLC-GC×GC/FID colour plots of the saturated fractions (A–D) and the unsaturated fractions (E–H) of the three motor oils and the RIVM oil: (A and E) Castrol SAE30, (B and F) Esso SAE15W40, (C and G) Elf LDX5W40 (D and H) RIVM-LOC1-001. Number 1–8 in B: C27, C29 ab, C30 ab, C31(S + R), C32 (S + R), C33 (S+R), C34 (S + R) and C35 (S + R) hopane biomarkers [188].

Each group can be further separated and divided by ring number or carbon number. Three selected motor oil samples were characterized by HPLC-GC×GC including two conventional motor oils and a synthetic motor oil (RIVM). Using a proprietary silver-modified HPLC column, the aromatic components in motor oils were baseline separated from the aliphatic UCM and were then further separated by GC×GC based on their aromatic ring numbers. Accordingly, the aliphatic components were separated by GC×GC based on their saturated ring numbers. This example illustrates the enhanced capabilities of HPLC-GC×GC for reliable and detailed quantitative group-type characterization of hydrocarbons present in motor oils [188].

1.6.1. GC×GC for quantification of petrochemicals

Quantification and characterization of petroleum hydrocarbon contaminations are carried out in routine environmental laboratories mostly using gas chromatography with flame ionization detection (GC-FID). The separation of petroleum samples in 1D-GC is limited and the individual identification of the entire sample is an unrealistic task, although this technique is very useful and widely used for total petroleum hydrocarbon (TPH) determination, *see Chapter 1, Section 1.1 and Chapter 2*.

GC×GC can be used in order to provide a more detailed composition of any type of petroleum samples, but it requires the careful adjustment of the technique and optimisation of parameters. The enhancement of resolution associated with the high peak capacity, the structure of the 2D-representation of the chromatograms and the higher sensitivity are the main advantages of GC×GC for a more detailed analysis of complex petrochemicals leading to a better understanding of the processes [189]. For chemical identification of organic compounds in petroleum products and petroleum-contaminated soil/water GC×GC usually is coupled to a mass spectrometer. The mass spectral data is used for confirmation of the compound class assignments. The potential of GC×GC/MS for group-type identification of oil samples was demonstrated by many authors, *see Section 1.6*. Coupling GC×GC with FID is preferred for quantification because of its wide linear range and simple calibration procedure.

The usefulness of GC×GC for the general characterization of complex petrochemical mixtures and applications of GC×GC in the field of petrochemical environmental pollution (oil spill source identification, crude oil biomarker analysis, characterization of unresolved complex mixtures in petroleum-contaminated sediments and screening of PAHs in soil) is enormous and has already been described in the *Section 1.6*. In spite of this, not so much attention has been spent on total petroleum hydrocarbon determination by GC × GC, with just few authors having dealt with this task [74,190].

Seelay *et al.* in 2007 [190], used the GC×GC system to analyse extracts of spiked water samples, wastewater, and soil. The accuracy of the method was compared to that of the MA EPH 2004 method (Method for the Determination of Extactable Petroleum Hydrocarbons (EPH), from the Massachusetts Department of Environmental Protection) [45] for analyzing extractable petroleum hydrocarbons, where the extract with petroleum compounds was concentrated and fractionated into aliphatic and aromatic

components. Five standard mixtures containing each of the 14 aliphatic (C9, even C-numbered C10-C36) compounds and 17 PAHs at different concentrations were used for calibration of the GC×GC method. The average response factors (i.e., peak area/component concentration) were calculated for each individual component. The concentrations of the hydrocarbons in the wastewater samples were calculated using the average response factors for each group and the relevant dilution factors. The GC×GC system generated a quantitative accuracy for the analysis of spiked water that was well within the MA EPH criterion of acceptability. The GC×GC method and the MA EPH method generated comparable levels of total hydrocarbons when wastewater was analyzed, but the GC×GC method detected a significantly higher aromatic content and lower aliphatic content. This could be caused by incomplete clean-up of non-petroleum related compounds prior to GC×GC analysis. It is also possible that the GC×GC method detected higher aromatic levels and lower aliphatic levels because the MA EPH results were in error. The observed discrepancy would occur if aromatics broke through into the aliphatic fraction during the MA EPH fractionation step. Both the GC×GC method and MA EPH method measured comparable levels of aromatics in the soil samples.

A similar study has been done by Van Den Weghe *et al.* [74], where the GC×GC/FID method was developed for the routine characterization of oil contaminations in the context of soil remediation. The obtained results on TPH were compared with results obtained according to the TPH determination method based on a protocol developed by the TPHCWG (Total Petroleum Hydrocarbon Criteria Working Group) [16] (including fractionation into aliphatic and aromatic components). A major advantage of the GC×GC analysis is that no sample preparation other than extraction is needed, thereby avoiding uncertainties associated with the TPH silica fractionation. Preliminary results show a better repeatability (1.6% against 14.7%) and a higher recovery (up to 30%) than the TPH method, probably as a consequence of the simplified sample preparation. For some samples substantial difference in aliphatic/aromatic ratio was found: the contribution of aliphatics is higher in the TPH result (76% against 61% with the GC×GC method). The difference, as has been proven, was attributed to the silica fractionation, when the monoaromatic hydrocarbons are partially eluted in the aliphatic extract (so called breakthrough of aromatic hydrocarbons). However, it is important to realize that this kind of problem remains unnoticed when performing a conventional TPH analysis, because the presence of aromatics in the aliphatic extract cannot be detected by GC-FID. With GC×GC, the silica breakthrough problem is completely avoided because the separation between aliphatic and aromatic hydrocarbons is realized in the second dimension GC-column, and not during sample preparation.

When integrating the group windows corresponding to the TPH classification (aliphatics: C8-C10, C10-C12, C12-C16, C16-C21, C21-C35 and aromatics: EC8-EC10, EC10-EC12, EC12-EC16, EC16-EC21, EC21-EC35), then the relative hydrocarbon distributions within the aliphatic and aromatic fractions (**Figure 1.38**) are in good agreement, with only slight differences for the heavier aromatic fractions in samples A and B [74].

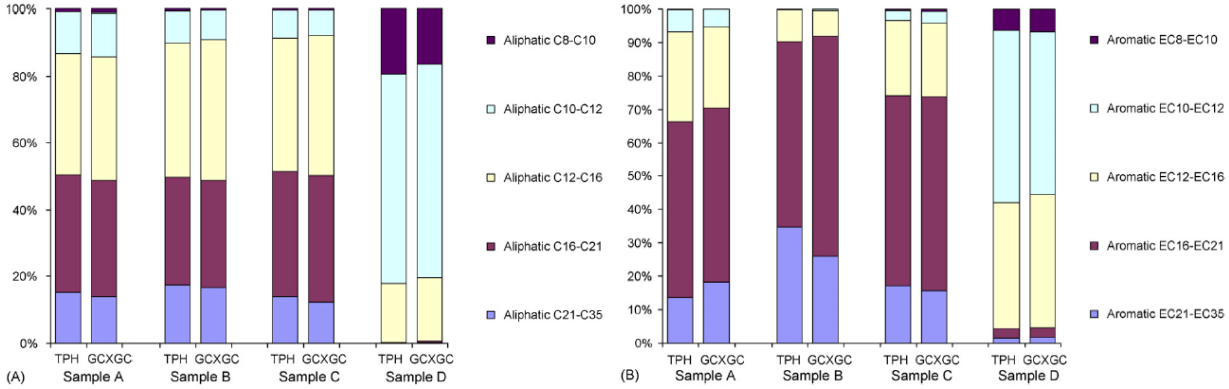


Figure 1.38 Comparison of the relative carbon distribution obtained with the conventional TPH method and with GC×GC for four soil samples collected from industrial polluted sites; (A) aliphatic hydrocarbons; (B) aromatic hydrocarbons [74].

The review of the opinions expressed in the literature (from the two mentioned examples) reveals that these are not consistent: While one says that GC×GC provides quantitative results for petroleum hydrocarbons comparable with those from 1D-GC; others report that results exhibit statistically significant deviations. Whether differences are considered as significant or not, depends on the reproducibility of the measurement, and thus on the ratio of the observed difference and the standard deviation of the measurement. Hence, at a higher standard deviation, two measurements may become statistically indistinguishable, while with a better measurement precision, these differences would have appeared as significant.

2. Quantitative one-dimensional GC analysis of petroleum hydrocarbons

2.1. Introduction

The determination of total petroleum hydrocarbons or, alternatively, hydrocarbon index in water and soil is one of the important analytical tasks to assess levels of environmental pollution. Clearly, different analytical methods used for the determination of the environmental concentration of petroleum and petroleum products will provide very different information. This is because they are designed to extract and measure slightly different subsets of petroleum hydrocarbons. No single method gives a precise and accurate measurement of the true *total* petroleum hydrocarbon (TPH) concentration for all types of contamination.

GC-based methods are currently the preferred methods for petroleum hydrocarbon measurement because they detect a broad range of hydrocarbons, they provide both sensitivity and selectivity, and they can be used for petroleum hydrocarbon identification as well as quantification. Although most laboratories appear to employ GC based methods for petroleum hydrocarbon determination, however, a study of interlaboratory comparisons by Saari [68] indicates that there is great variation in the GC operating settings which actually are applied in practice. The results show that the GC operating settings (especially injector settings) do have a significant influence on the performance of gas chromatographic systems for petroleum hydrocarbon determination.

Currently, there are two standard methodologies based on GC: DIN ISO 9377-2: 2000 method [49] and the OSPAR (Oslo-Paris commission) reference method [57]. **Figure 2.1** represents the diagram of the analytical procedures proposed by these two standards, which are described in detail in the *Section 1.3*.

Oil in produced water from petroleum installations can be dispersed or dissolved. Dissolved constituents are the more polar components like the aromatics, the acids and the phenols, while the dispersed components are the aliphatic, branched and cyclic apolar components in addition to higher molecular weight aromatics and fatty acids. In the extraction of produced water with an organic solvent, both the dispersed and the dissolved organic compounds are combined. According to the OSPAR definition (and the DIN ISO 9377-2:2000 method) only the non-polar hydrocarbons are defined as oil in water. Most other components are removed in the cleanup process. The TEX compounds are considered and reported separately in the OSPAR method.

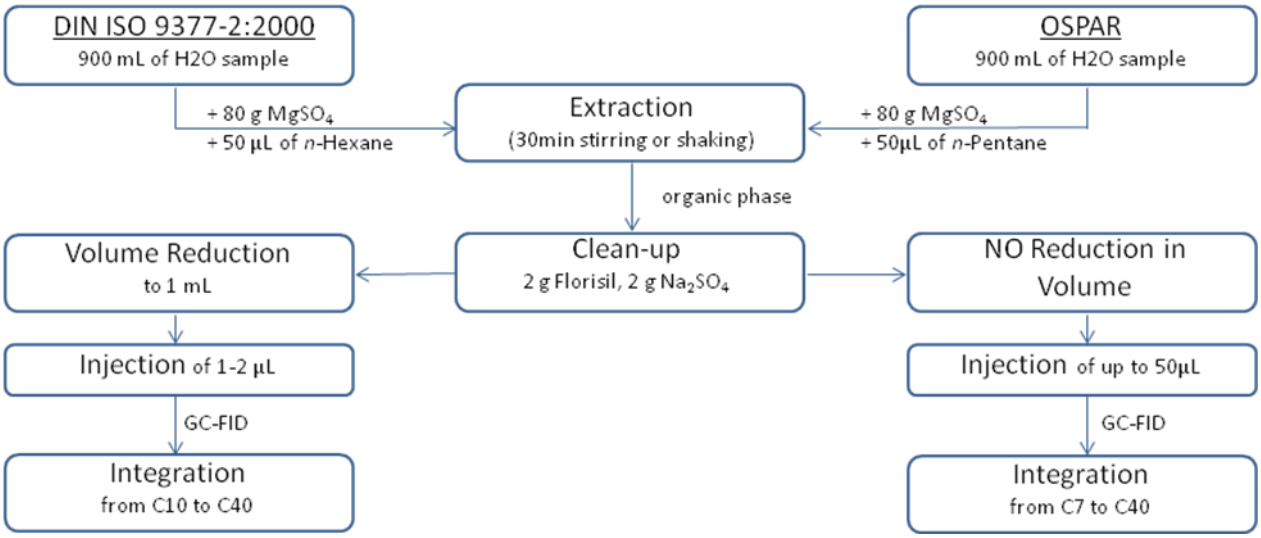


Figure 2.1 Diagram of the analytical procedures proposed by the two standards DIN ISO 9377-2:2000 standard and the OSPAR (Oslo-Paris commission) method for the analysis of petroleum hydrocarbon contaminations in water.

GC based methods are suitable for surface water, waste water and other types of waste water discharged from gas, concentrate and oil platforms and allow the determination of hydrocarbon oil concentration above 0.1 mg/L.

To reach the required detection limit, the method according to DIN ISO 9377-2:2000 foresees preconcentration of the extracts by solvent evaporation. In contrast to this, the OSPAR method does not allow for any external apparatus for preconcentration, for which reason the GC must be equipped with an injection system that allows the injection up to a volume of at least 50 µl of the extract. This is most easily realised with programmed-temperature vaporising-large volume injectors (PTV-LVI). This technique can reduce the loss of volatile analytes, increase sensitivity and is a viable, fast and automated alternative to an external preconcentration procedure. Nevertheless, the PTV-LVI technique requires careful optimisation (*see Chapter 1, Section 1.2 and Chapter 2*). It may also be for this reason that the two standard methods mentioned above do not prescribe fixed conditions of analysis, but rather performance criteria to be met in order for the analytical result to be considered valid.

In this chapter the two GC-based methods (DIN ISO 9377-2:2000 and OSPAR methods) are compared, and parameters for GC with PTV-LVI for the analysis of (total) petroleum hydrocarbons are adjusted. Particular challenges and limitations of the two methods are discussed. In addition to the chromatographic parameters addressed in this study, other important factors of the method were investigated which impact the trueness and precision of the method, such as recovery of the method for different types of oil, losses during the clean-up procedure, and choice of calibration standard. In the present work, it is not intended to (re-)optimise the two standard methods which can be assumed to have already been optimised during their development and validation. Instead, their appropriateness for the analysis of different oil samples and the mutual comparability of the results produced by these two standard methods is critically evaluated. The limitation of such procedural defined methods as to report a

"true" value, as was the case with the now defunct standard for total petroleum hydrocarbon (TPH) determination DIN 38409 H18 is hereby highlighted.

2.2. Experimental

2.2.1. Instrumentation and operating conditions

Analysis using *normal volume injection* was carried out on a 6890A GC system (Agilent Technologies, Palo Alto, CA), equipped with a 7673B automatic liquid sampler and flame ionisation detector (FID) operated at 340°C. Helium (purity >99.999%, Messer, Gumpoldskirchen, Austria) was used as the carrier gas at a flow rate 3 mL/min. Chromatographic separation was achieved on a RTX-5MS 30 m × 0.25 mm ID, 0.25 µm film thickness capillary column, coated with 95% dimethyl-/5% diphenylpolysiloxane polymer (Restek, Bellefonte, PA) and an uncoated 5 m × 0.32 mm ID, deactivated fused silica capillary as precolumn (Supelco, Bellefonte, PA) were used. The GC oven programme was kept at 40°C for 4 min, then increased at 35 °C/min to 340°C and was held for 6 min. Constant temperature splitless injection technique (at 340°C) was used for injections of 1 µL. A liner with a length of 78.5 mm and OD 6.5mm, with single taper and packed with deactivated borosilicate glass wool was used.

The work for *large volume injection* was carried out using an 7890A GC system, (Agilent Technologies, Palo Alto, CA) equipped with a UNIS 2100 large volume injector (Joint Analytical Systems, Moers, Germany), a flame ionisation detector (FID) operated at 340°C and with a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) allowing PTV-large volume injection. Carrier gas, gas flow and the analytical column were the same as in analysis using the normal volume injection. The GC oven temperature programme (for samples prepared in pentane) was kept at 35°C for 6 min, then increased at 50 °C/min to 340°C and was held for 6 min. The optimised parameters for programmed-temperature vaporiser large volume injector for samples in pentane as a solvent are: -20°C (hold 0 min), heated with 720 °C/min to 340°C (hold 15 min). Solvent vent flow is 100 mL/min; Solvent vent time after injection of 10 sec is 0 min; Vent pressure is 10 psi; Purge flow is 100 mL/min; Purge time is 1 min; Injection volume is 50 µL; Injection speed is 5 µL/sec. A liner with 88 mm length and 3 mm ID, packed with deactivated borosilicate glass wool was used.

Instrument control and data evaluation was done with the ChemStation software in both cases. The UNIS injection system is configured and controlled through the Agilent ChemStation software for which there exists an add-on supplied by JAS (Joint Analytical Systems). Limits of detection (LOD) and limits of quantitation (LOQ) were calculated from experimental data according to the calibration method (as outlined in DIN 32645 [191]) by the Excel macro [192] ValiData.

The *fast GC separation* was carried out using a Shimadzu GC2010-Plus gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an OPTIC3-PTV (ATAS, Eindhoven, The Nederland), AOC-5000 Plus (Shimadzu, Kyoto, Japan) and a flame ionisation detector (FID) operated at 340°C. Helium

(purity >99.999%, Messer) was used as the carrier gas at a flow rate 0.57 mL//min at inlet pressure of 354 kPa. The chromatographic separations were achieved on a SLB-5ms 10 m × 0.1 mm ID, film thickness 0.1 µm capillary column, coated with 95% dimethyl-/5% diphenylpolysiloxane polymer (Supelco, Bellefonte, PA). The GC oven initial temperature of 70°C was kept for 2 min, then increased at 40 °C/min to 300°C and at 10 °C/min to 330°C and was held for 9 min (total time 20 min). Constant temperature split injection technique (20:1) at 340°C was used for injections of 1 µL. A fritted liner (OPTIC liner with deactivated glass frit on 15 mm), packed with deactivated borosilicate glass wool was used. Instrument control and data evaluation was done with the GCsolution software. The OPTIC3 high performance injector is configured and controlled through ATAS Evolution Workstation software.

2.2.2. Reagents

n-Pentane puriss.p.a used as a solvent for standard and sample preparation, and Florisil used for clean-up were purchased from Sigma-Aldrich (Vienna, Austria). Alkane standard mixture C10-C40 for performance test of GC-systems in heptane (with each compound at 0.05 mg/mL); *n*-heptane (C_7H_{16}) puriss.p.a. standard for GC ≥99.8%, *n*-decane ($C_{10}H_{22}$) puriss.p.a. standard for GC, eicosane ($C_{20}H_{42}$) puriss.p.a. standard for GC ≥ 99.8% were from Fluka (Buchs, Switzerland). ($C_{40}H_{82}$) with purity 99.5% was from Supelco (Sigma-Aldrich). Toluene, ethylbenzene and *o*-/*p*-/*m*-xylene from Sigma-Aldrich were used. Different types of oil were obtained from the co-operation partner (QuantaRed Technologies GmbH, Vienna).

2.2.3. Standards and samples

BAM-K010 certified reference material for the determination of mineral hydrocarbons with a certified value of 1.00003 mg/mL was used (*Certified reference material for the determination of mineral hydrocarbons*, Bundesanstalt für Materialprüfung, Berlin).

System performance standards: The system performance check is done with a hydrocarbon mixture. Alkane standard mixture of C10-C40 in heptane, each at 0.05 mg/mL, is used as a standard for DIN ISO 9377-2:2000 method. A mixture of C7, C10, C20, C40 and TEX (toluene, ethylbenzene and o-/p-/m-xylene) compounds in pentane, each at 0.005 mg/mL, is used as a standard for OSPAR method.

BAM-K010 certified reference material for the determination of mineral hydrocarbons was a stock solution for preparation calibration standard solutions. Fourteen calibration standards were prepared in *n*-pentane at the levels from 0.1 to 80 mg/L (concentration of oil in water - OIW). The American Petroleum Institute gravity (API) is a measure of the specific gravity of a petroleum liquid compared to water (API 10). Medium oil is defined as having an API gravity between 22.3 API and 31.1 API. A mixture (1:1) of P-Oil (API 29.5) and A-Oil (API 23.9) was employed in order to simulate a typical crude oil. Spiked extraction solvent in different concentrations from 0.1 to 80 mg/L (OIW) was used as a stock

solution for calibration standards. Samples of different types of oil were prepared in duplicate at a concentration of 20 mg/L OIW. One half of the oil samples is cleaned-up through Florisil, while the other is not in order to study the influence of the clean-up step. To prepare standards and the extracts an alternative to the clean-up procedure through the column with Florisil, as prescribed by DIN ISO 9377-2:2000 method was used, where the total extract is shaken in a closed Erlenmeyer flask with 2 g of Florisil and 2 g of sodium sulphate during 30 min on a shaking apparatus. The purified aliquot is analysed by CG. To obtain a more concentrated final extract, samples are preconcentrated first using a rotary evaporator, and then under a stream of nitrogen as proposed by the DIN ISO 9377-2:2000 method to a volume of less than 1 mL, and then made up to a volume of exactly 1.0 mL with *n*-pentane before an aliquot is analysed.

For *fast GC analyses* the BAM-K010 certified reference material and samples of different types of oil were prepared at a concentration of 2 mg/mL.

2.2. Results and discussion

2.3.1. Determination of suitable GC and PTV-LVI parameters

The determination of total petroleum hydrocarbons (TPH), according to the DIN ISO 9377-2:2000 and OSPAR methods, requires that the peaks in the gas chromatogram of the system performance standard mixture of even-numbered *n*-alkanes shall be baseline-separated. Further, the relative response (peak area) of *n*-decane (C10) and *n*-tetracontane (C40) compared with *n*-eicosane (C20) should be at least 0.8 (later C10/C20 and C40/C20). If not, the discrimination of the injection system is too high and the injection system or technique shall be optimised or changed.

To adjust the system for constant temperature small volume injection usually is not a problem. For constant temperature splitless injection the choice of liner and packing material is important. In some studies it is suggested to use adsorbent-packed liners for the retention of very volatile compounds [80,193]. For retaining hydrocarbons in the C10-C40 range, there is no need for an adsorbent-filled liner. A plug of deactivated borosilicate glass wool, inserted in the top half of the liner is sufficient to ensure efficient evaporation and transfer of all hydrocarbons in the investigated volatility range with good recovery. The relative response of C10/C20 is 0.98 and of C40/C20 is 1.01 under these conditions, which perfectly fulfils the DIN ISO 9377-2:2000 standard method's requirements. Sample injections with an empty liner (without glass wool) were performed as well, and demonstrated that the recovery of C40 clearly decreased under these conditions. The glass wool helps to increase the contact area of solvent/analyte gas inside of the liner and results in good recoveries of compounds over a wide range of volatility for small volume injection.

The optimisation of PTV-LVI has to address problems such as injector initial temperature discrimination or loss of early eluting peaks, decreased recovery of late eluting compounds that may occur with unsuitable measurement conditions. The PTV parameters affecting the solvent vaporization and providing the transfer of analytes to the column should be optimised to maximum recovery of the analytes. The optimum values found for programmed-temperature vaporiser large volume injection are given in the experimental part. The recoveries of the components were determined experimentally, normalized peak areas were used for calculation of component recoveries. The system performance test performed under optimised conditions is fully satisfactory with the relative response of C7/C20 being 0.93, of C10/C20 being 0.90 and of C40/C20 being 1.04 which fully complies with the requirements of the OSPAR standard method (**Figure 2.2**).

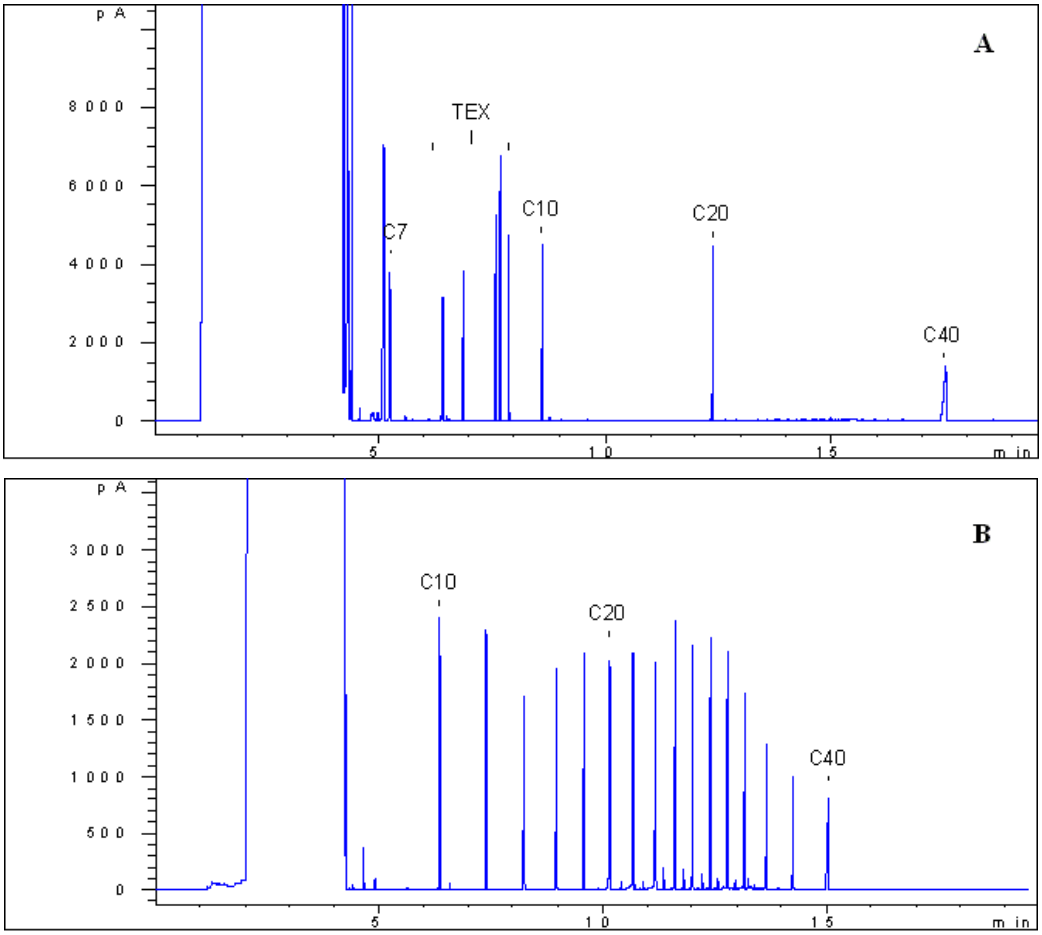


Figure 2.2 Chromatograms of the analysis of system performance test mixtures
(A) mixture of C7, C10, C20, C40 and TEX compounds in pentane, each at 0.005 mg/mL, obtained by the OSPAR method using LVI of 50 µL; relative responses: C7/C20 = 0.93; C10/C20 = 0.90; C40/C20 = 1.04,
(B) *n*-alkane standard mixture C10-C40 in heptane, each compound 0.05 mg/mL, obtained by the DIN ISO 9377-2:2000 method with 1 µL injection; relative responses: C10/C20 = 0.98; C40/C20 = 1.01.

2.3.2. Data evaluation

Due to the particular mode of data evaluation stipulated by the DIN-ISO 9377-2:2000 standard as well as by the OSPAR method, solvent purity and correct background subtraction become a critical issue. In fact, they can be limiting factors for the performance of the GC-based methods. A further important source of variation or error is the integration of a wide chromatographic time window of low signal intensity. While it is evident that the integration of chromatograms with different parameters can lead to varying results, this is so more pronounced for the determination of the hydrocarbon index due to the difficulty of correctly assigning the integration borders for a wide chromatographic signal of low intensity. The standard method specifies that the GC-FID signal is integrated in the time window starting immediately after the C10 peak until the start of the C40 peak at a signal level corresponding to the baseline in front of the solvent peak (*see **Figure 1.1** in Chapter 1, Section 1.1*). Depending on the nature of hydrocarbon contamination, the signal will typically not return to the baseline during the time window of C10-C40. This is both due to the fact that the hydrocarbon mixture may contain a significant fraction of high-boiling compounds with >C40, and of course due to column bleed which is unavoidable even with columns designed particularly for MS detection which show extra low column bleed. While background subtraction can compensate for the latter problem, it cannot account for the fraction of the TPH contamination that elutes outside of the integration window specified by the standard methods. The magnitude of this effect will be discussed further below. Considering that baseline-corrected chromatograms are used for integration, it becomes evident that the baseline has to be established with greatest care. Baseline shifts (e.g., as an effect of column ageing) have to be monitored regularly, and carryover must be prevented by running blank injections after the analysis of highly contaminated samples.

Since it is impossible to find hydrocarbon extraction solvents that are free of any higher homologues, the blank should actually not merely be a column blank, but the background resulting from both column and solvent must be subtracted. For this it is essential that the sample is extracted with a solvent of the same batch as that taken for the background measurement, since batch-to-batch variations of the impurities of typical solvents vary so strongly that quantitative measurements become impossible even at mg/L level concentrations.

Figure 2.3 illustrates the two points discussed just before, where the results are presented for a chromatogram from which *a)* the correct and *b)* an unsuitable background chromatogram (e.g. a background recorded immediately before or after the actual measurement in the former case, and at the beginning or the end of a longer sequence, respectively, in the latter case) were subtracted. “Unsuitable” in this context means that the background chromatogram has been recorded either with a solvent from a different batch or producer, or even with a solvent of the same batch, however, with a longer interval between the recording of the sample and the background. The difference may not be large for samples with a high concentration of oil, but it can be significant for low concentrations, due to the possible occurrence of negative signals in the subtracted chromatogram, which generally is problematic for

integration, and also falsifies the area sum value of the integrated signal. The reason for negative signals in the blank-subtracted chromatogram can not only be the selection of an unsuitable background chromatogram for subtraction, but also small shifts in the retention times of individual peaks. The subtraction of the two chromatograms then leads to a difference chromatogram with negative spikes of differential signal appearance at the position of large discrete peaks. As a more suitable alternative to the integration of the difference chromatograms as proposed in the DIN ISO 9377-2:2000 standard it is therefore proposed not to integrate the blank-subtracted chromatogram, but instead to integrate each chromatogram (blank and sample) separately in the integration borders stipulated by the standard, and to then subtract the area value of the blank chromatogram from the sample chromatogram. This method of background correction is clearly more robust than the initially proposed method and was used throughout in our experiments.

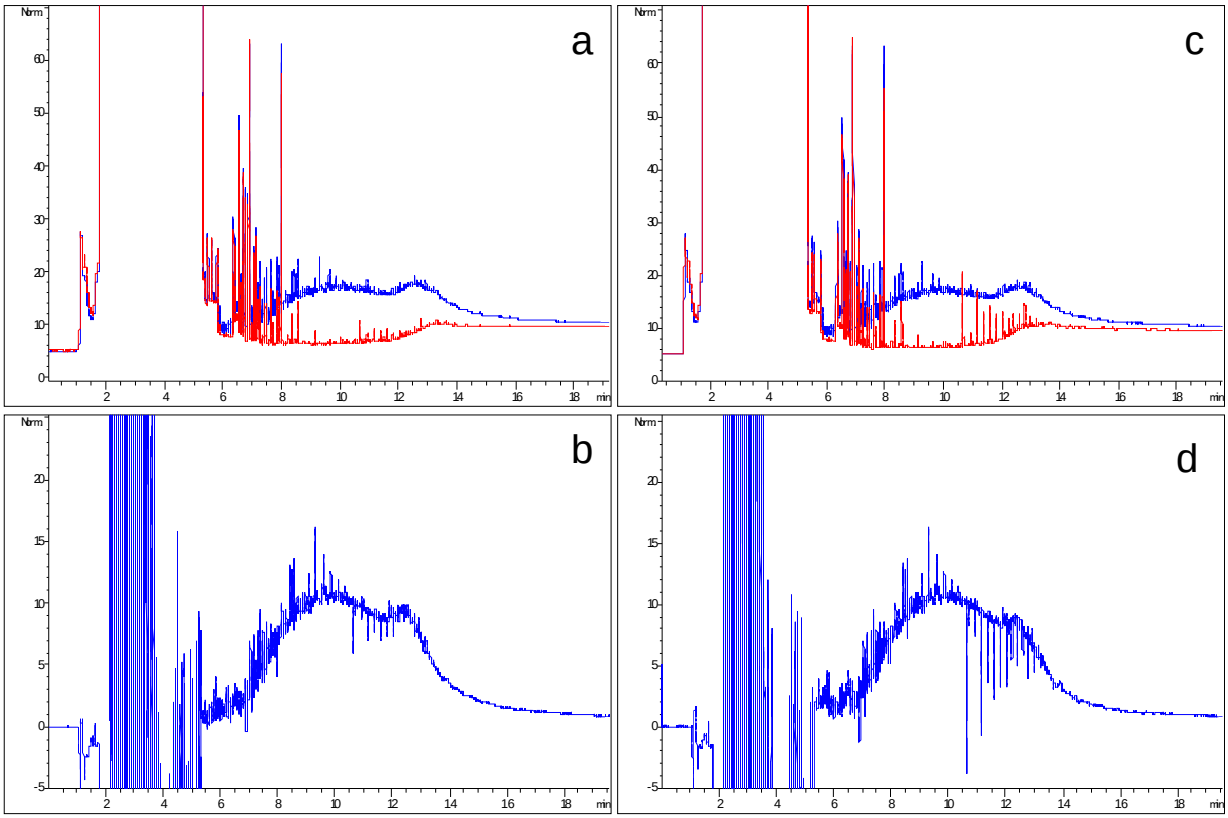


Figure 2.3 Chromatogram of A/P-Oil (1:1) with concentration 12.5 mg L^{-1} in water obtained according to DIN ISO-9377-2:2000 method (in blue), overlayed with *a)* a suitable and *c)* an unsuitable background. chromatogram (e.g. a background recorded much before or after the actual measurement; in red). The two chromatograms below are the difference chromatograms obtained by the subtraction of *b)* a suitable and *d)* an unsuitable background chromatogram, clearly identifiable from the large number of significant negative spikes in the difference chromatogram in graph *d)*.

2.3.3. Comparing the DIN ISO 9377-2:2000 and the OSPAR standard methods for total petroleum hydrocarbon (TPH) determination

The two standardized methods for the determination of total petroleum hydrocarbons in water, DIN ISO 9377-2:2000 and OSPAR, were compared under the optimised conditions presented above. The methods were compared on the basis of the analysis of mixtures of standards and different types of oil. Both methods show a linear response over the investigated range. The linearity range for constant temperature splitless injection (DIN ISO 9377-2:2000 method) was 0 – 80 mg/L of oil in water (OIW), and thus the same as for the PTV-LVI (OSPAR) method. The relative standard deviation (RSD) varies between 0.8% and 12.7% for DIN ISO 9377-2:2000 method, and between 1.0% and 7.7% for OSPAR method at different concentration levels. The limit of quantification (LOQ) of DIN ISO 9377-2:2000 method after preconcentration is 0.02 mg/L, while without preconcentration step it is 0.46 mg/L OIW (**Table 2.1**). The LOQ of the OSPAR method is 0.06 mg/L OIW, thus proving that the PTV-large volume injection technique is a suitable alternative to an external preconcentration procedure.

Table 2.1 Comparison of LODs and LOQs of DIN ISO 9377-2 and OSPAR method for BAM standard and an authentic oil mixture (A/P-Oil) used as a calibration standard. OIW: oil in water.

		Linearity range	R^2	Equations of calibration lines	Limit of Detection (LOD)	Limit of Quantitation (LOQ)
					OIW, mg/L	OIW, mg/L
ISO with preconcentration	BAM	0-50 mg/L	0.9998	y = 18013.4x + 69.3	0.01	0.02
	A/P-Oil	0-50 mg/L	0.999	y = 11398.5x + 234.4	0.01	0.04
ISO without preconcentration	BAM	0-80 mg/L	0.9993	y = 360.6x + 55.5	0.13	0.46
	A/P-Oil	0-80 mg/L	0.9994	y = 284.2x + 16.3	0.14	0.49
OSPAR	BAM	0-80 mg/L	0.9998	y = 20768.1x + 122.0	0.02	0.06
	A/P-Oil	0-80 mg/L	0.9999	y = 14404.0x + 198.3	0.03	0.09

While it is typically expected that the limit of detection proportionally improves with the amount or volume of sample introduced into the GC, this surprisingly is not the case with the OSPAR method to the expected extent: It turned out in our experiments that with the injection of even larger volumes of sample no gain in sensitivity could be realized: the noise level and interferences from the solvent blank increase with the injection volume and become the limiting factor in the detection of low petroleum hydrocarbon concentrations. As a consequence, the detection limit cannot be proportionally decreased by simply enlarging the injection volume.

It is clearly seen from the equations of the calibration lines given in **Table 2.1** for the BAM-standard and a mixture of A/P-Oil (1:1) that there is a significant difference in the response between these two sets of calibration samples. The BAM-standard which is a synthetic mixture of a diesel and a

lubricating oil shows 37% greater response than the standard of A/P-oil if samples are analysed by the DIN ISO 9377-2:2000 method and 30% - if samples are analysed by the OSPAR method.

Figure 2.4 represents the response ratio of the OSPAR method in relation to the method according to the standard DIN ISO 9377-2:2000. It is seen that while for most of the investigated samples, the response ratio of the two methods is close to 1 (or 100%) when the response relative to the BAM standard is used as common reference point for the two methods, there is a certain fraction of oils for which this response ratio is very significantly deviating from unity. The figure reveals that there are two, or perhaps even three different classes of hydrocarbon mixtures: The first group of hydrocarbon mixtures produces response ratios close to 100% and is the majority of investigated samples. Three further samples exhibit already significantly increased response ratios in the range of 140-180%. Then there is a group of three samples that have excessively high response ratios of more than 500%. This behavior can easily be interpreted when the particular conditions of measurements of the two methods being compared are taken into consideration: The DIN ISO method only considers the volatility range from C10 to C40 while the OSPAR method also includes the more volatile fraction of C7 to C10. This automatically leads to a relative attenuation of the results obtained for oils with a larger fraction of higher boiling compounds (C10-C40) which represent only a sub-fraction of the amount of petroleum hydrocarbons determined according to the OSPAR method (C7-C40). This is reflected by attributing to the majority of the heavier oils investigated here relative response factors of 100-130% , while for the volatile fuels with a higher content of <C10 hydrocarbons (normal and premium gasoline, and also Troll Condensate) the OSPAR/DIN ISO response ratios become very large, in the order of 520-820%.

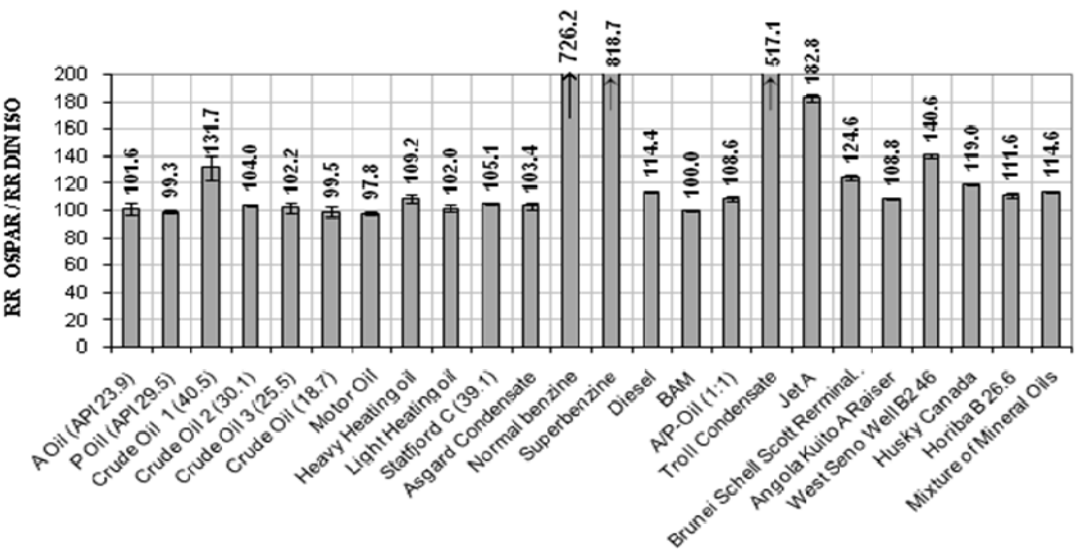


Figure 2.4 Relative response ratios (in %) of the results obtained with the DIN ISO 9377-2:2000 and the OSPAR standard methods for different oil samples (error bars represent ±1 standard deviation and are calculated from 3 measurements).

2.3.4. Dependence of response on oil types

The primary advantage of GC-based methods over e.g., a gravimetric method is that they do not only provide quantitative information, but also qualitative information which in the ideal case even allows the assignment of the source of contamination [194]. Identification of product type(s) is not always straightforward, however, and requires an experienced analyst of petroleum products. It is clearly seen from the difference in the slopes of the calibration curves that results based on the calculation with one particular standard (even if the BAM-standard, as suggested by DIN ISO 9377-2) do in general not provide a good estimate of the true concentration of oil in water for samples of different origin. This is proven by the comparison of chromatograms of different oil samples (**Figure 1.2** *in Chapter1, Section 1.1)*. Even for equal amounts of OIW (on the basis of their gravimetric preparation) qualitatively and quantitatively strongly differing results are obtained for the different types of oil.

Highly different responses/recoveries will result when different types of oil are analysed and only one type of oil is used as calibration standard for all samples (**Figure 2.5**). The recoveries or specific responses of real samples are always lower than the specific response of the BAM standard, and range from as low as 8% (for petrol fractions, analysed by DIN ISO 9377-2:2000 method) to ca. 95% (e.g., for diesel, analysed according to the OSPAR method). In contrast to this, the results for the response/recovery exceed 90% for all tested oil types (except heavy heating oil, due to a large loss in the clean-up step) when the same oil type is used for calibration as will be determined in the subsequent measurements.

The reason for this problem in quantification arises from the definition of the 'hydrocarbon index' according to the two standards quoted earlier, which is not equivalent to the amount of 'total petroleum hydrocarbons' nor to the gravimetric preparation of standards and samples, which does not take into account only the hydrocarbons eluting between C10 and C40, but all compounds present in the sample. Since, however, the fraction of hydrocarbons in an oil sample that elute in the chromatogram before C10 or after C40 and thus are disregarded may differ from type to type of oil, and may also be significant, a large and systematic deviation between the concentration of 'total petroleum hydrocarbons' and the 'hydrocarbon index' results. While for the analysis of one particular type of oil the results obtained with the two strategies should at least correlate, the GC method based on the DIN ISO 9377-2:2000 standard clearly fails to produce data that are comparable between different types of oil.

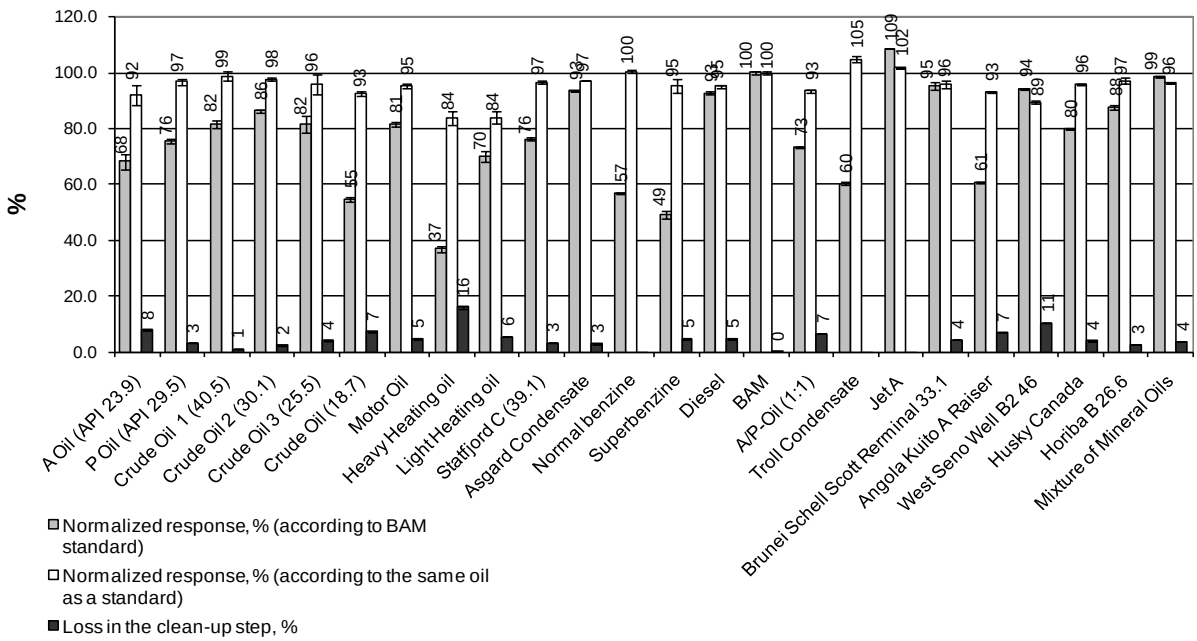


Figure 2.5 Normalized response (in %) for water samples spiked with different types of oil, based on calibration being performed either with the BAM standard (grey bars) or with the same type of oil (white bars) used for calibration and for the determination of the loss in the clean-up step (black bars), obtained by OSPAR method. Relative standard deviation (RSD) of repeated determinations varied between 0.1% and 4.0%.

The largest part of this bias is likely to be removed when standards are available for calibration of the same type of oil.

To support this statement, recovery data for seven different types of oils, analysed according to the DIN ISO 9377-2 and OSPAR methods are presented in **Table 2.2**, along with literature data [195,196] on the composition of these oils according to boiling range. It is clearly seen from these data that the recovery of the two methods is heavily depending on the boiling point range of the particular type of oil. The first oil in this table, Statfjord C (API 39.1) has both a significant fraction of low boiling constituents (<C7: 11.6% w/w) and high boiling constituents (>C40; 13.7% w/w). This is thus consistent with recoveries of only 72.6% according to the DIN ISO method and somewhat higher response according to the OSPAR method (relative to BAM standard). In the case of Crude Oil (API 18.7) Grane, the fraction of volatile and highly volatile compounds is relatively small (<C7: 0.9 and C7-C10: 3.0% w/w) while the fraction of high boilers (>C40: 32.8% w/w) is very significant. This explains why both standard methods only report ca. 55% related to BAM standard, and why the results of the two methods are in this case very well comparable, as the oil has only a small fraction of constituents in the C7-C10 range. The differences become very pronounced when the quantitative analysis of normal benzine (containing about 100% C5-C12) is attempted with these methods. While the OSPAR method still recovers ca. 57%, the DIN ISO method clearly fails to produce meaningful results with only ca. 8% recovery (w/w, related to BAM standard). The other extreme is the analysis of heavy heating oil whose boiling range only starts from 350°C (corresponding to C20). In this case, a large fraction of the sample elutes after the retention time of

C40, and thus the DIN ISO and the OSPAR method recover only ca. 34 and 37% w/w, related to BAM standard.

Table 2.2 Recovery data of different types of oil analysed according to the DIN ISO 9377-2 and OSPAR and comparison of these oils according to boiling range with literature data**.**

	Fractions				% Recovery	
	<C7	**C7 - C10**	**C10 - C40**	**C40<**	**DIN ISO**	**OSPAR**
	(%w/w)	(%w/w)	(%w/w)	(%w/w)	relative to BAM	
Statfjord C (39.1)[a]	11.6	18.1	56.6	13.7	72.6	76.3
Crude Oil (API 18.7) Grane[a]	0.9	3.0	63.2	32.8	55.0	54.7
Normal benzine[b]	~100 (C5-C12)				7.8	56.9
Diesel[b]		~100 (C10-C22)			81.0	92.7
Light heating oil[b]		~100 (C10-C22)			78.4	80.0
Motor oil[b]			~100 (C20-C35)		81.5	83.3
Heavy heating oil[b]				~100 (C20-C5(	33.9	37.0

a: [185], b: [186]

2.3.5. Chromatographic efficiency of the GC method for petroleum hydrocarbon determination (Fast GC analyses)

The results presented in *Section 2.3.4* demonstrate that the application of both the DIN ISO and the OSPAR methods to different types of oil does not yield a uniform relative response. Moreover, the recovery of the two investigated standard methods is strongly depending on the type of oil which is attributable to different integration borders. Both methods stipulate the integration of the unresolved signal up to the retention time of the *n*-alkane with C40. As the composition of oils is very different, many of them contain a substantial fraction with carbon number larger than C40. In order to check how much of the heavier fraction is not accounted for by the integration borders of C7 (or C10, respectively) to C40, the integration border must be extended to at least C50. This procedure could also help in evaluating the negative bias of the limited integration window for the TPH results obtained by one of the standard methods. Using the conventional GC separation with a column of 30 m × 0.25 mm and a maximum operation temperature of 350°C, the only way to extend the integration border is just to extend the analysis time at the final oven temperature and to have an isothermal phase of the separation of the hydrocarbons after the elution of C40. This makes the total analysis time very long. To overcome this problem Fast GC separation can be applied.

The primary aim of Fast GC is to maintain (compared to conventional GC) sufficient resolving power in a shorter time. Fast GC uses column and instrument improvements in combination with optimised run conditions to provide 3- to 10-times faster analysis times, while still providing acceptable

resolution. Fast GC is typically performed using short, 0.10 mm or 0.18 mm I.D. capillary columns with hydrogen carrier gas and rapid oven temperature ramp rates [197].

The use of a short column with small dimensions provides not only a fast separation, but also a better transfer of all analytes, especially the less volatile ones, to the detector, thereby improving their quantitative recovery. In this study, a SLB-5ms capillary column of 10 m and with ID of 0.1 mm (manufactured by Supelco, Bellefonte, ÜA) was used for the analysis of different oils. Helium was used as the carrier gas at a flow rate of 0.57 mL/min and at an inlet pressure of 354 kPa. The GC oven program was as follows: 70°C (2 min), then with 40°C/min to 300°C and further with 10°C/min to 330°C and hold time 9 min. As a result the analyses time was not as fast as would normally be expected for Fast GC – a total run time of 20 min was chosen –, but this time was enough for the elution of hydrocarbons until approximately C50, and allowed thus extending the integration borders.

BAM standard and samples of different types of oil before and after clean-up were analysed by Fast GC. Integration was extended for some samples until C43 (because the signal returns to baseline before C43 elutes), and for some others like motor oil and heavy heating oil even until C50, **Figure 2.6**. For samples like BAM standard and diesel there was no need to extend integration borders, because these oils have a composition which hardly contains any compounds higher boiling than C40 and the chromatographic signal that is to be integrated returns to baseline much earlier than C40. The relative responses/recoveries of the analysed oil samples before and after clean-up by the described Fast GC method and after extending the integration range are summarized and presented in the **Table 2.3**.

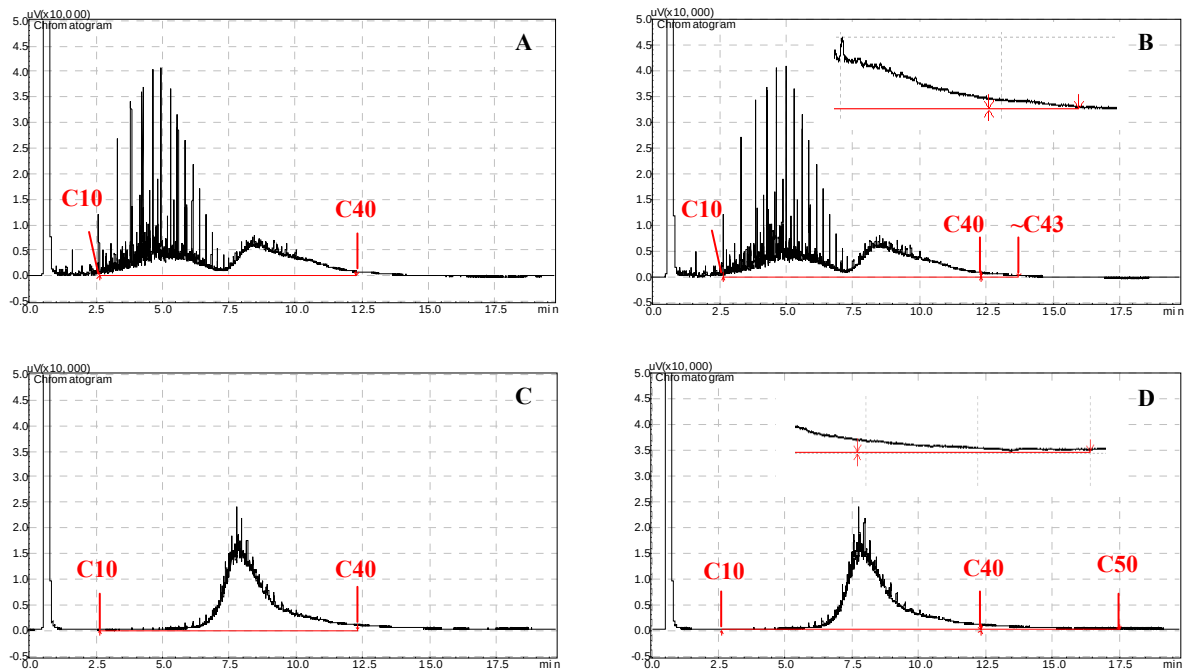


Figure 2.6 Integration of (A) mineral oil mixture and (C) motor oil chromatograms between C10 and C40 according to DIN ISO 9377-2:2000 method; and extension of the integration window of (B) mineral oil mixture chromatogram between C10-C43 and (D) motor oil chromatogram between C10 and C50. The inserts in figures (B) and (D) are magnified views of the tail of the chromatographic signal between the elution of C40 and the individually selected integration borders.

Table 2.3 Recoveries (R) before and after clean-up if the integration in an extended region is used.

Oil type	Integration borders	Recovery after clean-up, %	*RSD, %*	loss in integration, %	Recovery before clean-up, %	*RSD, %*	loss in integration +loss in clean-up, %
BAM							
	C10-C40	100	*0.1*	0	100	*1.1*	
Light heating oil							
	C10-C40	**74.3**	*1.1*	0.3	**81.3**	*0.4*	7.7
	C10-C43	**74.6**	*0.9*		**82.1**	*0.8*	
Motor oil							
	C10-C40	**82.2**	*1.2*	3.3	**88.1**	*1.8*	11.3
	C10-C50	**85.5**	*2.6*		**93.5**	*1.9*	
Diesel							
	C10-C40	**91.2**	*1.9*	0	**96.3**	*1.3*	5.1
A-/P-Oil							
	C10-C40	**73.2**	*2.3*	0.4	**86.7**	*1.3*	15.2
	C10-C43	**73.7**	*1.6*		**88.5**	*1.9*	
Heavy heating oil							
	C10-C40	**36.4**	*1.3*	2.6	**47.0**	*0.2*	18.7
	C10-C50	**39.0**	*0.3*		**55.1**	*2.5*	
Crude Oil API 40.5							
	C10-C40	**68.9**	*2.0*	1.1	**76.0**	*0.6*	7.9
	C10-C43	**70.0**	*2.5*		**76.8**	*0.3*	
Crude Oil API 18.7							
	C10-C40	**56.8**	*0.7*	1.9	**62.0**	*0.5*	8.2
	C10-C43	**58.6**	*0.8*		**64.9**	*0.3*	
Mineral oil mixture							
	C10-C40	**86.3**	*1.1*	1.1	**98.4**	*1.1*	12.4
	C10-C43	**87.5**	*1.4*		**98.8**	*2.0*	

If one compares the recoveries of all investigated samples with the integration in the chromatograms within different intervals, i.e. using standard integration between C10 and C40 according to DIN ISO 9377-2:2000 method and using the extended integration between C10 and C43 (of C50 depending on the sample), then the difference found in relative response/recoveries values is between 0 (for BAM standard and diesel) and 3.3% (for motor oil) as maximum. That allows to conclude that the loss in integration for the investigated samples is not so big that it could explain the different relative responses of different types of oil.

2.3.6. Evaluation of clean-up step

All common analytical methods for total petroleum hydrocarbon determination make use of a clean-up step. This is necessary to allow reliable measurements of TPH concentrations even with completely unselective techniques such as gravimetry, or with techniques that are only compound class selective, such as the IR-absorption method. Without appropriate clean-up, the latter method would also include any other type of CH-containing compound, irrespective of whether it falls under the chemical class of petroleum hydrocarbons or not.

The GC-based method also involves a clean-up step using Florisil and sodium sulphate (anhydrous) which essentially aims at removing the polar, oxygen or nitrogen-containing compounds of biological origin (which are not counted among the hydrocarbons in the strict sense) and remaining traces of water. This is both a protection for the GC column and also removes interfering compounds which otherwise would be also detected together with the TPH compounds. Moreover, it has to be stated clearly that the "hydrocarbon index" determined according to standard DIN ISO 9377-2:2000 is an operationally defined parameter, namely defined by the particular extraction, clean-up, separation and detection procedures proposed in this standard. The impact of the extraction and clean-up steps on the yield of total petroleum hydrocarbons was already evaluated for soils [53,198]. However, although it is known that the Florisil clean-up affects the recovery of the petroleum hydrocarbons in general, similar studies have not been undertaken for the impact of theses steps on the recovery of oil from water samples. Hence, neither the magnitude of this effect has been tested, nor is it known how big the differences are among a wider range of different oils.

It was therefore considered necessary to determine this loss for a variety of different oils. The results of this experiment are reported in **Figure 2.5**. It can be seen that the losses of hydrocarbon (evaluated from the total peak area of the samples, integrated between the C7 and C40 peaks) are up to 16% (note that the DIN ISO 9377-2:2000 standard classifies the recovery as "acceptable" as long as it is above 80%). The recovery of sample from the clean-up step clearly depends on the actual type of oil sample. This can be explained by the different composition of light, medium and heavy oils which also contain different fractions of oxygenated and polar compounds that are removed by the Florisil clean-up step. It can thus be concluded that there will always be a sample specific bias between the 'true' TPH value (if known, as in the case of a synthetic sample), and the result produced according to the DIN ISO 9377-2:2000 standard. As the OSPAR methods relies on the same clean-up step prior to analysis, this comment and shortcoming applies to it equally. Interestingly, the difference between the results for measurement with and without clean-up is negligible for the BAM standard (**Table 2.4**).

Table 2.4 Relative response ratio for the analysis of A/P-Oil at different concentration levels, and of BAM standard, measured with and without clean-up over Florisil (peak areas are the average from 3 determinations).

	OIW, mg/L	with clean-up		without clean-up		Rel. Ratio between response with and w/o clean-up
		Area	RSD, %	Area	RSD, %	
A/P-Oil	10	2678	2.0	2846	1.5	0.94
	20	5429	1.0	5904	0.5	0.92
	30	8191	0.4	8566	1.0	0.96
	40	10640	1.5	12217	1.6	0.87
	50	13068	0.2	15112	0.1	0.86
	60	16144	0.5	18584	0.3	0.87
	70	18432	1.0	21660	0.4	0.85
	80	21863	0.8	24216	0.8	0.90
				average:		0.90
BAM standard	30	12162	0.8	12185	1.2	1.00
	50	20566	0.1	20649	0.6	1.00

Furthermore, it appears that these effects are not depending on the concentration level. A study was performed for a mixture of A/P-oil measured at various concentrations (10-80 mg/L OIW) in which the results were compared for analyses with and without clean-up step. It turned out that for this particular type of oil, the ratio of the results from the two variations of the method were around 90% with only small statistical scatter (**Table 2.4**).

2.4. Conclusions

In a comparative study of the DIN ISO 9377-2:2000 and OSPAR standard methods for the determination of the "hydrocarbon index" their general feasibility for the determination of oil-in-water down to the sub-mg/L concentration range is demonstrated. The OSPAR method is inherently more sensitive due to the preconcentration of large volumes of sample (up to 50 µL) directly in the liner, while with the DIN ISO 9377-2:2000 method, this preconcentration must be done prior to analysis, if higher sensitivity is required. The OSPAR method is thus not only more efficient as it is faster, but also as it avoids potential losses of analyte as the preconcentration step is done automated and in-situ. The results of the two methods correlate well for individual types of oil, although there is a significant method-and oil-type specific bias. This can actually be explained by the fact that the OSPAR method has a wider time window for integration than the DIN ISO 9377-2:2000 method. For this reason, the results obtained with these two methods will hardly ever be identical, and even rarely be comparable in terms of absolute concentrations. They also never will provide the "total petroleum hydrocarbon" value, as may perhaps be known for synthetic standards from the gravimetric preparation. Both methods will inevitably lose those parts of the sample which are too volatile or too involatile at the beginning and at the end of the chromatogram and thus are outside of the integration borders set by the individual standard. For this reason, it must be understood that the "hydrocarbon index" is, as an operationally defined parameter, only an indicator value [199]. It can be expected that the hydrocarbon index and the total petroleum hydrocarbon (TPH) value should be strongly correlated for one particular type of oil; however, for oils very different in their composition this may not generally be so. The hydrocarbon index will thus be a good indicator of the (magnitude of the) relative contamination of oil in water; however, it will not be suitable to give a true representation of the actual concentration of total petroleum hydrocarbons in the water sample.

3. PTV-LVI optimisation

3.1. Introduction

Programmed-temperature vaporiser (PTV) injection is an attractive injection technique, which allows injection of larger sample volume and thus achieving significantly improved detection limits, without loss of volatile analytes. It may be applied instead of or in combination with an external preconcentration procedure in order to reach the detection limits stipulated by environmental legislation. The PTV technique is widely used in LVI applications. There are a large number of applications of gas chromatographic analysis with PTV-LVI in different fields, e.g. polyaromatic hydrocarbons, polybrominated diphenyl ethers, herbicides, pesticides [78,200,201], including the determination of total petroleum hydrocarbons (TPH) [72,73], The benefit of PTV consists in the fact that it allows to introduce a large amount of solvent which is efficiently eliminated during or after sample introduction. Due to the number and complexity of the operating parameters, PTV-LVI requires thorough optimisation of its relevant parameters.

While the efforts of Miñones Vázquez *et al.* [72] were already paving the way for an efficient PTV-LVI method for the analysis of petroleum hydrocarbons, an analytical method for the quantitative determination of petroleum hydrocarbons in water or soil samples must satisfy more stringent criteria and cover a wider range of sample constituents in order to comply with the accepted standards for the determination of total petroleum hydrocarbons (e.g., DIN ISO 9377-2:2000 or OSPAR method). For the latter, the analytical method must allow the quantitative determination of hydrocarbons from C7 to C40. In the present study I will focus on PTV-LVI parameter optimisation for the quantitative and unbiased determination of hydrocarbons in the range from C7 to C40 which significantly extends the range of applicability of this method.

The first part of this section reports the optimisation of parameters for GC with PTV-LVI for the analysis of petroleum hydrocarbons according to the OSPAR method. The effect of each parameter on the performance of the method (notably, the absolute and relative response (*i.e.*, discrimination) of all compounds of interests) is carefully investigated and discussed. The optimised parameters were applied for the analysis of oil samples; linearity, precision and limits of detection and quanitation of the method are presented as well.

In the second part of this chapter, further important factors for PTV operation, such as choice of the liner design and sample introduction speed of LVI, are investigated.

The initial work and optimisation of the the PTV injector parameters were performed for *n*-pentane as a sample solvent. In a subsequent step, an algorithm was developed to transfer operating conditions to other nonpolar solvents without the need of tedious re-optimisation for each new solvent. The background of this procedure is to introduce the solvent at the rate it is concurrently eliminated,

which is determined on the base of its vapor pressure at the conditions of sample introduction. The performance of the method when using different solvents is compared on the base of relative response of a set of *n*-alkanes as analytes.

3.2. Experimental

3.2.1. Instrumentation and operating conditions

The work was carried out using a 7890A GC system, (Agilent Technologies, Palo Alto, CA) equipped with a UNIS 2100 temperature-programmable vaporising inlet (Joint Analytical Systems, Moers, Germany), a flame ionisation detector (FID) operated at 340°C and with a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) allowing PTV-large volume injection. The relevant parameters of the UNIS 2100 temperature-programmable vaporising inlet for solvent vent mode are the following: *Inlet temperature* and *Oven temperature* are the inlet and oven initial temperatures, respectively; *Inlet initial time* is measured from start run, when the initial inlet temperature hold ends, it is usually greater than vent end time; *Vent pressure* is the inlet pressure during the vent period, with a range of 0 to 100 psi, if 0 is chosen, the inlet uses the lowest pressure possible at the given vent flow; *Vent flow* of carrier gas exits from the split vent during the vent period; *Vent end time* measured from start run, when solvent venting ends; during sample introduction the split vent is open, if vent end time 0 min is chosen, the solvent is vented only until the injection is completed, this time depends on injection speed; *Purge time* measured from start run, must be greater than vent end time.

Helium (purity >99.999%, Messer) was used as the carrier gas at a flow rate of 3 mL/min. The chromatographic separations were achieved on a RTX-5MS 30 m × 0.25 mm ID, film thickness 0.25 µm capillary column, coated with 95% dimethyl-/5% diphenylpolysiloxane polymer (Restek, Bellefonte, PA), connected to an uncoated 5 m × 0.32 mm ID, deactivated fused silica capillary as precolumn (Supelco, Bellefonte, PA). The GC oven initial temperature depends on the solvent and it was kept (for samples in *n*-pentane) at 35°C for 6 min, then increased at 50 °C/min to 340°C and was held for 6 min. The optimised parameters for programmed-temperature vaporiser large volume injection for samples in *n*-pentane as a solvent are: -20°C (0 min), heated with 720 °C/min to 340°C (15 min). Solvent vent flow: 100 mL/min; Solvent vent time: 0 min; Vent pressure: 10 psi; Purge flow: 100 mL/min; Purge time: 1 min; Injection volume: 50 µL; Injection speed: 5 µL/sec. A liner with 88 mm length and 3 mm ID, 1 ring restriction (denoted here and in the following as Liner N1), packed with deactivated borosilicate glass wool was used. Instrument control and data evaluation was done with the ChemStation software. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated from experimental data according to the calibration method (as outlined in DIN 32645 [49]) by the Excel macro ValiData [192].

3.2.2. Reagents

n-Pentane, Cyclopentane, *n*-Hexane, Cyclohexane and *n*-Heptane puriss.p.a used as a solvents were purchased from Sigma-Aldrich (Vienna, Austria). *n*-Heptane (C_7H_{16}) puriss p.a. standard for GC ≥99.8%, *n*-decane ($C_{10}H_{22}$) puriss.p.a. standard for GC, eicosane ($C_{20}H_{42}$) puriss.p.a. standard for GC ≥ 99.8% were from Fluka (Buchs, Switzerland). *n*-Tetracontane ($C_{40}H_{82}$) with purity 99.5% was from Supelco (Sigma-Aldrich).

3.2.3. Standards

System performance standards: The system performance check is done with a hydrocarbon mixture of C7, C10, C20, C40 compounds in *n*-pentane, each at 0.005 mg/mL is used. BAM-K010 certified reference material for the determination of mineral hydrocarbons with a certified value of 1.00003 mg/mL (*Certified reference material for the determination of mineral hydrocarbons*, Bundesanstalt für Materialprüfung, Berlin) was used as a stock solution for preparation calibration standard solutions. Fourteen calibration standards were prepared in *n*-pentane at the levels from 0.1 to 80 ppm (concentration of oil in water - OIW).

3.3. Results and discussion

Quantitative determination of petroleum hydrocarbons, according to the DIN ISO 9377-2:2000 and OSPAR methods, requires that the peaks in the gas chromatogram of the system performance standard mixture of even-numbered *n*-alkanes shall be baseline-separated. Further, the relative response (peak area ratio) of *n*-decane (C10) and *n*-tetracontane (C40) compared with *n*-eicosane (C20) should be at least 0.8. If not, the discrimination of the injection system is too high and the injection system or technique shall be optimised or changed. It has been already shown how the variation in GC splitless injection settings affects the accuracy of quantitative petroleum hydrocarbons results, which indicates that the injection parameters must be careful optimised [68].

When optimising the determination of petroleum hydrocarbons using LVI-PTV, not only the avoidance of sample discrimination (inhomogeneous relative response) must be a point to be considered, but also the maximization of the absolute response which is decisive to reach lower limits of detection and quantitation. A good separation of *n*-heptane (C7) peak from the *n*-pentane solvent peak in the chromatogram is necessary to allow the integration of the cumulative peak area of compounds eluted between the retention times of *n*-C7 and *n*-C40, as required by the OSPAR method. Clearly, the most critical components in the optimisation of PTV large volume injection are the more volatile solutes. Volatile components are easily lost during the elimination of the solvent. If these components have to be analyzed, the solvent vent conditions have to be chosen carefully.

The recoveries of the components were determined by means of the calculation of normalized peak areas and relative response of hydrocarbons injecting 50 µL of the system performance standard. Since the OSPAR method requires the demonstration of the analytical system's capability of injecting up to 50 µL of sample, this volume was chosen for all experiments in this work. The following parameters with regard to UNIS 2100 large volume injector and CombiPal autosampler were varied to optimise the method for determination of petroleum hydrocarbons using the PTV-LVI technique: initial column temperature, initial inlet temperature, inlet starting time, sample introduction rate (injection speed), solvent vent time, solvent vent pressure and flow, and purge time. According to the obtained results all variables studied have an effect. In **Figure 2** mean absolute responses of C7, C10, C20 and C40 under different PTV conditions are demonstrated and compared.

3.3.1 Effect of PTV-LVI parameters on method performance

The initial **column temperature** has a great importance for peak shape and resolution over the GC run. Introduction of the sample into the column at a temperature above the solvent boiling point can lead either to a loss of highly volatile compounds through the vapor exit together with the solvent vapor or to a coelution with the solvent, contrariwise introduction below the solvent boiling point can lead to solvent recondensation at the beginning of the analytical column, resulting a peak splitting in case of large volume injection [115]. In the present study there is an interest to retain early eluting peaks, such as *n*-heptane, to achieve a good resolution from the solvent peak and to recover quantitatively all hydrocarbons in the range C7-C40. Therefore with LVI the largest fraction of the solvent should be vented beforehand from the inlet and only the smaller rest together with the analytes be transferred to the analytical column, ideally without loss of the analytes. According to Grob the oven temperature ideally must be above the pressure-corrected boiling point of the solvent, in order to avoid preconcentration of the solvent in the column, but below the boiling point of the analyte, so that the analytes will be retained [202]. In our experiments, the temperature was set to 20, 30, 35 and 50°C. When the oven temperature was 50°C, which is corresponding to a vapor pressure of *n*-pentane of 159.3 kPa, losses of more volatile analytes occurred. In this way 20-35°C was proven experimentally to be the optimum initial oven temperature. These conditions maintain a good resolution between solvent (*n*-pentane) and C7 peaks and ensure the transfer of all hydrocarbons in the standard solution to the capillary column with reasonable responses relative to C20. Despite the fact that at 20°C the absolute response of peaks (C7, C10 and C20) is approximately 7-15% higher, this setting was not chosen as an optimum for oven temperature. This is both for performance reasons (since at this initial temperature incomplete recovery of C40 (with a ratio C40/C20 = 0.91 was observed), but also for practical reasons since at this temperature cryogenic cooling will be required to keep the GC oven at the temperature 20°C, thereby consuming large amounts of liquid nitrogen.

PTV-LVI as used in this study is a preconcentration technique of the sample solution performed directly in the injector, where the solvent is completely or partially evaporated through a vent. The evaporation temperature, which is the initial **inlet temperature**, is one of the important parameters to be considered in the optimisation procedure. In our experiments temperature settings of -20, 0, 20 and 35°C were tested. As it is seen in **Figure 3.1**, increasing the inlet temperature reduces the recovery of volatile compounds and eventually leads to their complete loss if the temperature is too high. After changing the inlet temperature from -20°C to 35°C the recovery of C40, as an example, has not changed significantly (decrease by only 4%) while the peak of C7 disappeared, either because this compound was lost completely via split vent, or it could no longer be separated from the huge solvent peak. The injector heating ramp was at the instrument's maximum (720 °C/min) and started immediately after the injection was completed (**inlet initial time**). Using a slower heating rate and keeping the injector longer at the initial temperature caused the recoveries of less volatile hydrocarbons to fall below acceptable values. If, for example, the inlet stays at the initial temperature for 0.5 min the recovery of C40 drops to 14%, and keeping the initial temperature for 1 min results in almost complete loss of all hydrocarbons (**Figure 3.1**). The longer interaction of analytes with the liner packing material leads to their retention in the liner and difficulties in reaching their quantitative transfer to the analytical column. Therefore, it is recommended to use the maximum applicable heating rate of the injector, and to start this ramp immediately after completion of the sample introduction for the analysis of petroleum hydrocarbons.

Speed controlled LVI requires the elimination of the largest part of the solvent to prevent column overload, while avoiding losses of the analytes. To establish equilibrium between solvent introduction and evaporation rate, the sample is introduced at a constant rate. While in theory a sample introduction rate (**injection speed**) equivalent to the solvent evaporation rate has to be chosen, in practice it should be set slightly higher in order not to lose the most volatile analytes together with the solvent. The injection speed will be discussed more detailed in this contribution later. However, if the sample is injected too quickly or too slowly into the PTV injector, no equilibrium can be established and solvent (and analyte) evaporation will occur under difficult-to-control conditions. A too fast injection into a cold not packed liner bears the risk of flooding the injector and thus losing the sample through the solvent vent exit of the injector due to the pressure pulse created at the onset of evaporation. If, on the other hand, the injection is too slow, losses of the volatile range of analytes will occur, as these are evaporated concurrently with the solvent (**Figure 3.1**).

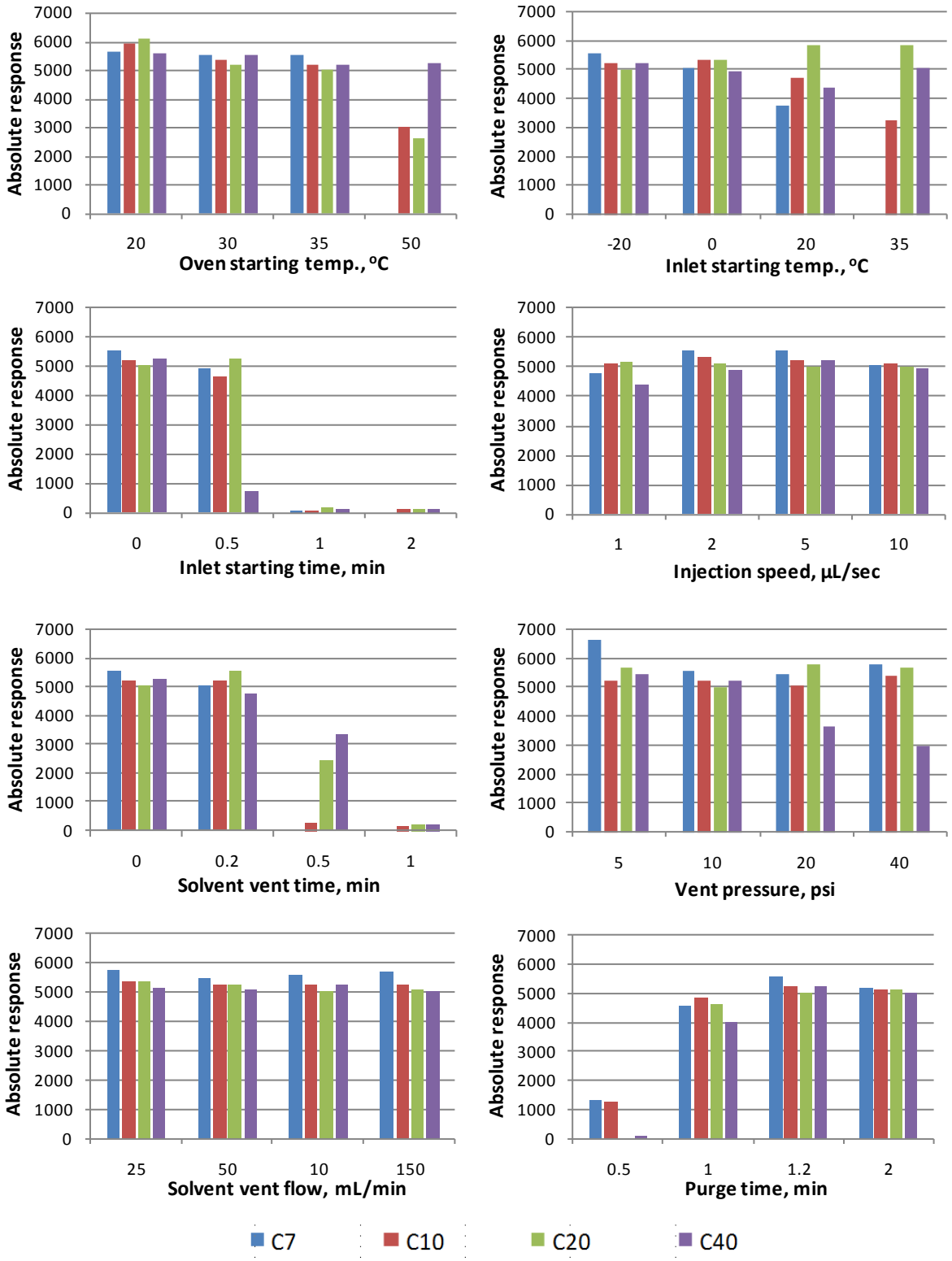


Figure 3.1 Mean absolute responses of C7, C10, C20 and C40 under different PTV parameters. All measurements were done in triplicates, with RSD varying between 0.1 and 4.0%.

A further critical parameter in the optimisation of PTV injection conditions is the solvent **vent time** which is the time for how long the split valve is kept open after the end of the (large volume) injection. To recover all hydrocarbons from C7-C40 (when dissolved in *n*-pentane) the most suitable vent time was 0 min, which means that the split valve was in fact closed immediately after the end of sample injection. When the split exit remains open for a too long time, discrimination of the more volatile analytes will occur, resulting in a loss of early eluting compounds. Note that until the injection of sample into the liner is complete the solvent vent valve is opened. Considering that the injection of 50 µL at an injection speed of 5 µL/sec takes 10 seconds, this is already enough time to eliminate the largest part of the solvent, while the analytes are retained in the liner. In contrast, the recovery of late eluting compounds can also decrease significantly if the **vent pressure** is too high (**Figure 3.1**), while the influence of split **vent flow** was not significant for all hydrocarbons. For the determination of petroleum hydrocarbons, a vent pressure of 10 psi and a vent flow of 100 mL/min have shown the best performance.

After all analytes are transferred into the analytical column, the split exit (vent line) is reopened to remove the remaining solvent and low volatile matrix constituents from the inlet. Early start of purging can lead to analyte discrimination with loss of the less volatile hydrocarbons, and in more pronounced cases with relative recoveries dropping below acceptable values even for all analytes. A **purge time >** 1.2 min shows satisfactory results, while purge times longer then 2 min are not recommended since in this case the background noise can be increased and the contamination from matrix can enter the analytical column.

It can be seen that PTV-LVI parameters are mutually dependent and in order to obtain good accuracy of analytical data, careful optimisation of all parameters has to be carried out. Once the optimum values of all parameter of PTV-LVI for the determination of TPH according to the OSPAR method were established, BAM standard and oil samples spiked in *n*-pentane were analysed. Optimised parameters are summarized in the experimental part. The chromatograms of a system performance standard and the BAM standard obtained under these conditions are shown in **Figure 3.2**. The system performance test yielded under optimised conditions very satisfactory results with the relative response of C7/C20 = 1.11, C10/C20 = 1.04 and C40/C20 = 1.05 which fully complies with the requirements of the OSPAR standard method. The optimised method shows a linear response over the investigated range for BAM standard solutions. The linearity range for the PTV-LVI (OSPAR) method was 0 – 80 ppm. The precision of the measurement, expressed as a relative standard deviation (RSD) varies between 1.0% and 7.7% at different concentration levels. The LOQ of the OSPAR method is 0.06 ppm OIW, which proves that the PTV-large volume injection technique is a suitable alternative to an external preconcentration procedure. Additionally, the precision of C7, C10, C20, C40 was calculated using a mixture of the previously mentioned alkane compounds in *n*-pentane, leading to a RSD < 1.7% (n=9) for all of them.

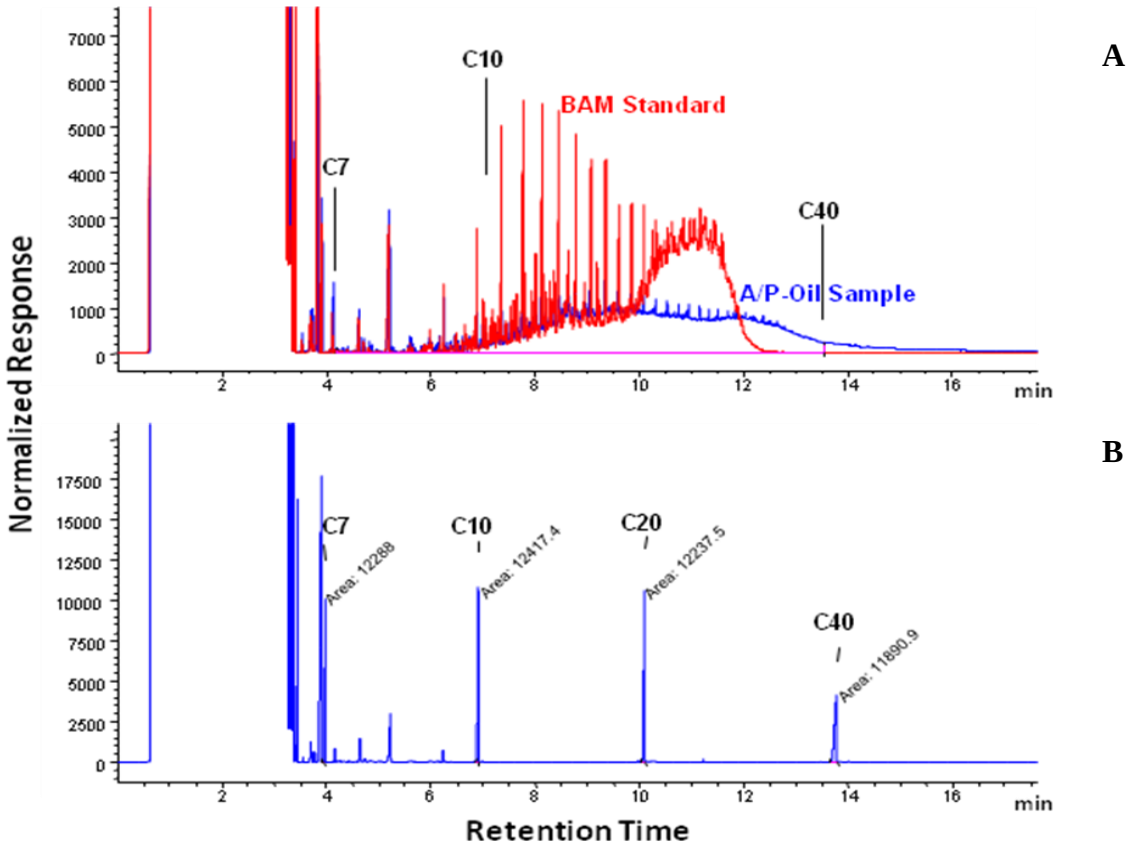


Figure 3.2 Chromatograms obtained under optimised conditions: (A) BAM standard solution and A/P-oil sample in *n*-pentane, with concentration 0.35 mg/mL, with 50 µL injection, and (B) mixture of C7, C10, C20, C40 in *n*-pentane, each at 0.005 mg/mL; relative responses: C7/C20=1.11; C10/C20=1.04; C40/C20=1.05.

3.3.2. Influence of liner design

Liner geometry and the choice of the packing material have an important effect on analyte trapping in GC, independent of the injection technique. The importance of liner diameter and density of the packing (glass wool) was already pointed out by Mol *et al.* [113]. These authors discussed the relevance of these parameters for the injected sample volume. Tollbäck *et al.* [201] investigated different baffled liners for the analysis of polybrominated diphenyl ethers, and the multi-baffled liner as well as sintered glass liners gave excellent trapping of the analytes with lower boiling point in the mixture (*mono*-, *di*-, *tri*-brominated diphenyl ethers).

In the frame of this study for the determination of petroleum hydrocarbons, we have also investigated the effect of different liner designs on the absolute recoveries of *n*-alkanes between C7 and C40 using LVI. Six liners with different design have been tested under identical conditions, where two of them were packed with deactivated borosilicate glass wool (**Table 3.1**).

Table 3.1 Different types of liners used in this work

Liner N1	88mm, ID 3mm, 1 ring restriction, filled with deactivated borosilicate wool, *for liquid injection*	
Liner N2	88mm, ID 3mm, 4 ring restrictions, *deactivated surface*	
Liner N3	88mm, ID 3mm, 1 ring restriction;	
Liner N4	88mm, ID 3mm, 5 ring restrictions, filled with deactivated borosilicate wool, *Large Volume*	
Liner N5	88mm, ID 3mm, 8 ring restrictions	
Liner N6	88mm, ID 3mm, 1 base ring + 4 ring restrictions	

Significant differences were observed in the analysis of the system performance test solution with the six different liners tested. Usually the choice of packing material depends on the case of each analysis. For retaining hydrocarbons in the C10-C40 range, there is no need for an adsorbent-filled liner. A plug of deactivated borosilicate glass wool, inserted in the top half of the liner is sufficient to ensure efficient evaporation and transfer of all hydrocarbons in the investigated volatility range with good recovery. For liner of type N1, a liner with one ring restriction filled with deactivated borosilicate wool, quantitative trapping is observed for all components under the operating conditions given in the experimental part compared to the liner of type N3 with the same geometry but without glass wool (**Figure 3.3**). Liner N3 did not give satisfactory results concerning the recovery of less volatile C40. C40 was always trapped more efficiently in the liners packed with deactivated borosilicate wool (Liner N1 and N4). The use of glass wool helps to increase the contact area of solvent/analyte vapor inside the liner while reducing the liner volume only to a very limited degree and results in improved recoveries of compounds over a wide range of volatility. The solvent droplets formed in the injection phase impact on the glass wool, and are eventually evaporated in the first phase of PTV injection, while the analytes stay behind, and are only transferred to the column when the injector temperature is increased. For the liner with 5 restrictions and filled with glass wool (Liner N4) under the same conditions a lower recovery of the more volatile C7 and C10 compared to less volatile C20 and C40 is observed. The glass wool helped quantitatively recover less volatile compounds. It is, however, assumed that the presence of several restrictions in this liner leads to a particular flow pattern than results in a higher discrimination of more volatile compounds than observed with other liners.

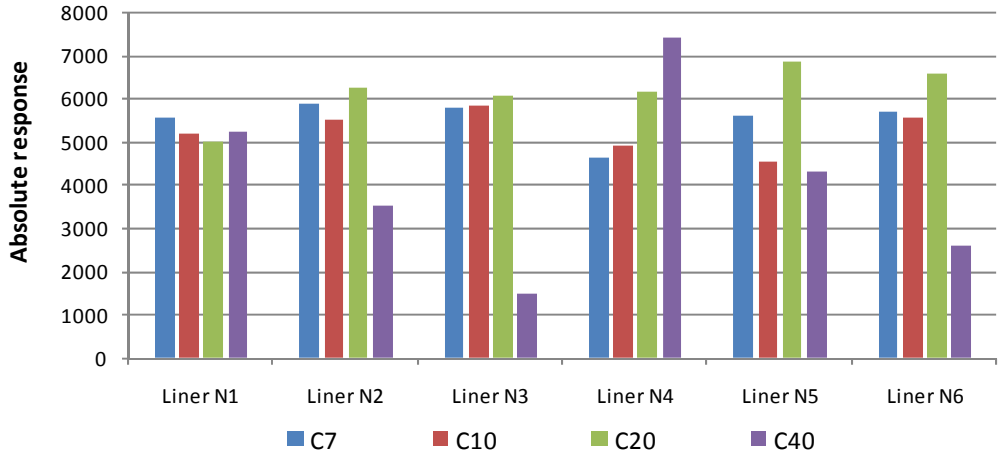


Figure 3.3 Comparison of absolute responses of C7, C10, C20 and C40 for different liners at the same operating conditions. Measurements are repeated twice, RSD are from 0.2 to 3.2%.

Under the same PTV parameters liners N 2, 5 and 6 with restrictions in the different parts of the liner showed slightly different performance. It appears that the geometry of these three liners assists in trapping the more volatile hydrocarbons with reasonable response, however the recovery of C40 is worse.

The advantage of using the liner with the glass wool in comparison with the liner without glass wool for oil samples (BAM standard in *n*-pentane with concentration 0.35 mg/mL) can be seen from the chromatograms presented in **Figure 3.4**. The difference between the total peak areas of compounds eluted between C7 and C40 in these two chromatograms is significant – 22%, which may be a reason for underestimation of petroleum hydrocarbon concentrations when calibration and actual measurement are performed with two different liner designs. The choice of the suitable liner together with optimised PTV-LVI parameters is especially important for the determination of petroleum hydrocarbons. However, the small discrimination at the beginning or at the end of chromatogram can have a significant effect for the quantitative evaluation and this effect becomes more noticeable if largely different types of oil or samples of highly different volatility ranges are analysed. This is not only due to the fact that the chromatographic pattern varies strongly among different samples, but also that these show highly variable responses/recoveries. The challenges and some difficulties related to different response/recoveries in the determination of the petroleum hydrocarbons in water by GC are investigated and discussed in our previous study (*Chapter 2*).

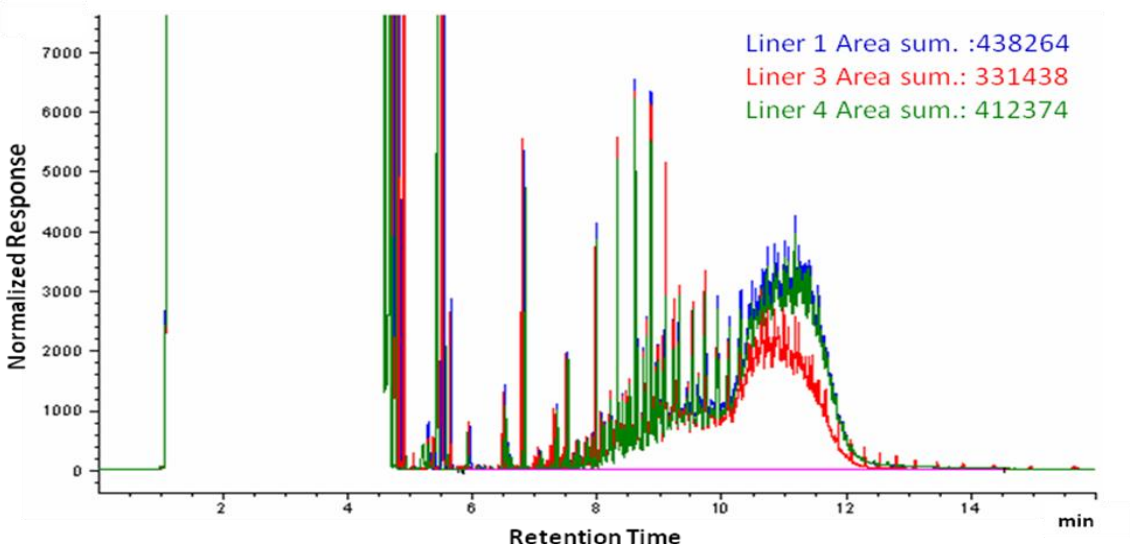


Figure 3.4 Chromatograms obtained from a 50 µL injection of BAM standard (0.35 mg/mL) in *n*-pentane using the Liners N1 and N3.

3.3.3. Injection speed

As previously explained, the introduction of large volumes is based on introducing of the liquid sample into the liner at a controlled speed. Introduction actually involves two steps: evaporation, first of all of the solvent, and separation of the solute material from the solvent vapors before the latter are discharged. Solvent-solute separation occurs by gas chromatographic retention power that keeps the sample constituents as sharp initial bands in the column inlet while the solvent vapors are released [112]. The conditions for the injection of large volume of sample, particularly injection speed, must be determined so that good chromatography and analysis reproducibility are not sacrificed.

When using the speed controlled PTV large volume injection method, the volume that can be injected is (in theory) unrestricted. During sample introduction an equlibrium state is reached. In this state the speed of introduction of the liquid sample into the liner equals the evaporation rate. This steady state is described by the following equation:

Liquid sample flow into the liner = gaseous vent flow out of the liner

This equation might look very simple, however in reality the PTV system is more complex. Some important parameters like the inlet temperature, nature of solvent, actual inlet pressure, solvent venting flow and pressure, affect the described equilibrium state. When considering these parameters, the injection speed can be estimated using the **Equation 1.2** for calculation of the solvent elimination rate [*v*, µL/min] calculation. Solvent evaporation is, in first instance, considered an isothermal process. In practice, the calculated sample introduction rate is too high for solvent elimination, because the calculation does not include effects that slow down the evaporation: the effect of cooling of the liner that occurs during the evaporation of a large volume of the solvent [114,115] and the effect of liner design and

packing which is determining the resulting flow pattern. For these reasons, a lower value for the solvent introduction rate then the calculated optimum speed must be used in practice.

PTV-LVI equipments used nowadays, and particularly the one used in this work, are capable of keeping the injection port at constant temperature, despite the cooling effect from solvent evaporation. While there is no need to introduce a correction of the determined value for injection speed because of the cooling effect, there still remain other factors unaccounted by the model that require an experimental adjustment of the theoretically derived values.

Table 3.2 Injection speed and time for samples in *n*-pentane, calculated according **Equation 1.2** at different inlet temperatures, vent pressure – 10 psi and vent flow – 100mL/min.

Inlet temperature ºC	***n*-Pentane vapor pressure** *kPa*	**Calculated injection speed** *µL/sec*	**Injection time** *sec*
-20 ºC	9.0	**0.45**	111.1
0 ºC	24.4	**1.23**	40.7
20 ºC	56.6	**2.65**	18.9
35 ºC	97.7	**4.36**	11.5
50 ºC	159.3		

Injecting at the calculated values of sample introduction speed using the **Equation 1.2** for four different inlet temperatures (**Table 3.2**) did not show satisfactory results of the system performance test for petroleum hydrocarbon determination in the range between C7 and C40 (**Figure 3.5**). The most critical components in petroleum hydrocarbon determination by OSPAR method are the more volatile solutes (hydrocarbons starting from C7), which elute immediately after the solvent peak. Volatile components are easily lost during the elimination of the solvent if the injection is too slow or if inlet temperature is too high. Fine tuning of the sample introduction rate has to be done experimentally in this case.

The calculated speed appears to be too slow for sample introduction at the initially chosen PTV parameters and liner. Relative responses of C7, C10 and C40 to C20 in the **Figure 3.5** show, that if the injection speed is too low, the loss of highly volatile C7 happens. The sample introduction time during which the solvent exit is open, depends on the injection speed. The slow rate is accompanied by longer solvent evaporation and this leads to a loss of more volatile analytes in the sample. At higher sample introduction rates the injection time, respectively the solvent venting time, is shorter. This leads to better recoveries of higher volatile compounds. This behavior was observed for all tested inlet temperatures.

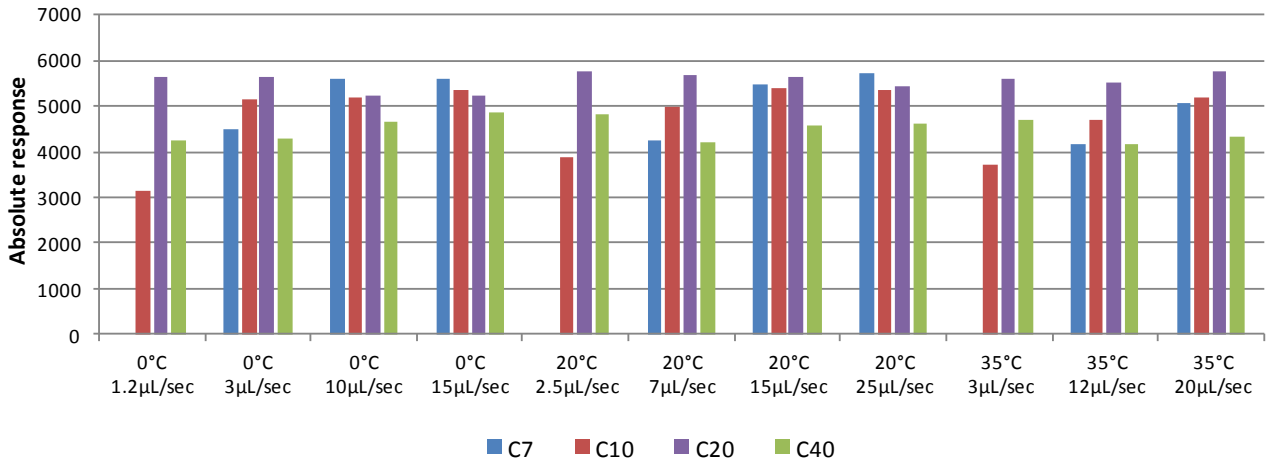


Figure 3.5 Comparison of absolute responses of C7, C10, C40 to C20 at different inlet temperatures (given in the first row) and sample injection speeds (given in the second row); vent pressure: 10 psi, vent flow: 100mL/min, vent time 0 min, oven temp 35°C; Liner N1 (with 1 restriction, filled with deactivated borosilicate glass wool). RSD values are between 0.5 and 2.7% for all measurements done in triplicates.

3.3.4. Parameter transfer to other solvents

In order to obtain good recoveries of all analysed compounds during the PTV-LVI, according to **Equation 3.2** the equilibrium between the introduced volume of liquid and eliminated solvent vapor must be established. As different solvents have different vapor pressure, this has to be the prime consideration in selecting optimal PTV parameters. Notably, the inlet temperature has to be chosen in a way that allows efficient solvent evaporation while the analytes are initially retained and then, during the fast temperature increase of the injector, quantitatively transferred to the GC column. Once PTV parameters have been optimised for one solvent, they can be transferred – on the basis of the above considerations – to other solvents while maintaining the analytical performance. This can be realized when the vapor pressure of the solvent in the inlet during the injection is adjusted to the same value. Such parameter transfer facilitates and/or eliminates the optimisation procedure for other solvents used for the same analytes. The optimised PTV-LVI parameters (which are summarized in the experimental part) for petroleum hydrocarbons when *n*-pentane is used as a solvent were chosen as a reference and transferred to cyclopentane, *n*-hexane, cyclohexane and *n*-heptane.

Solvent vapor pressure in the inlet depends on the inlet temperature. The initial inlet temperature of -20°C showed the best performance for the determination of petroleum hydrocarbons if *n*-pentane is used. Vapor pressure of *n*-pentane at -20°C is 9.0 kPa [203]. As an example, cyclopentane has the same vapor pressure at -9°C. If the inlet temperature is -9°C, then the vapor pressure of cyclopentane will be the same as the vapor pressure of *n*-pentane at -20°C. Thus the elimination rate of the solvent during the PTV-LVI must be similar for both solvents. To transfer PTV-LVI parameters from one solvent to another, the inlet temperature must be recalculated, according to the vapor pressure of the first solvent at optimised inlet temperature.

Not only the inlet temperature has an important role in PTV-LVI parameter transfer. It is also the initial oven temperature whose importance has been shown above, and which must be considered in PTV parameter transfer process as well. The same recalculation for the initial oven temperature, as for the initial inlet temperature, based on the vapor pressure of the solvent should be applied. For samples in *n*-pentane the method showed comparable performance in the initial oven temperature range of 20 to 35°C (which is just below the boiling point of *n*-pentane of 36.1°C). The initial oven temperature for another solvent must be set to have similar conditions as for *n*-pentane at 20 or 35°C accordingly, that is, an initial temperature of up to 15°C below the atmospheric boiling point is recommended.

The initial temperatures for inlet and oven were recalculated for four other solvents used in the analysis of petroleum hydrocarbons, as it is shown in **Table 3.3**. All other PTV parameters, such as injection speed, venting and purge parameters remained unchanged.

Table 3.3 PTV and GC oven temperatures for LVI using different sample solvents and corresponding vapor pressures.

Solvent	**Boiling point, °C**	**PTV initial temp. , ºC** (temp. program: (0 min), 720 ºC /min → 340ºC(15 min)	**Vapor pressure**[a] at PTV initial temp., **kPa**	**Oven initial temp**. ºC (temp. program: (6 min), 60 ºC /min → 340ºC (5 min)	**Vapor pressure**[a] at oven initial temp., **kPa**
n-Pentane	36.1 ºC	**-20** ºC	9.0	**35** ºC	97.7
				20 ºC	56.6
Cyclopentane	49 ºC	**-9** ºC	9.0	**49** ºC	100.5
				33 ºC	57.4
Hexane	69 ºC	**+8** ºC	9.1	**69** ºC	102.2
				51 ºC	56.0
Cyclohexane	80.7 ºC	**+17** ºC	9.0	**81** ºC	101.2
				63 ºC	57.5
Heptane	98.4 ºC	**+33** ºC	9.0	**98** ºC	101.3
				80 ºC	57.0

[a] Numerical inconstancies due to the mathematical approximation in the calculation of vapor pressure according to [203]

Figure 3.6 compares results for the relative response of C7/C20, C10/C20 and C40/C20 in chromatograms of the system performance standard solutions in different solvents under conditions re-calculated according to our proposed model for parameter transfer. The method can be successfully transferred for all solvents used with satisfactory recoveries only for C10, C20 and C40. The quantitative recovery of C7 is only possible if *n*-pentane or cyclopentane are used, due to their higher volatility compared to the other solvents. Despite the fact that hexane (bp 69°C) and cyclohexane (bp 80.7°C) have lower boiling point then C7 (bp 98.4°C), it is already not possible to effectively separate the C7 peak from the solvent peak. As the difference between volatility of solvent and C7 becomes smaller, the C7 peak just disappears under the huge solvent peak. Furthermore the solvent peak size gets wider if the oven initial temperature decreases, what was observed for all solvents. When decreasing the oven temperature from 49°C to 33°C, the cyclopentane peak gets wider, which causes the C7 peak to be hidden under the

solvent peak. Hence in the case, where cyclopentane is the sample solvent the oven temperature becomes a critical parameter.

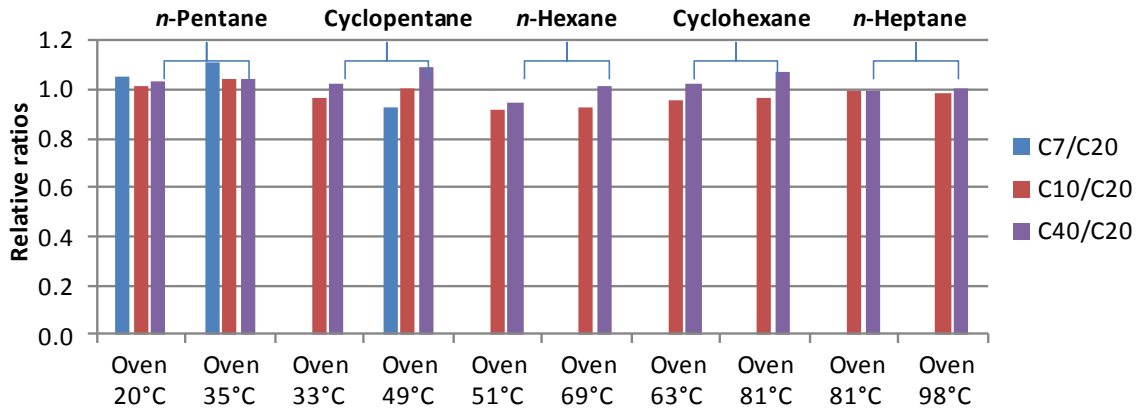


Figure 3.6 Comparison of relative responses of C7, C10, C40 to C40 under conditions optimised for *n*-pentane and transferred to different solvents. RSD values are between 0.2 and 2.8% for all measurements done in triplicates.

When hydrocarbons in the range C7-C10 cannot be detected without interference from the solvent peak, the method is not suitable for hydrocarbon determination by the OSPAR method. Nevertheless, the proposed method for the transfer of PTV-LVI parameters from one solvent to another can be applied almost without loss in performance for hydrocarbons in the range C10-C40 (C10/C20=0.92-1.00; C40/C20=0.99-1.07). For hydrocarbon samples extracted into cyclopentane, the parameter transfer is completely successful as it yield satisfactory results of system performance test not only for the recoveries of hydrocarbons in the range C10-C20, but also from C7 (C7/C20=0.92; C10/C20=1.00; C40/C20=1.09). The chosen parameters are thus allowing to successfully use cyclopentane instead of pentane for the determination of petroleum hydrocarbons according to the OSPAR method by means of large volume injection.

3.4. Conclusions

To avoid discrimination of sample constituents in the analysis of samples that spread over a wide range of volatility, careful optimisation of PTV-LVI injection parameters is required. Although there is some guidance in the selection of useful PTV-LVI parameters through the available literature and the models presented therein, there is always some experimental fine tuning required due to the particular conditions existing with different instruments or components (e.g. liners). I have presented in this work a rational approach for the optimisation of PTV-LVI parameters for petroleum hydrocarbons with a wide range of volatility (C7-C40). The most critical components in petroleum hydrocarbon analysis are the more volatile solutes (hydrocarbons starting from C7), which elute immediately after the solvent peak.

There is a difference between theoretically calculated and practical values of the sample introduction rate for this particular application. Theoretical calculations are focused on the effective solvent elimination during the speed-controlled introduction of the large sample volumes, but they do not include other certain effects and requirements of the analytical procedure (i.e., the recoveries that are found for volatile hydrocarbons relative to those found for less volatile hydrocarbons must be equal).

Alternatively to speed-controlled LVI, which requires careful optimisation, at-once large volume injection could be a feasible option. While the latter may be simpler to optimise, it requires a special packed liner with large surface area. In contrast, speed-controlled LVI offers more flexibility. Once PTV-LVI parameters have been optimised for one solvent, then they can be easily transferred to other solvents on the base of solvent's vapor pressure at the conditions of sample introduction, with maintaining the method's performance and without the need of tedious re-optimisation.

4. Quantitative analysis of petroleum hydrocarbon contaminations by GC×GC

4.1. Introduction

In the comparative study of the two 1D-GC standard methods currently used for the determination of the TPH (*see Chapter 2*), their principle feasibility for the determination of petroleum contaminations was already demonstrated. Particular challenges and limitations of the two methods, recovery of the method for different types of oil, losses during the clean-up procedure, and choice of calibration standard were discussed. It was observed that the results of the two methods correlate well for individual types of oil, although there is a significant method-and oil-type specific bias.

The appropriateness of the two standard methods for the analysis of different oil samples and the mutual comparability of the results produced by these two standard methods were critically evaluated. The limitation of such procedural defined methods as to report a "true" value is hereby highlighted. It can be expected that the hydrocarbon index and the total petroleum hydrocarbon (TPH) value should be strongly correlated for one particular type of oil; however, for oils very different in their composition this may not generally be so.

The subsequently described study for the quantitative determination of petroleum hydrocarbons by GC×GC/FID was designed for the following purposes: to check the trueness and precision of investigated 1D-GC methods, to explain the different recoveries for different oils and losses during the clean-up, and to support or refute our results and conclusion from comparative study of two 1D-GCstandard methods.

To bring some insight into why there is such a strongly deviation in relative response requires thus a detailed analysis of the petroleum hydrocarbon mixture, based on individual chemical compound classes, e.g. linear and branched aliphatics, (poly-)aromatics, alkyl-substituted aromatics, heterocycles, etc., under the assumption that there are compound-class specific response factors for the different classes of hydrocarbons. Unfortunately, classical, that is, one-dimensional chromatography is not able to provide this information, even with more selective detection, such as mass spectrometry (MS). The reason for this is the chromatographic overlap of the countless compounds contained in the sample that does not allow to detect separately and identify the constituents of such a hydrocarbon sample. When staying in one dimension, chromatographic separation efficiency cannot be increased to that degree needed to resolve the several thousand compounds contained in a typical petroleum hydrocarbon sample.

Comprehensive two-dimensional gas chromatography (GC×GC), in contrast, is a valuable tool to resolve a large fraction of the compounds occurring in natural petroleum hydrocarbon samples. The use of GC×GC with MS detection is expected to not only allow the separation of the various constituents of complex TPH samples, but also to identify them based on MS detection. Moreover, it is known that a

certain class of chemical compounds (a series of 'homologues') is forming a very distinct, clearly identifiable pattern in the two-dimensional space of the GC×GC separation. This will be essential for correlating the FID response with individual compound classes in the TPH sample, and is expected to provide a more accurate calibration than currently possible.

Two important aspects will be subject to our further GC×GC research for the quantification of petroleum hydrocarbons. The first aspect is establishing the GC×GC method for petroleum hydrocarbon characterization. GC×GC with MS detection will be used for qualitative analysis and to define the individual classes of chemical compounds (group-types) present in the analysed oils of different type. A second aspect to be addressed is the accuracy and precision of the quantitative response of the 2D chromatogram. Quantitative analysis in (one dimensional) chromatography is normally performed under the assumption that the peak area of the signal is proportional to the concentration of the analyte to which the peak corresponds. This is certainly also true in GC×GC, however, an analyte will not produce a two-dimensional peak in GC×GC, whose area is easy to determine by integration, but rather a three-dimensional signal whose volume must be determined precisely.

In this work GC×GC will be applied for the compound-class specific analysis of hydrocarbons present in the samples of different oil types and for quantitative determination of total petroleum hydrocarbons. The objective will be to test the applicability of a model using compound-class specific response factors for quantitative analysis to obtain a more uniform quantitative response when different types of oil are analysed, eventually making universal calibration feasible.

4.2. Experimental

4.2.1. Instrumentation and reagents

The work was carried out using a Shimadzu GC2010-Plus gas chromatograph equipped with a QP-2010 Ultra quadrupole mass spectrometer (Shimadzu, Kyoto, Japan), a flame ionisation detector (FID), an (ATAS, Eindhoven, The Nederland), AOC-5000 Plus (Shimadzu, Kyoto, Japan).

Helium (purity >99.999%, Messer) was used as the carrier gas at a flow rate 1.05 mL/min and head pressure of 169.0 kPa for GC×GC/MS and at a flow rate 1.47 mL/min and head pressure of 235.2 kPa for GC×GC/FID. The GC×GC analyses were carried out with a nP stationary phase system, where the first dimension separation was performed on an apolar DB-5MS 30 m × 0.25 mm × 0.25 µm capillary column, coated with 95% dimethyl-/5% diphenylpolysiloxane polymer (Agilent J&W GC Columns, USA), and the second dimension separation on a BPX-50 capillary column 1.2 m × 0.1mm × 0.1µm , coated with 50% dimethyl-/50% diphenylpolysiloxane polymer (SGE Analytical Science), situated in the same oven. An uncoated 1 m × 0.2 mm ID, deactivated fused silica capillary (Supelco, Bellefonte, PA) was used as a loop between the two separation columns. The GC oven initial temperature of 70°C was

kept for 0 min, then increased at 2 °C/min to 340°C and was held for 10 min (total time 140 min). Constant temperature split (2:1) injection technique (at 340°C) was used for injections of 1 µL. A fritted liner (OPTIC liner with deactivated glass frit at 15 mm above the liner bottom end), packed with deactivated borosilicate glass wool was used. For cryomodulation, a dual jet modulator (ZOEX, ZX1, Houston, USA) operated with liquid nitrogen with modulation time of 8 seconds and hot jet (at 340°C) with pulse duration of 600 msec was applied. For the MS detection the scan range 40-650 m/z and event time 0.04 sec (25 Hz) were used. FID was operated at 340°C and sampling rate 4 msec (250Hz).

Instrument control and data evaluation was done with the GCsolution and GCMSsolution softwares (Shimadzu). GC Image software, Version 2.2b0 (Lincoln, University Nebraska) - Software for Multidimensional Chromatography – was used for GC×GC data processing. The OPTIC3 high performance injector is configured and controlled through the ATAS Evolution Workstation software. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated from experimental data according to the calibration method (as outlined in DIN 32645 [191]) by the Excel macro ValiData [192].

4.2.2. Reagents

n-Hexane used as a solvents was purchased from Sigma-Aldrich (Vienna, Austria). *n*-Alkane standard mixture of C10-C40 in heptane, each at 0.05 mg/mL from Fluka (Buchs, Switzerland); EPA 610PAH mixture at 100-2000 µg/mL in methanol/dichlormethane (2:1) from Sigma-Aldrich (Steinheim, Germany), butylcyclohexane, ≥99%, biphenyl, 99.5%, 2,2,4-trimethylpentane, 99+%, 1,2,3-trimethylbenzene techn., 90%, 1,2,4-trimethylbenzene, 98%, 5α-cholestane, 5α-androstane, cyclodecane, 1-phenyldodecane, 1,6-dimethylnaphthalene from Sigma-Aldrich (Steinheim, Germany), pentylbenzene, ≥97%, 1,1-bicyclohexyl, pur from Fluka (Buchs, Switzerland), decalin, 99% from Riedel-de Haen (Germany). Different types of oil were obtained from the co-operation partner (QuantaRed Technologies GmbH, Vienna): light heating oil, motor oil (Castroil), diesel, a mixture (1:1) of P-Oil (API 29.5) and A-Oil (API 23.9), heavy heating oil, OMV Crude Oil API 40.5, OMV crude oil API 18.7, mineral oil mixture. Alkane standard mixture C10-C40 for performance test of GC-systems in heptane (with each compound at 0.05 mg/mL) from Supelco (Sigma-Aldrich).

For fractionation of oil *n*-hexane, dichloromethane and methanol from Sigma-Aldrich (Vienna, Austria) were used as solvent for elution and fractionation was performed on silica gel 60 (0.040 – 0.063 mm particle size) from Merck (Darmstadt, Germany).

4.2.3. Standards and samples

BAM-K010 certified reference material for the determination of mineral hydrocarbons with a certified value of 1.00003 mg/mL (Certified reference material for the determination of mineral hydrocarbons, Bundesanstalt für Materialprüfung, Berlin) was used as a stock solution for the preparation of calibration standard solutions in a range from 0.005 to 2 mg/mL. Samples of different types of oil were prepared in duplicate at a concentration of 2 mg/mL. One half of the oil samples is cleaned-up through Florisil, while the other is not in order to study the influence of the clean-up step. To prepare standards and the extracts an alternative to the clean-up procedure through the column with Florisil, as prescribed by DIN ISO 9377-2:2000 method was used, which is more practical if a larger number of samples is to be processed in parallel: To this aim the combined extract (from the two extraction steps) of 50 mL is shaken in a closed Erlenmeyer flask with 2 g of Florisil and 2 g of sodium sulphate during 30 min on a shaking apparatus. The purified aliquot (sample after clean-up) and the sample before clean-up were analysed by GC×GC.

The standard solution for determination of response factors was prepared from all reagents listed above to have concentration of 0.01 mg/mL for each component and 0.002-0.04 mg/mL for PAHs.

4.3. Setup of GC×GC/MS and FID method for petroleum hydrocarbon analyses

In the introduction part, *Section 1.5.4.*, is has been shown that GC×GC method set up and optimisation is not straightforward. The main GC×GC operational conditions that must be established are the stationary phase chemistry, column dimensions, carrier gas flow, modulation parameters, temperature programs and detector settings. Based on our experience and on literature studies the most important recommendations and guidance for set up optimisation of GC×GC parameters are summered and presented in the **Table 1.12** (*Section 1.5.4.*).

The experimental work was carried out on the one oven GC system equipped with FID, and the dual-jet (hot and cold) dual-stage loop-type (ZOEX) modulator. The GC × GC experimental conditions were optimised by analyzing a standard mixture of *n*-alkanes, standard BAM samples and samples of different oils. Following to recommendations in the mentioned table the instrumental conditions presented below were found to be the optimal for petroleum hydrocarbon analyses by GC×GC/MS and GC×GC/FID.

Instrumental Conditions

1D: DB-5MS 30 m × 0.25 mm × 0.25 µm
95% dimethyl-/5% diphenylpolysiloxane
loop: 1 m × 0.2 mm
non polar fused silica
2D: BPX-50 1.20 m × 0.1mm
50% dimethyl-/50% diphenylpolysiloxane

} **Equivalent to a column of 79.3 m × 0.25 mm × 0.25 µm**

Head Pressure:

For FID – 235.2 kPa → total column flow 1.47 mL/min, linear velocity 24.4 cm/sec

1D: linear velocity 19.2 cm/sec 2D: linear velocity 185.3 cm/sec

For MS –169.0 kPa → total column flow 1.05 mL/min, linear velocity 23.1 cm/sec

1D: linear velocity 17.2 cm/sec 2D: linear velocity 186.4 cm/sec

GC Oven: 70 ºC (0 min), 2 ºC/min → 330 ºC (10 min), total 140 min

FID: 330 ºC, sampling rate 4 msec

MS: 40-650 m/z; event time 0.04 sec (25 Hz)

ZOEX dual jet modulator:

8 sec – modulation period; hot jet 600 msec; hot jet 340 ºC

Cold N_2: pressure 3 – 3.5 bar, flow 10 – 12 L/min

Since there is a lot of work and research done on petroleum hydrocarbon analyses by GC × GC, the choice of stationary phases and dimensions for both columns was not difficult. For our GC×GC analyses the normal orthogonal separation has been chosen, where non-polar DB-5MS 30 m × 0.25 mm × 0.25 µm column was used in the first dimension (1D), the semi-polar BPX-50 1.20 m × 0.1 mm column in the second dimension (2D). This column set offers a good separation between alkanes, cycloalkanes, monoaromatic and polyaromatic hydrocarbons. Although, the reverse orthogonal column set offers equally good (but different) separation between aliphatic and aromatic hydrocarbons, it was not a choice for our initial experiments.

The non-polar deactivated fused silica of 1 m × 0.2 mm was used as a delay loop between two columns. 1 m is a usual length of the loop for the dual-stage loop-type modulator. The internal diameter of the loop has been chosen to be similar to the internal diameter of the 1D column, as to reduce the pressure difference in the midpoint (the point between two columns of different dimensions) and to have the linear velocities in 1D near to optimum ~ 20-35 cm/sec and in 2D in the range of ~ 160-330 cm/sec (which is far above the optimum, but a necessary concession if no split is used after the first column). **Table 4.1** reports the relation between the linear velocities in both dimensions and the internal diameter of the loop. A loop of 1 m × 0.2 mm at head pressure 235.2 kPa provides reasonable linear velocities in both columns together with a satisfactory hold up time of 0.6 sec in the second dimension. The midpoint

in this particular case is just before the 2D column of 1.2 m × 0.1 mm. In contrast, a loop with the internal diameter 0.1 mm which is equal to the i.d. of the 2D column (resulting in (1 m loop + 1.2 m 2D column) × 0.1 mm → 2.2 m × 0.1 mm of column after the midpoint) at the same head pressure changes linear velocities, so the flows in the 1D and 2D are slower and below the respective optimum. Such (relatively) slow flow in the second dimension increases the hold up time to 1.9 sec, which can cause wrap-around if the modulation period stays the same. It is possible to change the head pressure in case of a loop of smaller ID, in order to have optimum or close to optimum linear velocities, but then a higher pressure will result at midpoint and in some cases depending on the type of connectors used, a leak can appear.

Table 4.1 The effect of the internal diameter (ID) of the loop on linear velocities in the 1D and 2D columns, on midpoint pressure and on hold up time in each dimension.

	Pressure		**Linear velocity, cm/sec**		**Total flow**	**Hold up time**	
1m loop ID	**Head**	**Midpoint**	**1D**	**2D**	**mL/mim**	**1D, min**	**2D, sec**
1m × 0.2 mm	235.2 kPa	165.4 kPa	**19.2**	**185.3**	1.47	2.8	0.6
1m × 0.1 mm	235.2 kPa	193.0 kPa	**12.6**	**116.1**	1.01	4.0	1.9
1m × 0.1 mm	384.0 kPa	319.7 kPa	**19.2**	**185.3**	2.20	2.6	1.2

**at 70 ºC, 1D: 30 m x 0.25 mm x 0.25 µm; 2D: 1.20 m x 0.1 mm; FID as a detector*

For comprehensive two-dimensional GC, which uses two columns of largely different lengths and internal diameter, it is important to calculate the column equivalent for this column set in order to set the correct pressure or flow rate and to achieve good performance of the system, as judged by the controlled linear velocities and hold up times in both dimensions. Once suitable parameters have been found for one type of detector, for example the ambient-pressure operated FID, then parameters can easily be converted to another detector such as the MS operated in vacuum, by recalculating the column head pressure and flow taking into the consideration the vacuum at the MS which increases the actual pressure drop over the column set by 1 atmosphere. Recalculation must be done with respect to linear velocity in the 2D, as to maintain the separation and the modulation period, **Table 4.2**.

Table 4.2 Calculation of the column equivalent parameter for the used column set and linear velocities and head pressure if FID or MS are used.

Columns	**1D + loop**	**1D + loop + 2D**	**Linear velocity, cm/sec**	
			FID	**MS**
			235.2 kPa*	169.0 kPa*
1D: 30 m × 0.25 mm **loop**: 1 m × 0.2 mm	32.4 m	79.3 m	19.2	17.2
2D: 1.20 m × 0.1mm			185.3	186.4
		Hold up time in 2D:	0.6 sec	0.6 sec

**head pressure at 70 ºC*

The important parameter modulation period was adjusted so as to provide a good focusing of the compounds and to avoid a wrap-around (**Figure 1.26**, *Section 1.5.4*). The modulation period of 8 sec (time of the cold jet) was found to be the most suitable for the used column set and GC × GC settings. Wrap-around was observed only for the highest boiling PAHs (this is seen, for example, in the contour plot of one of the last heavy heating oil fractions, **Figure 4.18**) but because they eluted early in the next modulation cycle no coelution with other analytes occurred.

Attention must also be paid to the other modulation parameters. The temperature of the cold jet must be cold enough to trap the analytes (also highly volatile analytes) and to avoid breakthrough (**Figure 1.28**, *Section 1.5.4.*). At the same time the cold jet must not be too cold in order to avoid the overcooling effect (**Figure 1.27**, Section 1.5.4). The hot jet temperature and the hot jet duration must be appropriate for the efficient desorption and reinjection of the trapped analytes. Thus the optimum nitrogen coolant gas flow was found to be between 10-12 L/min at a pressure of 3.5 bar and the hot jet duration time 600 msec at 340 ºC (with the ZOEX modulator system, the flow rate of the hot jet is not regulated separately).

Both columns are placed in one oven. The oven starting temperature was 70ºC and the temperature programming rate was 2 ºC/min until 330ºC. The widths at 5% high of the *n*-alkane peaks (from the standard solution) in 1D at the beginning of the run were 12 sec and 24 sec at the end of the run, which allows to have between 2 and 3 modulations per peak if the modulation period is 8 sec, resulting in peaks of 600 msec in 2D.

The parameters for detectors were set so to collect at least 10 data points per 2D peak. A high sampling rate of 4 msec was used for FID and an event time of 0.04 sec (25 Hz) was applied for MS detector.

The column set and the parameters described above were applied for all following experiments for petroleum hydrocarbon characterization and quantification. **Figure 4.1** represents an example of the GC×GC separation of the petroleum hydrocarbon mixture, like light heating oil. The chromatogram of the one-dimensional separation is reproduced in order to compare it with the much improved separation, after comprehensive two dimensional GC separation.

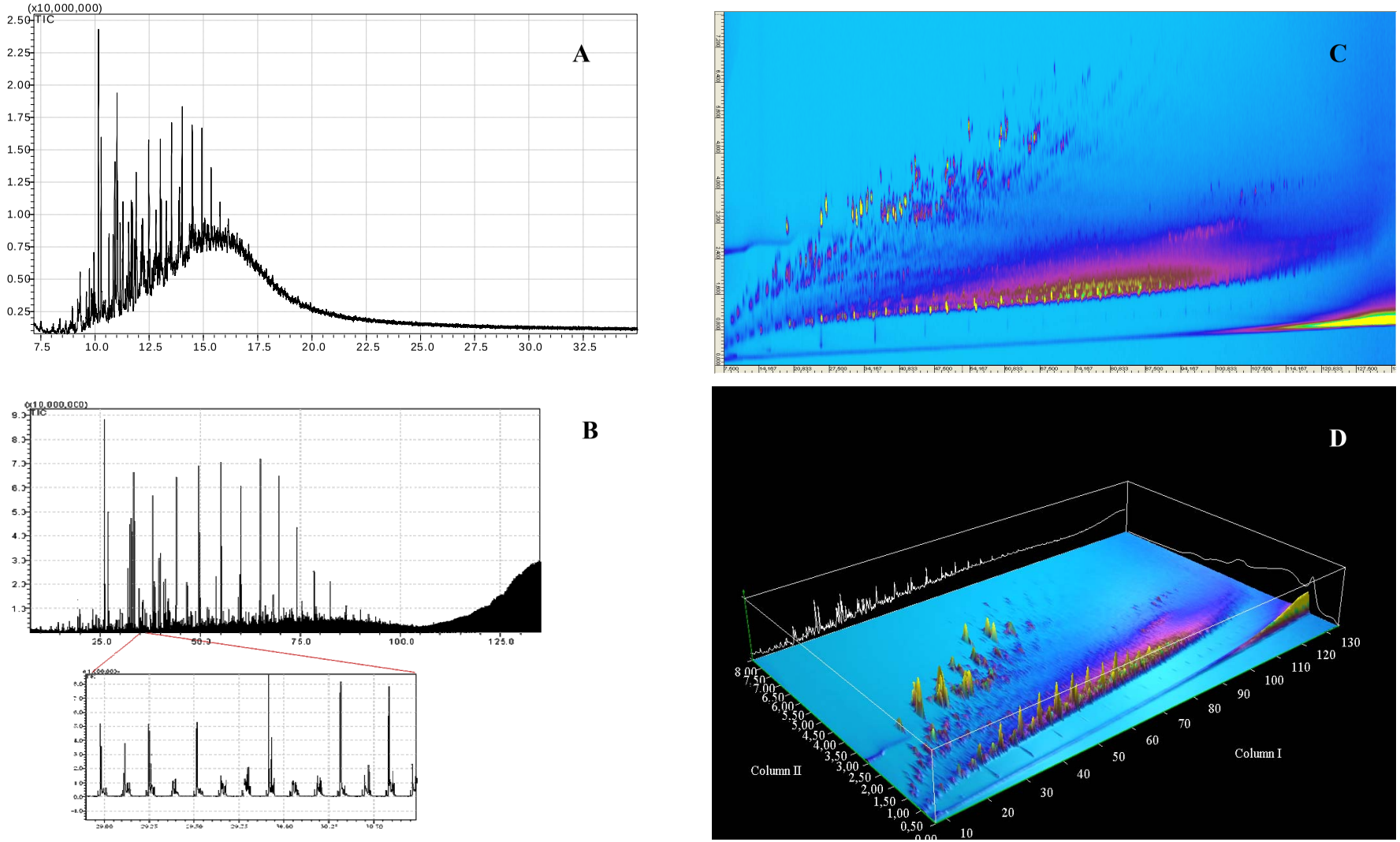


Figure 4.1 (A) 1D GC chromatography of light heating oil (SLB-5ms 10 m × 0.1 mm ID); GC×GC chromatography of light heating oil: (B) raw data from the detector positioned at the end of the second column; (C) contour plot of a 2D chromatogram after data visualization (time windows of x-axis: 7-130 min, y-axis: 0-8 sec); and (D) 3D plot of the same 2D chromatogram.

4.4. Compound-class specific analysis of petroleum hydrocarbons by GC×GC

Comprehensive multidimensional separation coupled to a FID or MS provides a detailed characterization of petroleum fractions, allowing the separation of different classes of structurally related compounds. Given the fact that a mass spectrum is obtained for each data point in the 2D separation space, the chemical information in each spectrum was exploited to decide whether the compound is, for example, an aliphatic or an aromatic hydrocarbon. Also in order to obtain a better separation between compound classes, and to be able to identify them and define the belonging of compounds to a certain group of compounds, one particular hydrocarbon sample (crude oil API 40.5) was subjected to fractionation on a silica gel column. The individual fractions obtained by this column chromatographic fractionation were individually subjected to comprehensive GC×GC separation, expecting that the fractionation had led to an efficient separation of the different hydrocarbon classes.

For the fractionation, an aliquot of the crude oil (200 mg) was subjected to liquid chromatography using 3 g of silica gel (0.040 – 0.063 mm particle size) as a stationary phase. The crude oil sample was dissolved in hexane (2 ml) and applied to the top of a column (of 2 cm inner diameter) filled with silica gel slurry. Saturated hydrocarbon fractions were eluted with hexane (30 mL) and aromatic fractions were eluted with hexane:dichloromethane (8:2) (30 mL) while 10 mL of dichlormethane:methanol (1:1) was applied on the column in order to elute polar compounds if such existed in the petroleum hydrocarbon mixture. The fractionation procedure described in [204] and [205] was taken as a reference for the performed fractionation. Several fractions were collected and consequentially analysed by GC×GC/MS. The contour plots with identified compounds in different fractions are represented in the **Figure 4.2**.

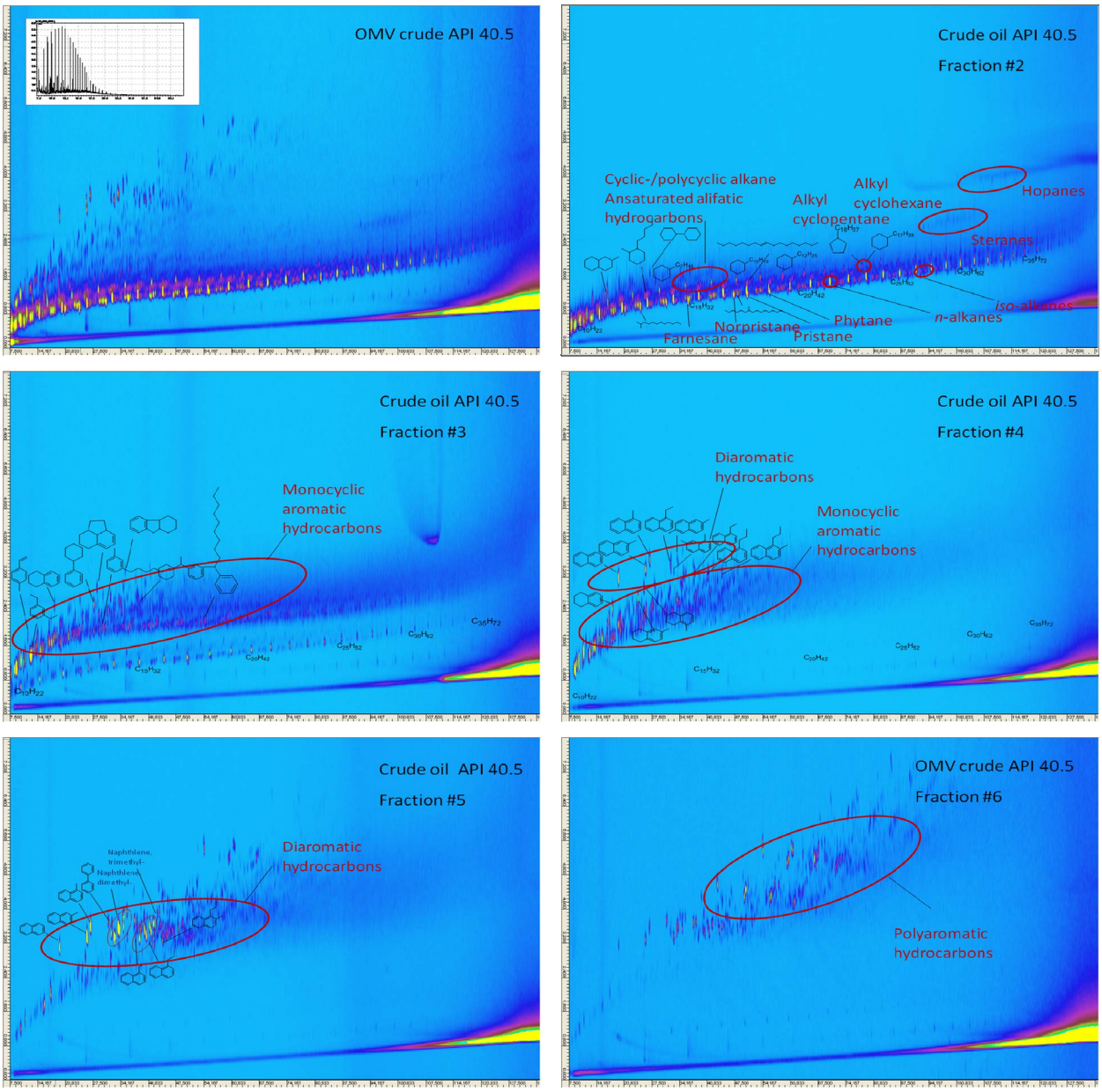


Figure 4.2 GC×GC/MS contour plots of crude oil (API 40.5) fractions after fractionation on silicagel column (time windows of x-axis: 7-130 min, y-axis: 0-8 sec).

The information was extracted from the mass spectra and the 2D data array and used for the construction of a 2D-plot of the data with a chemical map for each investigated sample that indicates where representatives of a certain class of compounds are located in the 2D separation space, see **Figures 4.3**, **4.4** and **4.5**. With the 1D-GC method only a boiling point distribution is obtained (when using an apolar column for separation), while the GC×GC results provide more information about the nature of the compounds which allows to discriminate between major chemical classes of hydrocarbons. Different compound-classes, e.g. linear and branched alkanes, unsaturated alkanes, alkyl cyclopentanes and cyclohexane, polycycloalkanes, monoaromatics, di-/tri-/tertra-/penta-aromatic hydrocarbons, were detected and identified in all analysed oil mixtures. From this variety of different chemical compound classes, five groups of compound classes were separated for the following quantitative experiments: *n*-

alkanes; *iso*-alkanes; cyclic-/polycyclic alkanes; monoaromatic hydrocarbons and polyaromatic hydrocarbons.

Once the regions in the 2D separation space have been defined where individual compound classes of the analysed hydrocarbon mixture are eluted, the integration of these compound-classes can be performed. For GC×GC data processing the GC Image software has been used, which offers the possibility of integrating the peak volumes. The FID is used as a detector for quantitative analyses, since it is a universal detector the response of which is directly proportional to the analyte's mass. When compound-classes in the GC×GC chromatograms were defined and integrated the absolute percentage composition of each oil type was calculated on the basis of the total peak volume of all (detected and integrated) peaks in each sample, see **Tables 4.3** and **4.4**. The compound-class compositions of different oils presented in the table demonstrate and prove the – in some cases significant – difference in chemical composition. GC×GC is a unique technique which allows already from the contour plot a first visual interpretation of the data. It furthermore provides a detailed picture of the qualitative composition of the sample which only in rare cases (where dominant marker peaks are present) can also be obtained from 1D chromatography. Furthermore, quantitative evaluation of the GC×GC chromatogram gives quantitative information about the analysed oil composition even without prior fractionation and may, as some authors have pointed out, even make the clean-up over Florisil unnecessary.

Before proceeding to further quantitative GC×GC analyses of complex petroleum hydrocarbon mixtures, it was decided, however, to check the quantitative possibilities of the comprehensive two-dimensional GC on a less complicated mixture of compounds. A mixture of fatty acid methyl esters (FAME) was chosen for this purpose, see the next Section.

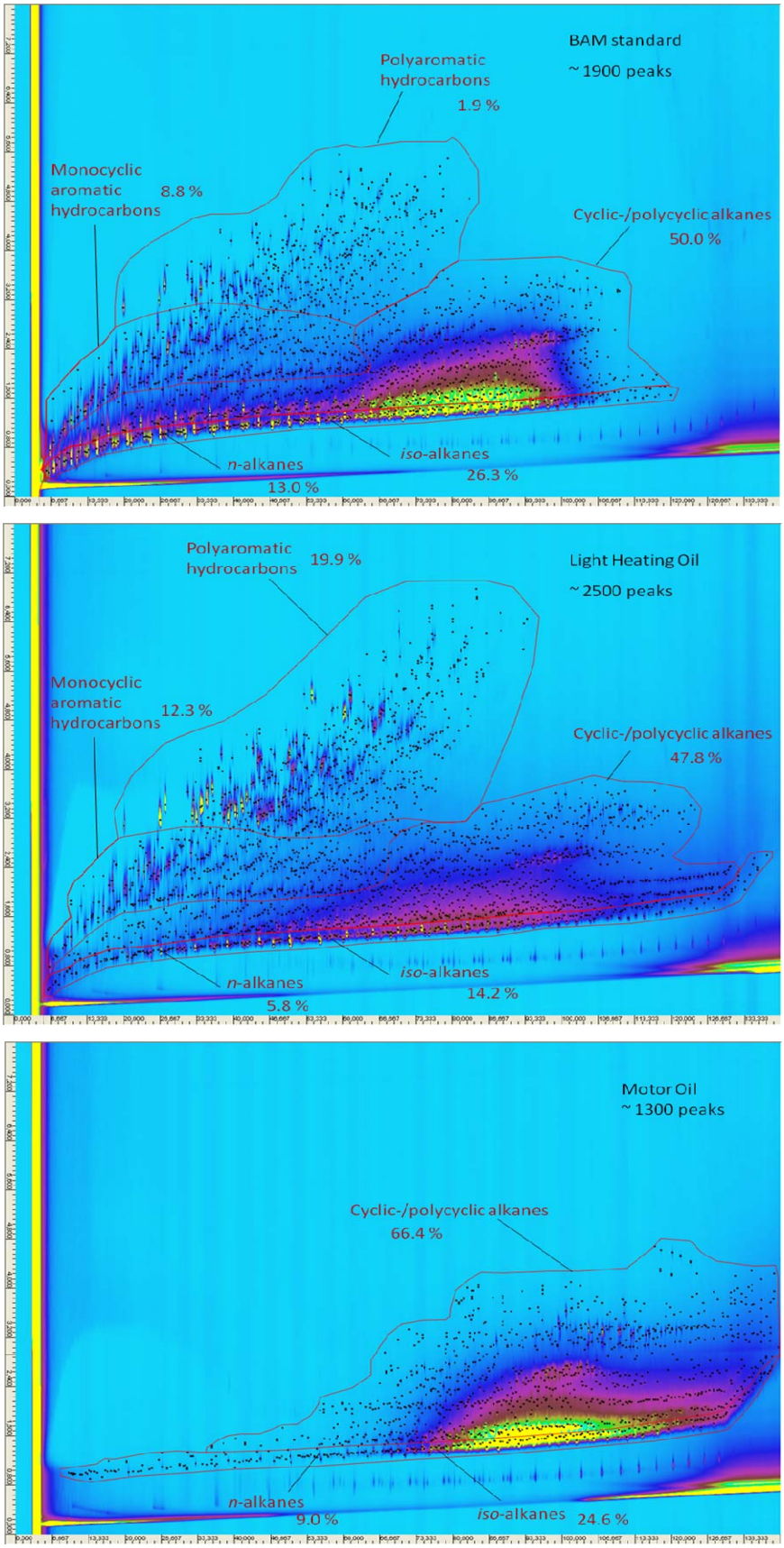


Figure 4.3 GC×GC/FID colorful plots with detailed compound-class separation of the BAM standard, light heating oil and motor oil (percentage composition is calculated on the base of total peak volume), time windows of x-axis: 7-140 min, y-axis: 0-8 sec.

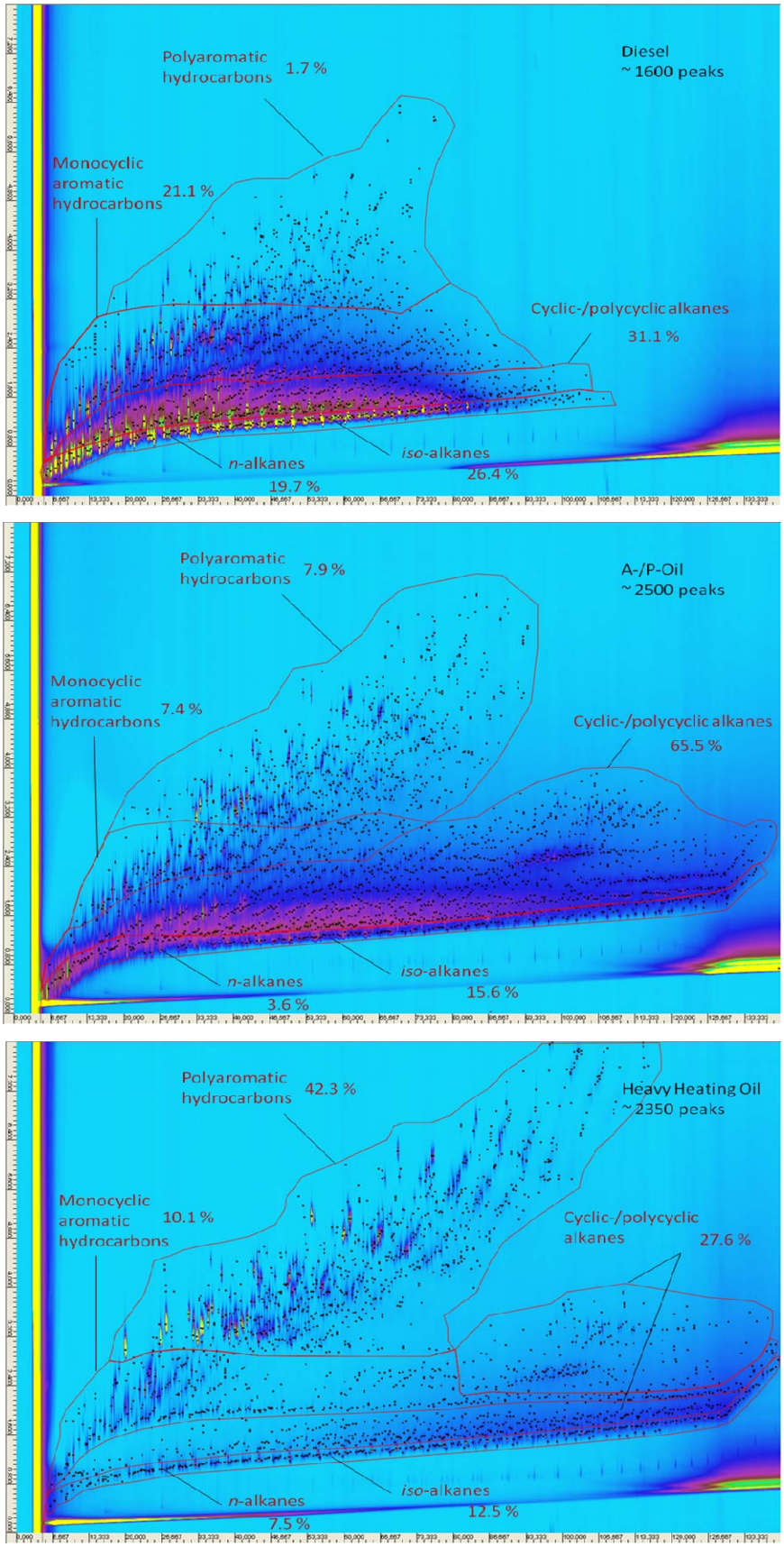


Figure 4.4 GC×GC/FID colorful plots with detailed compound-class separation of diesel, A-/P-oil and heavy heating oil (percentage composition is calculated on the base of total peak volume), time windows of x-axis: 7-140 min, y-axis: 0-8 sec.

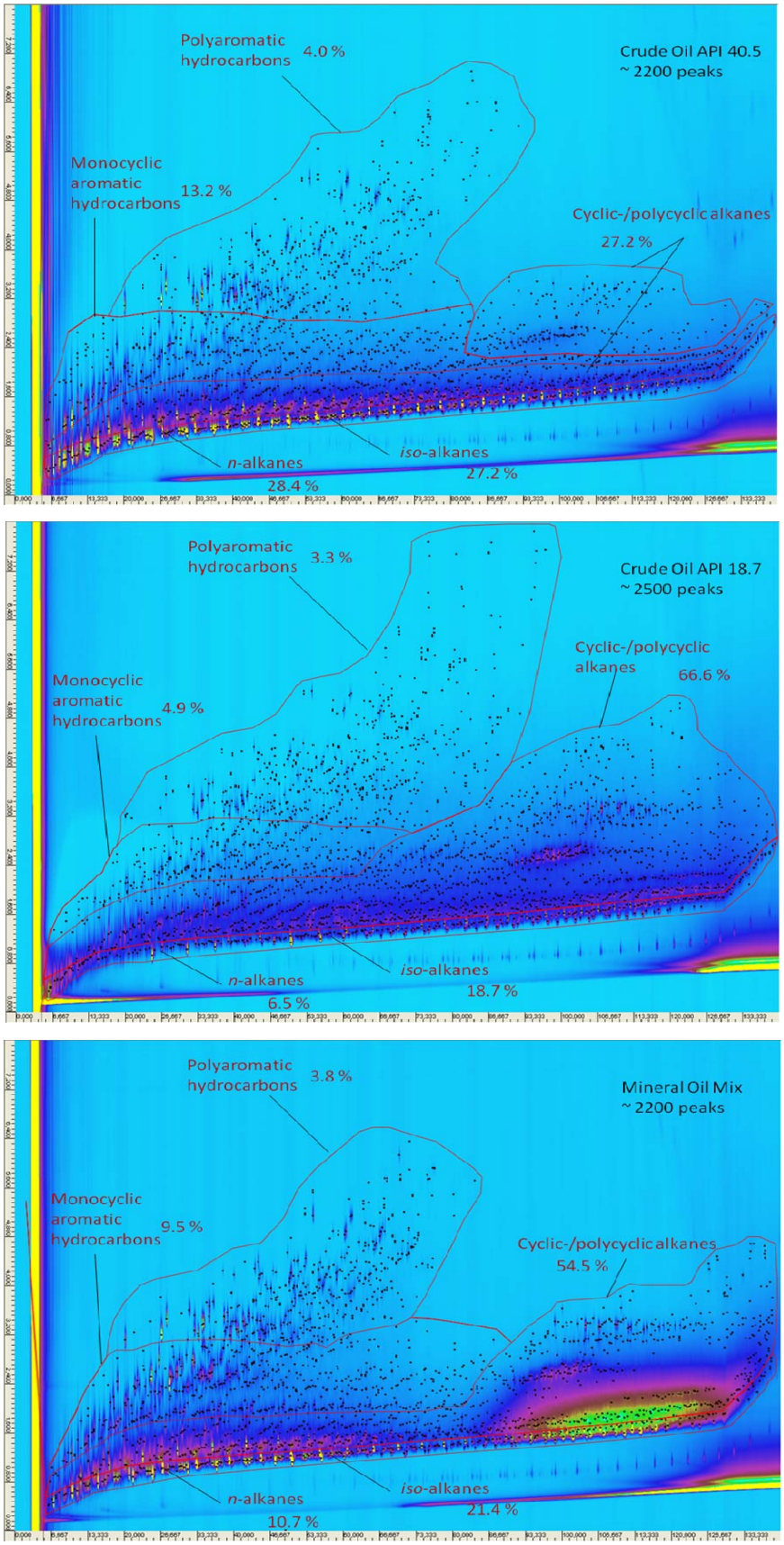


Figure 4.5 GC×GC/FID colorful plots with detailed compound-class separation of crude oil API 40.5, crude oil API 18.7 and mineral oil mixture (percentage composition is calculated on the base of total peak volume), time windows of x-axis: 7-140 min, y-axis: 0-8 sec.

Table 4.3 Compound-class composition of different oils after clean-up based on their GC×GC/FID response.

	n-alkanes	iso-alkanes	Cyclic-/polycyclic alkanes	Monocyclic aromatic HC	Polyaromatic HC	Other	Total Peak Volume	Normalised response according to BAM, %
	compound-class absolute percentage composition, %							
BAM	13.0	26.3	50.0	8.8	1.9		4523446223	100
Light heating oil	5.8	14.2	47.8	12.3	19.9		2913681211	64.9
Motor oil	9.0	24.6	66.4	0.0	0.0		3638210951	81.7
Diesel	19.7	26.4	31.1	21.1	1.7		3970255065	87.9
A-/P-Oil	3.6	15.6	65.5	7.4	7.9		2923307958	63.2
Heavy heating oil	7.5	12.5	27.6	10.1	42.3		1217442500	26.0
Crude oil API 40.5	28.4	27.2	27.2	13.2	4.0		3160917315	69.5
Crude oil API 18.7	6.5	18.7	66.6	4.9	3.3		2208411956	50.1
Mineral oil mix	10.7	21.4	54.5	9.5	3.8		4478867104	93.8

** : All Peak Volume values have been normalised to the same reference concentration, 2 mg/mL*

Table 4.4 Compound-class composition of different oils before clean-up based on their GC×GC/FID response.

	n-alkanes	iso-alkanes	Cyclic-/polycyclic alkanes	Monocyclic aromatic HC	Polyaromatic HC	Other	Total Peak Volume	Normalised response according to BAM, %
	compound-class absolute percentage composition, %							
BAM	13.0	26.2	50.1	8.7	1.9		4532706166	100
Light heating oil	5.8	12.8	44.0	12.9	24.6		3180980696	70.7
Motor oil	8.8	23.1	68.1	0.0	0.0		3781657284	84.7
Diesel	18.1	24.6	30.0	20.6	2.0	4.6	4291812132	94.9
A-/P-Oil	3.4	14.8	62.1	8.9	10.7		3257229389	70.3
Heavy heating oil	6.7	11.2	23.5	9.6	49.0		1472490627	31.4
Crude oil API 40.5	27.0	26.0	26.9	14.3	5.7		3323532115	72.9
Crude oil API 18.7	6.3	18.2	65.3	5.4	4.7		2283174849	51.6
Mineral oil mix	10.1	21.9	52.7	10.0	5.4		4754401569	99.4

** : All Peak Volume values have been normalised to the same reference concentration, 2 mg/mL*

4.5. GC/MS Analysis of trace fatty acid methyl esters (FAME) in jet fuel using one- and two-dimensional gas chromatography

Before evaluating the ability of GC×GC for quantitative analysis of complex samples, a simpler example was chosen which should allow to better understand the parameters that affect quantitative evaluation in 2D-GC, and also to assess potential differences in the results obtained with the different modes of quantitative analysis as available with the ChromSquare and the GC Image softwares. The determination of trace fatty acid methyl esters (FAME) in Jet Fuel was chosen for this purpose; moreover, it is of practical value and of industrial interest.

4.5.1. Introduction

In an effort to reduce the emission of greenhouse gases, and to increase fuel supply security, biofuel production is developing rapidly. Biodiesel is produced by the trans-esterificaton of vegetable oil to form fatty acid methyl esters (FAME). Biodiesel is an advanced biofuel that nowadays is typically blended with petroleum diesel to be as a fuel in Europe and the US.

On the other hand, jet fuel – being of very similar chemical composition to diesel – is the transportation fuel with the most stringent specifications, while it is sharing its production, transportation and distribution facilities with bio- or blended diesel. While zero cross-contamination of jet fuels with FAME is desired in the aviation industry, it has been recognized that a small amount of cross-contamination must be tolerated for jet fuel in shared fuel systems. A particular problem contributing to potential contamination of jet fuels with FAMEs is that the FAMEs are surface-active. This means that they can adhere to pipe and tank walls as the diesel passes through, and then eventually be released from the walls into the following product, which may be jet fuel. A further source of cross-contamination may arise from small amounts of diesel containing FAME remaining within distribution manifolds, tanks, vehicles and pipes which are also used for jet fuel transport and handling. As these are typical situations, the risk of cross-contamination of aviation fuel with biodiesel or biodiesel-containing products is high, and must be understood at every single handling step from the refinery to the airport. At high enough concentrations, FAME can impact the thermal stability of jet fuel, leading to coke deposits in the fuel system. FAME contamination can also impact the solidification point of jet fuel, resulting in fuel gelling. Such conditions can result in engine operability problems and possible engine flameout.

The aircraft and engine manufacturers have conceded that levels of FAME less than 5 ppm are acceptable. This limit is extremely low, which poses some large operational challenges (optimising product sequencing, using specific cleaning procedures and defining appropriate interfaces).

Another challenge is the ability to identify and quantify this trace amount of FAME. Since a concentration of 5 ppm is barely detectable in a highly complex mixture such as aviation fuel, only

dedicated methods and instrumentation are capable of this task. A specially configured gas chromatograph is used as one of the industry accepted methods (IP-585/10) for detection, [206]. Concurrently, aviation industry is working on an emergency limit of 30 ppm. This limit would stay in place until the 100 ppm approval is granted and implies that the aircraft having between 5-30 ppm FAME does not have to be diverted to the closest airport but can fly to its final destination.

Currently used standard method IP-585/10 is based on gas chromatoghraphy – mas spectrometry (GC-MS) for the identification and determination of specified FAMEs in the concentration range 4.5 ppm to 150 ppm, where the net of jet fuel is run together with an internal standard on the GC-MS with a polar column to separate polar FAME from the hydrocarbon matrix of the jet fuel. Qualitative and quantitative analysis is performed by a simultaneous Scan and Selective Ion Monitoring (SIM) measurement.

GC×GC has been shown to be an effective approach for the characterization of petroleum–based fuels such as gasoline and diesel. GC×GC has been applied to the analysis of FAMEs in a variety of works [207,208]. The majority of studies have focused on characterizing the distribution of fats in food samples, some of them also have demonstrated that FAMEs could be monitored with GC×GC in the presence of petroleum hydrocarbons; however, they did not provide detailed quantitative GC×GC results in comparison with the results of 1D-GC following a standard method for determination of biodiesel blends. **Figure 4.6** presents 1D-GC/MS and GC×GC/MS chromatograms of a jet fuel sample (without contaminations of FAMEs). The region where FAME compound elute is marked as a circle. FAMEs elute after the main fraction of the jet fuel, what allows determination of FAME traces in jet fuel samples without any interference.

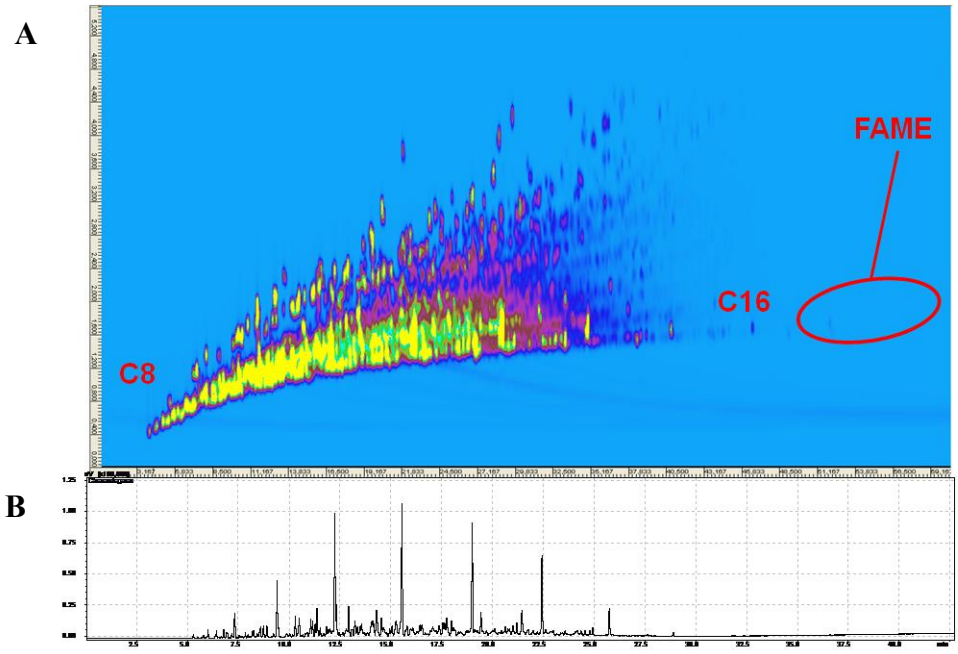


Figure 4.6 A: GC×GC/MS chromatogram of Jet Fuel (time windows of x-axis: 7-60 min, y-axis: 0-8 sec.), B: 1D-GC/MS chromatogram of Jet Fuel.

The primary goal of the experiments presented in this section was to evaluate the usefulness and applicability of GC×GC for FAME determination in jet fuel and to compare quantitative results obtained by one- and two-dimensional GC.

4.5.2. Experimental

One-dimensional chromatographic analyses were carried out with a Shimadzu GC2010-Plus gas chromatograph equipped with a QP-2010 Ultra quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) a flame ionisation detector (FID), an OPTIC3 programmed-temperature vaporising injector (ATAS, Eindhoven, The Nederland), an AOC-5000 Plus programmable autosampler (Shimadzu, Kyoto, Japan). The column used was an Innowax polyethylene glycol (PEG) column (Agilent J&W GC Columns, USA) 50 m × 0.2 mm × 0.4 µm , with the helium (purity >99.999%, Messer) carrier gas flow of approximately 0.6 mL/min. GC oven program: 150°C (5 min), 12°C/min → 200°C (17 min), 3°C/min → 252 °C (6.5 min). 1µL injection in splitless mode at 260°C. A fritted liner (OPTIC liner with deactivated glass frit at 15 mm above the liner bottom end), packed with deactivated borosilicate glass wool was used. MS ion source: 200 °C. Interface: 250 °C. Solvent cut time: 20 min. MS detection was operated in simultaneous scan and selected ion monitoring (SIM) mode. MS detection in the scan range 40-650 m/z and event time 0.3 sec (3.3 Hz); with equal event time of 0.3 sec for SIM mode detection.

The standard IP-585/10 requires that six defined FAME species are measured. The ions of the individual FAMEs used in the SIM detection mode are the following:

Methyl hexadecanoate	(methyl palmitate)	$C_{17}H_{34}O_2$	C16:0	SIM ions : 227, 239, 270, 271
Methyl heptadecanoate	(methyl margarate)	$C_{18}H_{36}O_2$	C17:0	SIM ions : 241, 253, 284
Methyl octadecanoate	(methyl stearate)	$C_{19}H_{38}O_2$	C18:0	SIM ions : 255, 267, 298
Methyl octadecenoate	(methyl oleatestearate)	$C_{19}H_{36}O_2$	C18:1	SIM ions : 264, 265, 296
Methyl octadecadienoate	(linoleate)	$C_{19}H_{34}O_2$	C18:2	SIM ions:262, 263, 264, 294, 295
Methyl octadecatrienoate	(linolenate)	$C_{19}H_{32}O_2$	C18:3	SIM ions : 236, 263, 292, 293

The deuterated methyl heptadecanoate-d33 (C17:0 (d33)) is used as an internal standard (SIM ions 317).

The GC × GC analyses were carried out with the same system, where the first dimension separation was performed on an apolar DB-5MS 30 m × 0.25 mm × 0.25 µm capillary column, coated with 95% dimethyl-/5% diphenylpolysiloxane polymer (Agilent J&W GC Columns, USA), and the second dimension separation by a BPX-50 capillary column, 1.45 m x 0.1 mm x 0.1µm, coated with 50% dimethyl-/50% diphenylpolysiloxane polymer (SGE Analytical Science), situated in the same oven. An uncoated 1 m × 0.2 mm ID, deactivated fused silica capillary (Supelco, Bellefonte, PA) was used as a loop between the two separation columns. Helium (purity >99.999%, Messer) was used as the carrier gas at a flow rate of 1.3 mL/min and head pressure of 279.8 kPa. GC oven program: 150°C (0 min), 2°C/min

→ 230°C (0 min), 70 °C/min → 330 °C (5 min). 1 µL injection in split mode (1/50) at 260°C for samples with high FAME content. The splitless mode was applied for FAME standards with low concentration of FAMEs for the determination of limit of detection and limit of quantitation of the GC × GC method A fritted liner (OPTIC liner with deactivated glass frit at 15 mm above the liner bottom end), packed with deactivated borosilicate glass wool was used. Solvent cut time: 23.5 min. MS detection was operated in the scan range 40-650 m/z with an event time of 0.04 sec (25 Hz). For cryomodulation, a dual jet modulator (ZOEX) with liquid nitrogen with modulation time of 8 seconds and a hot jet (at 340°C) with pulse duration of 600 msec was applied.

Instrument control and data evaluation was done in both cases with the GCsolution and GCMSsolution softwares (Shimadzu). GC Image software, Version 2.2b0 (Lincoln, University Nebraska) - Software for Multidimensional Chromatography – was used for GC×GC data processing. The OPTIC3 high performance injector is configured and controlled through the ATAS Evolution Workstation software. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated from experimental data according to the calibration method (as outlined in DIN 32645 [191]) by the Excel macro ValiData [192].

All FAME standards were purchased from Sigma-Aldrich (Steinheim, Germany), *n*-dodecane used as a solvents was purchased from Sigma-Aldrich (Vienna, Austria). Jet Fuel used for spiking was provided by OMV (Vienna, Austria): Sample 1 and Sample 2 are spiked with ~50-60 ppm of FAME, Sample 3 with ~200 ppm of FAME and Sample 4 with ~ 200 ppm C18:1. Unknown jet fuel sample was provided by QuantaRed (Vienna, Austria).

Calibration with the internal standard was performed in the calibration range 0.2-100 ppm by linear regression. *n*-Dodecane was used as a solvent. Calibration standard solutions for determination of limit of quantitation and limit of detection of both methods were prepared in a range from 0.1 to 2 ppm in *n*-dodecane.

4.5.3. Results and discussion

Six FAME species have been identified by Energy Institute (EI – is the professional body for the energy industry, [209]) as FAMEs that represent 95% of the potential sources of jet fuel contamination: methyl palmitate (C16:0), methyl margarate (C17:0), methyl stearate (C18:0), methyl oleate (C18:1), methyl linoleate (C18:2) and methyl linolenate (C18:3). The listed FAMEs are used for standard sample preparation in order to perform quantitative analysis. The deuterated methyl heptadecanoate-d33 (C17:0 (d33)) is used as an internal standard.

Standards and jet fuel spiked with FAMEs were first analysed by one-dimensional GC according to the standard method IP-585/10, 1D-GC chromatograms of FAME standard and one spiked sample are presented in the **Figure 4.7**.

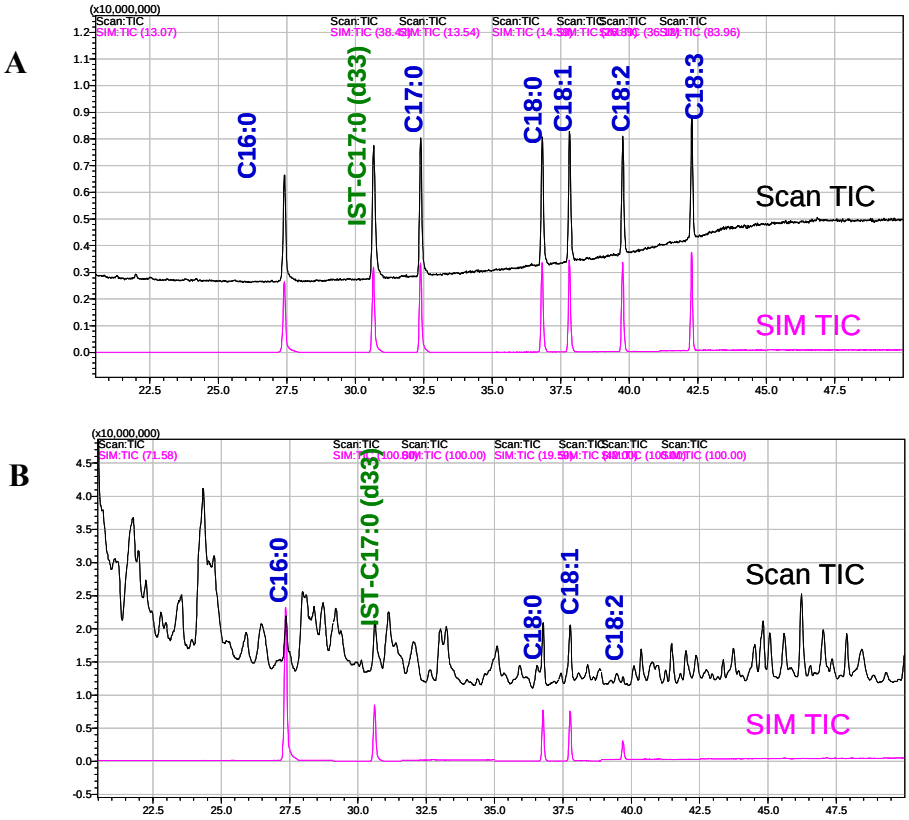


Figure 4.7 1D-GC chromatograms of A: FAME standard solution (10 ppm) and B: Sample 2 (Jet fuel A1 + FAME ~50.095 ppm).

The same samples (FAME standard and jet fuel spiked with FAMEs) were analysed by GC×GC/MS. Data acquisition was started at 23.5 min, after the main fraction of jet fuel was eluted. **Figure 4.8** presents the GC×GC chromatograms obtained from the standards and jet fuel spiked with FAME compounds.

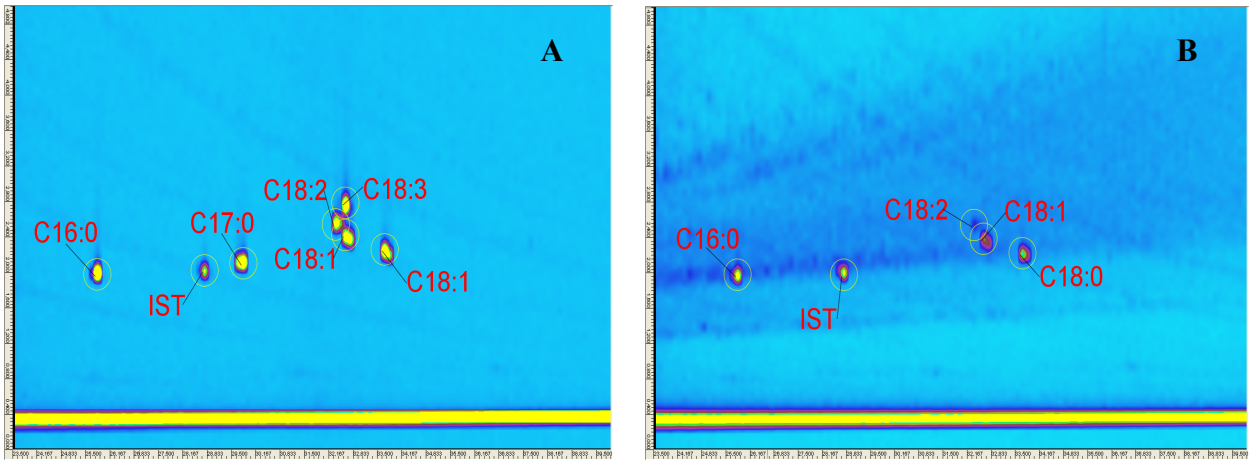


Figure 4.8 A: GC×GC/MS chromatograms of the FAME standard sample of FAME at 40 ppm.
B: GC×GC/MS chromatogram of the Sample 2 with total FAME concentration ~ 50 ppm **(**time windows for both plots are x-axis: 23.5-40 min, y-axis: 0-4.9 sec).

In order to compare the performance of the two methods, the same spiked jet fuel samples were analysed and quantified in the same calibration range. Split injection with split ratio 1:50 was used in the case of GC×GC analyses, as to prevent the saturation of the detector for the more abundant sample constituents. Quantitative GC×GC shows well comparable results to the ones obtained according to IP-585/10 (**Table 4.5**). The observed difference in total FAME concentration for all analysed samples is between 2 and 17 ppm. The larger difference is obtained for the sample with a high amount of FAME, which actually is outside of the calibrated range. Relatively small and thus acceptable differences, from 0.1-3 ppm, versus the reference values according to standard method IP 585/10 are obtained for the individual FAME species. That indicates that the used GC×GC method is able to successfully identify and quantify FAME components in jet fuel with high precision.

Table 4.5 Quantitative results of FAME concentrations found in various jet fuel samples analysed by one- and two-dimensional GC.

	Sample 1 FAME, ppm		**Sample 2** FAME, ppm		**Sample 3** FAME, ppm		**Sample 4** FAME, ppm	
	GC×GC	*IP-585/10*	*GC×GC*	*IP-585/10*	*GC×GC*	*IP-585/10*	*GC×GC*	*IP-585/10*
C16:0	27.2	29.2	13.5	17.5	8.4	10.2		1.1
C17:0								
C18:2	5.7	4.5	5.1	3.4	38.3	39.6		
C18:3					21.3	15.4		1.1
C18:1	13.8	16.5	13.8	13.7	115.1	116.9	197.0	211.2
C18:0	17.5	16.8	16.0	15.9	5.9	4.3		1.0
Sum	**64.1**	**67.0**	**48.4**	**50.5**	**189.0**	**186.4**	**197.0**	**214.3**

The proposed method is capable of analysing and detecting much smaller concentrations of FAMEs in the splitless injection mode. **Table 4.6** represents the limits of detection obtained in the splitless mode. In comparison with the ones obtained according to standard IP-585/10 they are lower by more than one order of magnitude.

Table 4.6 Limits of detection and coefficient of determination of 1D-GC and GC×GC method for FAME traces determination, calibration range 2-100 ppm.

FAME	**IP-585/10**		**GC×GC**	
	LOD, ppm	**R2**	**LOD, ppm**	**R2**
C16:0	0.40	0.9997	0.06	0.9977
C17:0	0.24	0.9995	0.03	0.9963
C18:0	0.22	0.9993	0.04	0.9979
C18:1	0.23	0.9993	0.05	0.9975
C18:2	0.24	0.9995	0.03	0.9894
C18:3	0.25	0.9994	0.07	0.9962

Another advantage of the GC×GC/MS method lies in its principle suitability as a screening detector for unknown or unexpected compounds: Despite the large number of peaks that are obtained in

the 2D separation space, it is still possible to easily spot compounds that are not expected to be present in a given sample due to the appearance of signals at unusual or unexpected places in the 2D separation space. The **Figure 4.10** presents a GC×GC/MS chromatogram of an unknown jet fuel sample, where the presence of FAMEs is not detected. Only the peak of the internal standard together with other unknown peaks is seen, which are most likely the traces of other unexpected compounds present in the analysed sample. GC×GC/MS is a very powerful tool, it is capable to detect and identify not only target compounds, in our case FAMEs, but also other unwanted compounds, that make this method more informative. With the help of the MS detector the unknown peaks were identified and it was found that the analysed jet fuel sample contain some traces of sulfurous acids, disulfides, some remains of alkanes with long and branched chains and oleanitrile as individual compound.

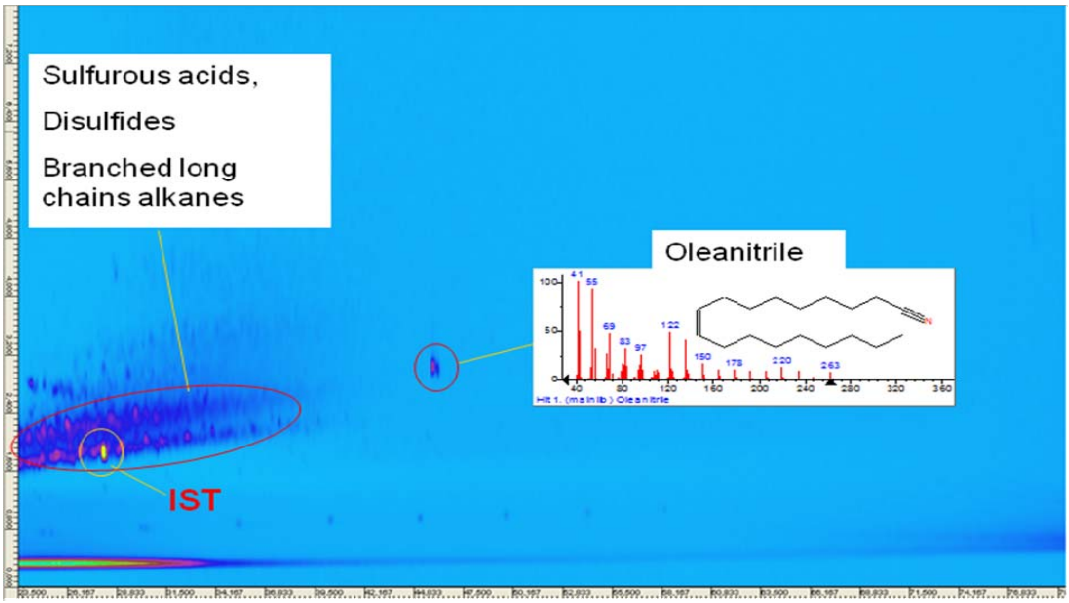


Figure 4.10 GC × GC/MS chromatograms of unknown jet fuel sample (IST: internal standard, deuterated methyl heptadecanoate-d33, time windows of x-axis: 23.5-40 min, y-axis: 0-4.9 sec).

4.5.3. Conclusions

In this section the applicability of GC×GC for quantitative analaysis of FAMEs in jet fuels was assessed. It was demonstrated that this method produces quantitative results that compare well with the standard method and that do not appear to have a systematic bias. The method is well suited for quantitative analysis of FAMEs in jet fuels and has the capacity of detecting concentrations significantly lower than stipulated by the currently accepted standard IP-585/10. Moreover, GC×GC provides the advantages of increased selectivity, separation power, sensitivity, and improved interpretability of the data in comparison to one-dimensional GC.

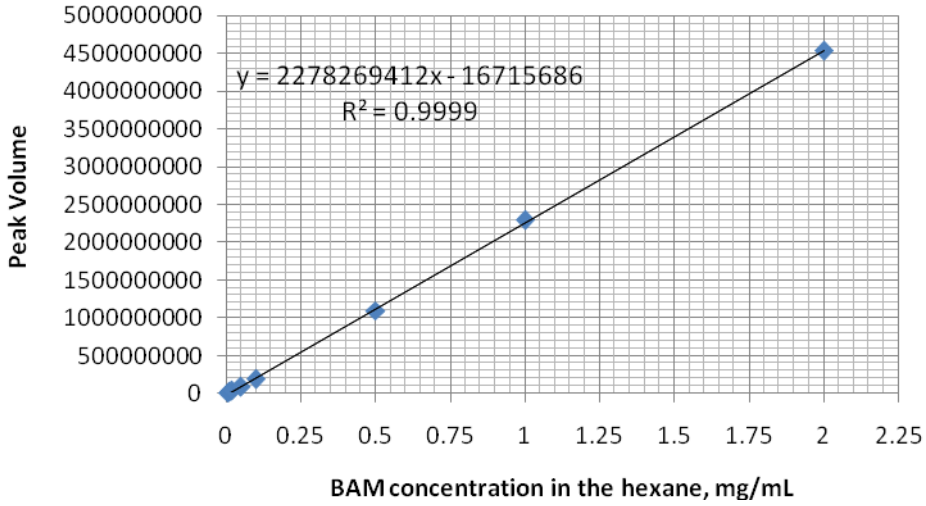
These results underline the general ability of GC×GC for quantitative analysis.

4.6. Quantitative determination of petroleum hydrocarbons by GC × GC/FID

4.6.1. Calibration and LOQ / LOD of GC×GC method for petroleum hydrocarbon determination

In order to determine the practical detection limit (LOD) and quantitation limit (LOQ) for oil in water, a series of BAM calibration standards was prepared at 9 calibration levels in the range 0.005 – 2 mg/mL in hexane, which corresponds to 0.25 – 100 ppm of oil in water (OIW by DIN ISO 9377-2:2000 or OSPAR methods). These standards were analysed by the previously described GC×GC/FID method. The calibration showed excellent linearity over the investigated range, **Figure 4.10**. The relative standard deviation (RSD) was calculated only for one calibration level (1 mg/mL) and was 0.8 % for 6 repetitive injections. Since the analysis time of each sample injected is 140 min, measurements were not done in triplicate, but only single injections at each calibration level were done, otherwise the total analysis time would have amounted to 3 days. The long analysis time for a single run is not the only one concern. The consumption of liquid nitrogen used for a cryogenic dual-jet dual-stage loop-type modulator is usually high. From our experience a 120 L dewar of liquid nitrogen can be consumed in 1.5 – 2 days if the system is continuously in operation.

A



B

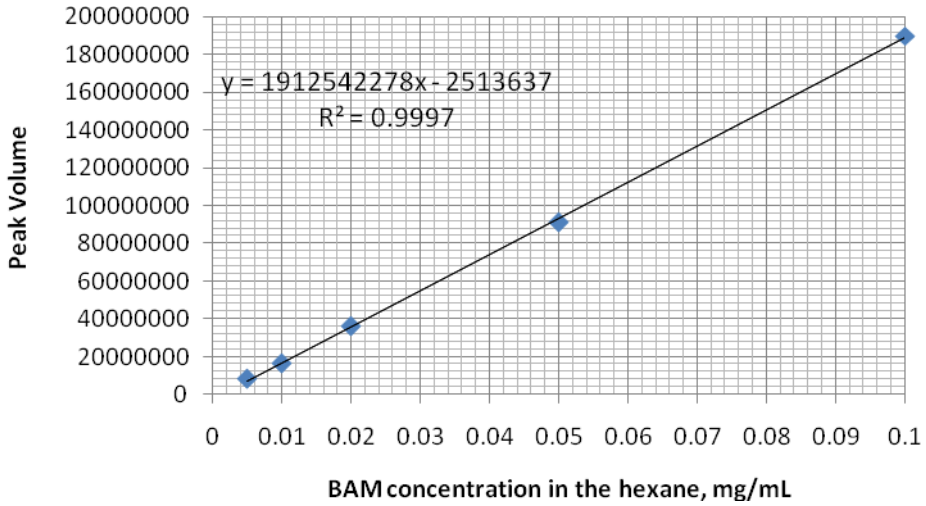


Figure 4.10 BAM standard calibration graphs for GC×GC/FID analyses, (A) linearity range 0.005 – 2 mg/mL in hexane, (B) lineariy range 0.005 – 0.1 mg/mL in hexane for LOD and LOQ calculation

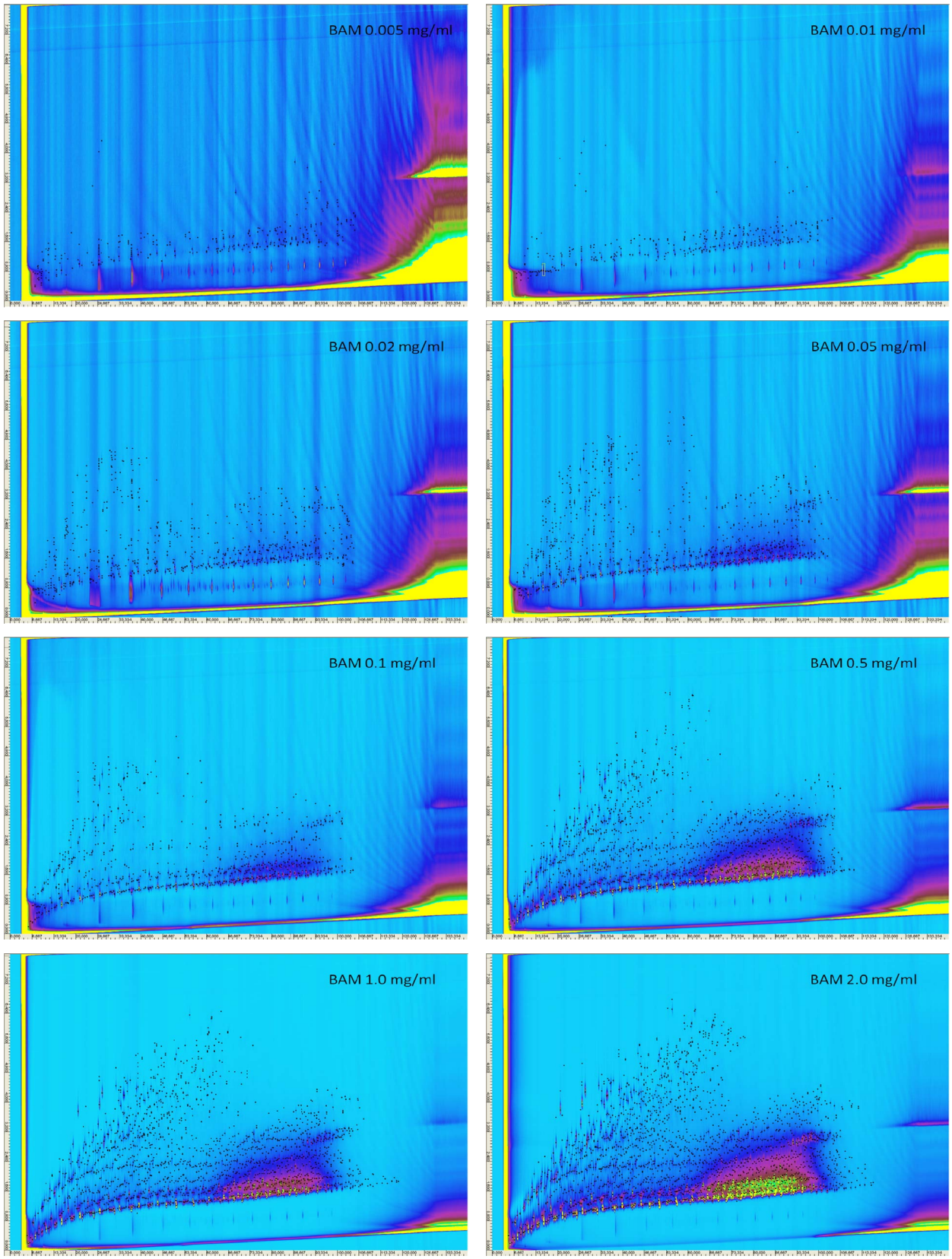


Figure 4.11 GC×GC/FID chromatograms of BAM calibration standards at different calibration levels in a range 0.005 – 2 mg/mL in hexane. The colour scale was adjusted for each graph individually to produce maximum contrast in the contour plot (time windows of x-axis: 0-140 min, y-axis: 0-8 sec).

GC×GC chromatograms of each calibration level have been integrated and the total peak volume was used for construction of the calibration line. The integration of GC×GC chromatograms is not a difficult procedure. With a help of the software (GC Image) all peaks can be integrated and integrated peak volumes are summed into the total peak volume, **Figure 4.11**. Only for the very low oil concentrations more attention must be paid to correctly set parameters of the integration procedure.

The importance of correct integration in 1D-GC petroleum hydrocarbon analyses has been already discussed in *Section 2.3*. The correctly defined integration windows (from C10 to C40) and correct background subtraction has a big importance in 1D-GC analyses, especially for a chromatographic signal of low intensity. The only difficult point in the integration of petroleum hydrocarbon signals in GC (in general) is that it is difficult to get consistent results in integration of low intensity signals, since the peak that is to be integrated is a very wide signal. Comparing the peak areas in 1D-GC and GC×GC, it must further be considered that the one-dimensional signal is a sum of unresolved peaks, which overlay and together finally become detectable, while in GC×GC the significantly higher chromatographic resolution allows detecting peaks individually. Whether these peaks can be integrated or not, depends on the size of the peak, meaning that the smaller peaks will probably remain undetected and do thus not contribute to the total area sum.

The detection of small total petroleum hydrocarbon concentrations in GC × GC can thus be problematic, because, unlike in 1D-GC, where the limit of detection (LOD) is depending on the ability to reproducibly integrate a small signal over a wide time window, in GC×GC many individual compounds have to be detected, which may or may not be above the LOD. The limit of quantification (LOQ) of GC×GC/FID for BAM standard was found to be 0.008 mg/mL (0.42 ppm OIW) and the limit of detection 0.002 mg/mL (0.11 ppm OIW), **Table 4.7**. These LOQ and LOD values are comparable, or actually are the same, as the results obtained by DIN ISO 9377-2:2000 without preconcentration. Therefore GC×GC, apart from improved selectivity, cannot offer a higher sensitivity in the determination of total petroleum hydrocarbons if preconcentration or large volume injection (LVI) is not applied. This finding is surprising and complementary to the observation in the quantitative analysis of FAMEs by one- and two-dimensional GC, where the GC×GC method offers the higher sensitivity for individual FAMEs. This apparent contradiction is explained by the fact that in individual compound analysis, the re-focusing of the analytes in the modulation step of GC×GC, together with the fast separation in the 2nd dimension leads to very narrow peaks, having a better signal-to-noise ratio than the original peak after 1D-GC. In the analysis of unresolved complex mixtures, however, 1D GC benefits from the coaddition of the intensities of the many co-eluting substances. When these are – at least partially – resolved in GC×GC, they must also be detected separately, therefore exceed the LOQ for each individual compound. Since this will not be the case for all compounds, sensitivity suffers from the compounds that go undetected, even though the ones that are detetected are detectable at lower levels than in 1D-GC.

Table 4.7 Comparison of LODs and LOQs of DIN ISO 9377-2, OSPAR and GC×GC/FID method for BAM standard. OIW: oil in water.

	Linearity range	R^2	Equations of calibration lines	Limit of Detection (LOD)	Limit of Quantitation (LOQ)
				OIW, mg/L	OIW, mg/L
ISO with preconcentration	0-50 mg/L	0.9998	y = 18013x + 69	0.01	0.02
ISO without preconcentration	0-80 mg/L	0.9993	y = 360x + 55	0.13	0.46
OSPAR	0-80 mg/L	0.9998	y = 20768x + 122	0.02	0.06
GC×GC-FID	0-100 mg/mL	0.9999	y = 2278269411x + 16715656	**0.11**	**0.42**

4.6.2. Determination of response factors for different petroleum hydrocarbons in GC×GC

The main advantage of the GC×GC method in the analysis of petroleum hydrocarbon is that it provides easily interpretable qualitative information. This is proven by the comparison of chromatograms of different oil samples (**Figures 4.3 – 4.5**). In contrast to 1D-GC based methods where the identification of product type(s) is not always straightforward and requires an experienced analyst of petroleum products, in GC×GC it becomes a relatively easy procedure. A unique outcome of GC×GC is the well-structured chromatograms that show a clear separation of different classes of chemical compounds within the 2D separation space. The presence of an ordered structure in the GC×GC plot allows the classification of chemical compounds and their assignment to such a class through visual inspection of the contour plot.

When analyzing different types of oil, the difference in the 2D-contour plot – being a representation of the difference in chemical composition – becomes evident. Less evident, however, is the fact that also the quantitative response of different types of oil (that is, the specific chromatographic response per unit mass) can differ largely. For this reason, a more detailed study was performed to assess whether the different specific response observed for different types of oil is a result of the differences in composition, assuming that the GC/FID response factors are different for different classes of compounds. This required the calculation of the relative response for each analysed oil type from the total peak volume in GC × GC. The response of BAM standard was taken as a reference. The specific responses of the analysed samples are always lower than the specific response of the BAM standard, and range from as low as 26 % (for heavy heating oil) to ca. 93.8% (for mineral oil mixture). The difference in relative response values of different types of oil obtained by GC×GC method is comparable with the difference obtained by 1D-GC method for the same types of oil, **Table 4.8**.

Table 4.8 Normalised relative response for different types of oil obtained by GC×GC/FID, ISO 9377-2 and OSPAR methods.

	Normalised relative response according to BAM, %		
	GC×GC-FID	**ISO 9377-2**	**OSPAR**
BAM	100	99.7	98.0
Light Heating Oil	64.9	68.4	70.0
Motor Oil	81.7	83.3	81.5
Diesel	87.9	81.0	92.7
A-/P-Oil	63.2	67.5	73.3
Heavy Heating Oil	26.0	33.9	37.0
OMV Crude Oil API 40.5	69.5	62.0	81.7
OMV Crude Oil API 18.7	50.1	55.0	54.7
Mineral Oil Mix	93.8	86.0	98.5

**: All peak integration values have been normalised to the same reference concentration*

Since so strongly different responses have been observed in 1D-GC (and also GC×GC) analyses an explanation to this observation was searched. . When extending the integration range, it could be concluded that the loss in response by not integrating part of the sample signal was not so big for the investigated samples that it could explain the different relative responses observed for different types of oil. The presence and effect of heteroatom-containing petroleum hydrocarbons is unlikely, because they should be removed during the clean-up step. The losses in the clean-up step in some cases were high, but not high enough to explain the difference in relative responses observed in some cases.

While all mentioned factors have been shown to have only a minor effect on the relative response, another factor, such as the different response factors of the FID for different compound classes remains to be checked. Since the FID is a carbon-selective detector, and as such, it is not responsive to the entire hydrocarbon molecule, it is therefore possible that different compound classes have different response factors. The FID response to hydrocarbons is generally proportional to the mass of carbon present in the sample in reduced form (that is, bound to either carbon or hydrogen atoms). FID response to hydrocarbons is generally proportional to the mass of carbon present in the sample in reduced form. This response – normally assumed to be uniform throughout different compound classes – is however reduced due to the presence of partially oxidized carbon atoms in compounds containing heteroelements and varies markedly with heteroatom and bond types. Compounds that contain, for example, oxygen, nitrogen, or halogens give varied responses but can be quantified by the FID if corrections to the experimental response are made or if calibration is done with the known compounds. For a detailed information about response factors, the reader is referred to the literature [210,211,212,213], while only a brief overview is given here.

The response factor (RF) is essentially a "correction" factor which measures the normalized response of a given compound at the detecting device and takes into consideration either chemical, instrumental or other factors that cause a non-uniform response. Dietz first tabulated the RFs, defined by **Equation 4.1**, for various compounds for the FID (flame ionization detectors), using *n*-heptane as a standard with a defined RF of 1.0. In **Equation 4.1**, (area of compound) and (area of standard) denote the

areas of the respective GC peaks, and (mass of compound) and (mass of standard), the respective masses prepared as standard solutions [211]. The RF factor found in this way will be called the practical RF.

$$RF_{practical} = \frac{(area\ of\ standard) \cdot (mass\ of\ compound)}{(mass\ of standard) \cdot (area\ of\ compound)} \qquad (4.1)$$

On this basis, the numerical value of the relative response factor is the weight of the component necessary to produce the same response (area counts) as the reference component. As defined by **Equation 4.1**, a response factor greater than 1.0 means that the detector is less sensitive to the component than to the reference material and thus the observed area must be multiplied by a number greater than 1.0 to give the same corrected area per unit weight for the component and the reference compound.

To predict response factor in cases of nonavailability of pure standards, the "effective carbon number" (ECN) method can be used. Only nonoxidized carbon atoms effectively produce a response in the FID, so the “effective” carbon atom is defined as one connected only to either other carbon or hydrogen atoms. The ECN for many compounds can be calculated from the heteroatoms and functional groups in the molecule and be used to obtain the RF from **Equation 4.2** However, even for those classes of compounds for which the ECN can be calculated, both calculated and measured RF's can vary by 25% [211] .

$$RF_{calculated} = \frac{(MW\ of\ compound) \cdot (ECN\ of\ standard)}{(MW\ of\ standard) \cdot (ECN\ of\ compound)} \qquad (4.2)$$

The RF found from the **Equation 4.2** will be called the calculated RF.

Using the **Equation 4.2** the FID response factors for different hydrocarbons were calculated relative to eicosane (C20), because the investigated range in GC×GC of hydrocarbons is large (from ~ C8 to ~C43) and C20 is the middle point of this range. RF change depending on the number of effective carbon is shown in **Figure 4.12**. This figure shows that hydrocarbons have different RF, depending on the compound-class they belong to. Theoretical RF for monoaromatic and polyaromatic hydrocarbons are lower than for aliphatic hydrocarbons, e.g. 10% smaller some tri-/tetra- and pentaaromatic hydrocarbons.

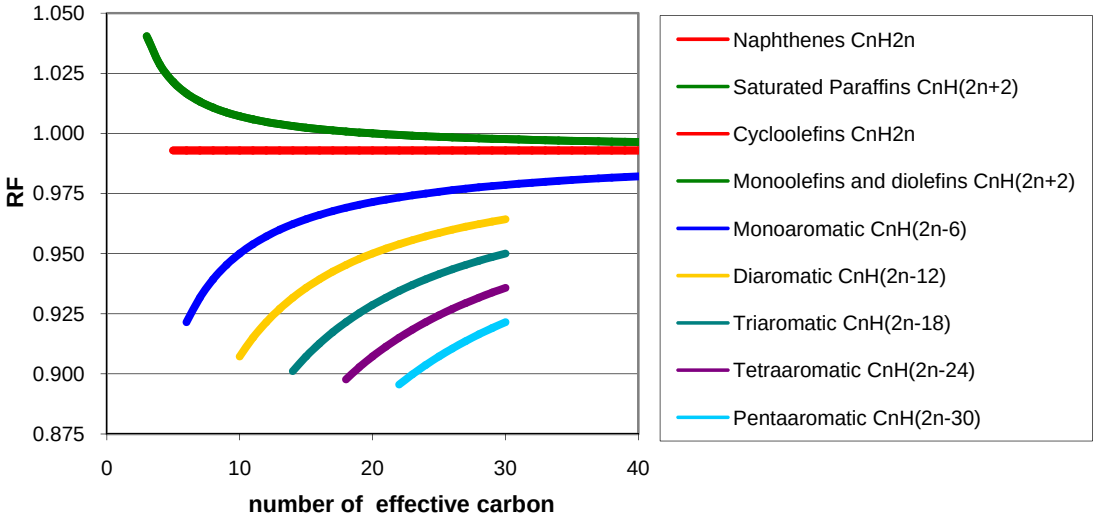


Figure 4.12 Calculated FID response factors for hydrocarbons, based on percentage by mass carbon, normalized to eicosane = 1 (Cycloolefins and mono-/di-olefins are corrected for hydrogenation of olefins).

As **Figure 4.12** indicates, there are indeed – at least in theory – differences in the FID response factors for different petroleum hydrocarbon classes. It should then be possible to correct the variable overall FID response of petroleum hydrocarbons if the largely variable composition of different oils is precisely known. To achieve this, it is necessary to perform compound-class specific analysis which, however, is only possible with GC × GC.

A mixture of *n*-alkanes, polyaromatic hydrocarbons (PAH) and some representatives of cyclic alkanes and monocyclic aromatic hydrocarbons was used for practical determination of specific response factors for individual compound-classes in petroleum samples analysed by GC×GC/FID. The GC×GC/FID chromatogram of this mixture is represented in the **Figure 4.13**.

The RF was calculated for each individual compound and normalized relative to the response of the classes of *n*-alkanes and *iso*-alkanes, whose response factors were considered as 1. All other representatives of petroleum hydrocarbons showed to have the same average response factor as *n*-alkanes with variation for some individuals from 0.83 as minimum to 1.18 as maximum (the highest RF 1.24 and 1.48 are not included because of inadequate peak shape), while the theoretical values, presented above, vary and deviate from 1.0 for some representatives, **Table 4.9**. The practical results for RFs in most cases matches with calculated RFs. It is, however, seen that the introduction of compound-class specific response factors (which are relatively close to 1.0) to correct the largely variable composition of different oils would not explain the experimentally observed difference (of 20 – 70%) in relative response for different types of oil. Thus, the RF can only explain to a small part the observed non-uniform response of different petroleum hydrocarbons.

Other potential sources are investigated in the following section.

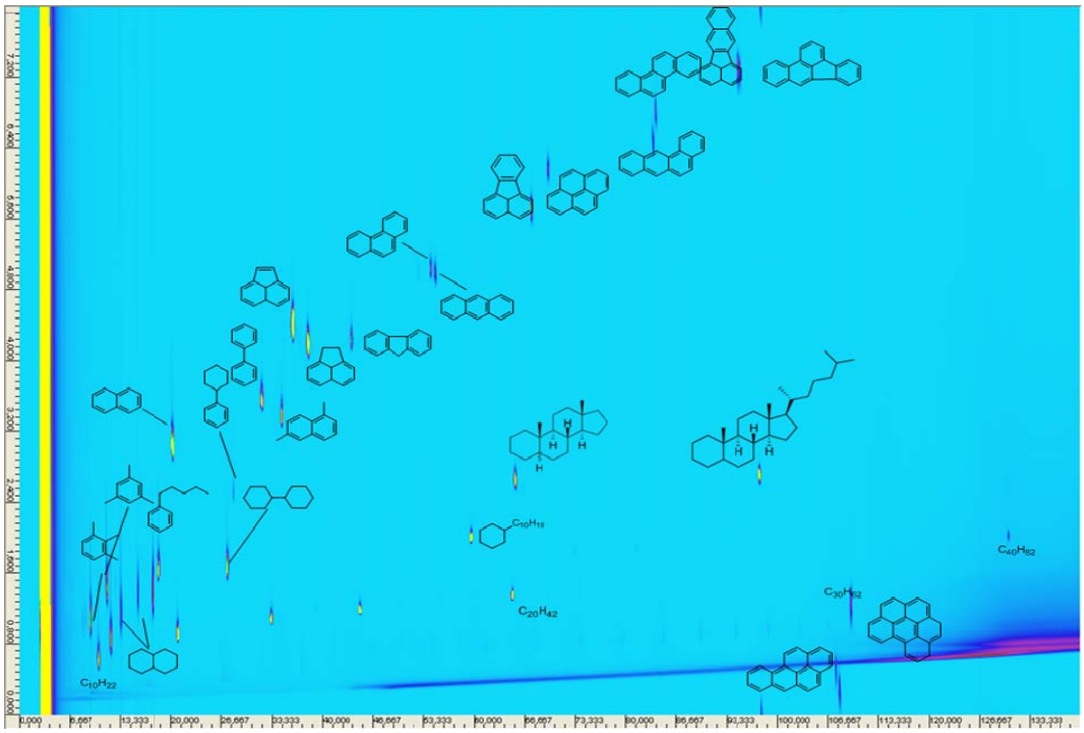


Figure 4.13 Mixture of *n*-alkanes, cyclyc alkanes, monoaromatics and PAH for the determination of the specific response factors of individual compound-classes in petroleum samples (time windows of x-axis: 0-140 min, y-axis: 0-8 sec).

Table 4.9 Experimentally obtained compound-class specific response factors normalized relative to the response of eicosane (*n*-C20).

n-alkanes	Experimental RF to C20	RSD, %	Calculated RF to C20
Decane	**0.98**	3.2	**0.94**
Dodecane	**1.04**	4.0	**1.00**
Tetradecane	**1.06**	4.9	**1.00**
Hexadecane	**0.99**	1.8	**1.00**
Eicosane	**1.00**	0.0	**1.00**
C40H82	**1.00**	0.0	**1.00**
Average:	**1.01**		**0.99**
Cycloalkanes			
Butylcyclohexyl	**1.04**	5.7	**0.99**
Naphthalene, decahydro-, trans-	**1.01**	0.6	**0.98**
Cyclodecane	**1.07**	1.2	**0.99**
1,1'-Bicyclohexyl	**1.04**	3.8	**0.98**
5a-Androstane	**0.96**	2.1	**0.97**
5a-Cholestane	**0.98**	2.0	**0.98**
Average:	**1.02**		**0.98**
Monoaromatics			
Benzene, 1,2,4-trimethyl-	**1.13**	7.68	**0.95**
Benzene, 1,2,3-trimethyl-	**1.09**	5.45	**0.95**
Benzene, pentyl-	**1.02**	4.59	**0.95**
Biphenyl	**1.02**	2.84	**0.91**
1-Phenyldodecane	**0.99**	2.04	**0.97**
Average:	**1.05**		**0.94**
PAH			
Naphthalene	**0.86**	2.2	**0.91**
1,6-Dimethylnaphthalene	**0.86**	4.4	**0.92**
Acenaphthylene	**0.83**	1.5	**0.90**
Acenaphthene	**0.85**	1.7	**0.91**
Fluorene	**0.93**	0.9	**0.91**
Phenanthrene	**0.94**	2.3	**0.90**
Anthracene	**0.96**	2.8	**0.90**
Fluoranthene	**0.89**	1.9	**0.89**
Pyrene	**0.93**	2.8	**0.89**
Benz[a]anthracene	**1.03**	3.1	**0.90**
Chrysene	**1.03**	3.4	**0.90**
Benzo[b]fluoranthene	**1.00**	5.5	**0.89**
Benzo[k]fluoranthene	**0.94**	10.6	**0.89**
Benzo[a]pyrene	**1.24**	2.0	**0.89**
Indeno[1,2,3-cd]pyrene	**1.18**	11.9	**0.89**
Dibenz[a,h]anthracene	**1.48**	9.3	**0.90**
Benzo[ghi]perylene	**1.01**	5.2	**0.89**
Average:	**1.00**		**0.90**

4.6.3. Fractionation of oils and determination of compound-class specific correction factors for quantitative analysis of petroleum hydrocarbons by GC×GC/FID

In the previous section we concluded that the different FID response factors (RFs) for individual petroleum hydrocarbon compound-classes have a less important contribution to the observed differences in the quantitative determination of total petroleum hydrocarbons. This conclusion was made on the basis of calculated and experimentaly obtained RFs for different representatives substances of petroleum

hydrocarbons, which were found to be close to 1.0. Thus, even if such a correction were applied, this would hardly improve quantitative results for total petroleum hydrocarbon measurements.

Neverthless, experiments have been continued in order to check whether it is still possible to find other potential sources or ways to explain and correct different relative responses for different types of oil. To be able to correct the specific response of petroleum hydrocarbon compound-classes and thus bringing the practically determined value of total petroleum hydrocarbons close to the true value, correction factors for each compound-class must be introduced. This will be an empirical correction factor, a sum of the factors which are difficult to explain. This is a pragmatic approach which should not be mixed with the response factor (RF) of FID.

Empirical data are data produced by an observation or experiment, and thus the following experiments were performed in order to find the empirical correction factors for five petroleum hydrocarbon compound classes: (*n*-alkanes; *iso*-alkanes; cyclic-/polycyclic alkanes; monoaromatic hydrocarbons and polyaromatic hydrocarbons).

Three oils, heavy heating oil – with larger content of polyaromatic hydrocarbons (42.3%), motor oil – with larger content of cyclic-polycyclic alkanes (66.4%) and crude oil API 40.5 (contain all 5 compound-classes), were subjected to fractionation over silica gel column (the procedure is described in *Section 4.4*). The exact amount (~200 mg) of oil was applied on the column and fractionated. Fractions were collected, preconcentrated (solvent was evaporated) and the exact weight of the fractions was taken. To each fraction obtained from the fractionation procedure different volumes of hexane solvent were added to bring the samples to a concentration of oil fraction in hexane of ~ 1 mg/mL. Samples of the individual fractions were analysed by GC×GC-FID and quantitatively evaluated. The GC×GC contour plots of each analyzed fraction are presented in **the Figures 4.14**, **4.15** and **4.16**. The total absolute response of detected petroleum hydrocarbons was compared with the response of *n*-alkanes with total concentration of 0.8 mg/mL. The absolute weight recoveries after fractionation are satisfactory (for crude oil API 40.5 they were not reported) and are presented together with relative response results relative to *n*-alkanes in the **Table 4.10**. The empirical correction factor for the integrated petroleum hydrocarbons was calculated for each fraction, using the **Equation 4.1** for practical RF calculations. In the Equation 4.1 instead of "area of compound" and "area of standard" the total peak volume of all detected compounds in the analysed sample of the fraction and the total peak volume of *n*-alkanes (from C10 to C40) respectively were used. In turn instead of "mass of compound" and "mass of standard" the concentration of hydrocarbons in the sample of the fraction and total concentration of *n*-alkanes respectively were used.

Experimentally found correction factors for fractions of the three oils are presented in the **Table 4.10**. Some fractions contain hydrocarbons of more than one compound-class, as it can be seen in the GC×GC contour plots, but hydrocarbons from two or three compound-classes, e.g. Fraction #4 in the fractionation of crude oil API 40.5 (**Figure 4.16**) contains monoaromatic and di-aromatic hydrocarbons. Fractions #2 in fractionation of all three oils contain *n*-alkanes and *iso*-alkanes and cyclic-/polycyclic alkanes. It is also difficult to separate *n*-alkanes and *iso*-alkanes from cyclic-/polycyclic alkanes, **Figure**

4.16, Fraction #2. Nevertheless, it was possible to collect fractions with hydrocarbons of one compound-class, e.g. Fractions #3, 4 and 5 from motor oil represent cyclic-/polycyclic hydrocarbons, Fraction #3 from Crude Oil API 40.5 represents monoaromatic hydrocarbons and Fraction #5 from the same oil represents polyaromatic hydrocarbons. The correction factors found for these fractions were used as the correction factors for the respective compound-classes and applied for the correction of compound-class specific responses in the quantification of petroleum hydrocarbons, **Table 4.11**. The correction factors of the classes of *n*-alkanes, *iso*-alkanes and others (like FAME) was considered as 1. Thus, in order to obtain a corrected absolute response, the obtained total peak volume for each considered compound-class must be multiplied by the corresponding correction factor.

The compound-class specific quantitative evaluation performed in the following is based on the response factors from the **Table 4.11**.

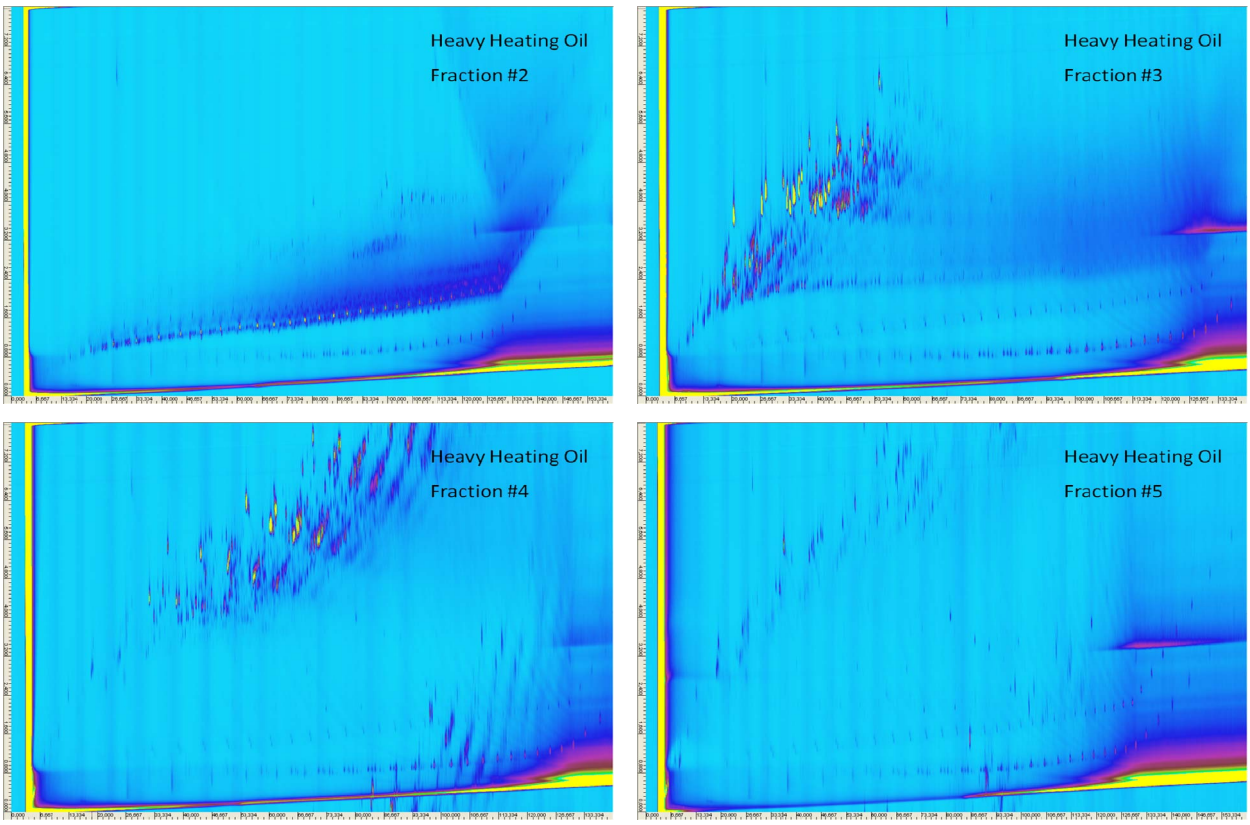


Figure 4.14 GC×GC/FID contour plots of Heavy Heating oil fractions after fractionation on silicagel column. Fraction #2 – alkanes, *iso*-alkanes, cyclic-polycyclic alkanes; Fraction#3 – monoaromatic, di-aromatic; Fraction#4 – polyaromatic (time windows of x-axis: 0-140 min, y-axis: 0-8 sec).

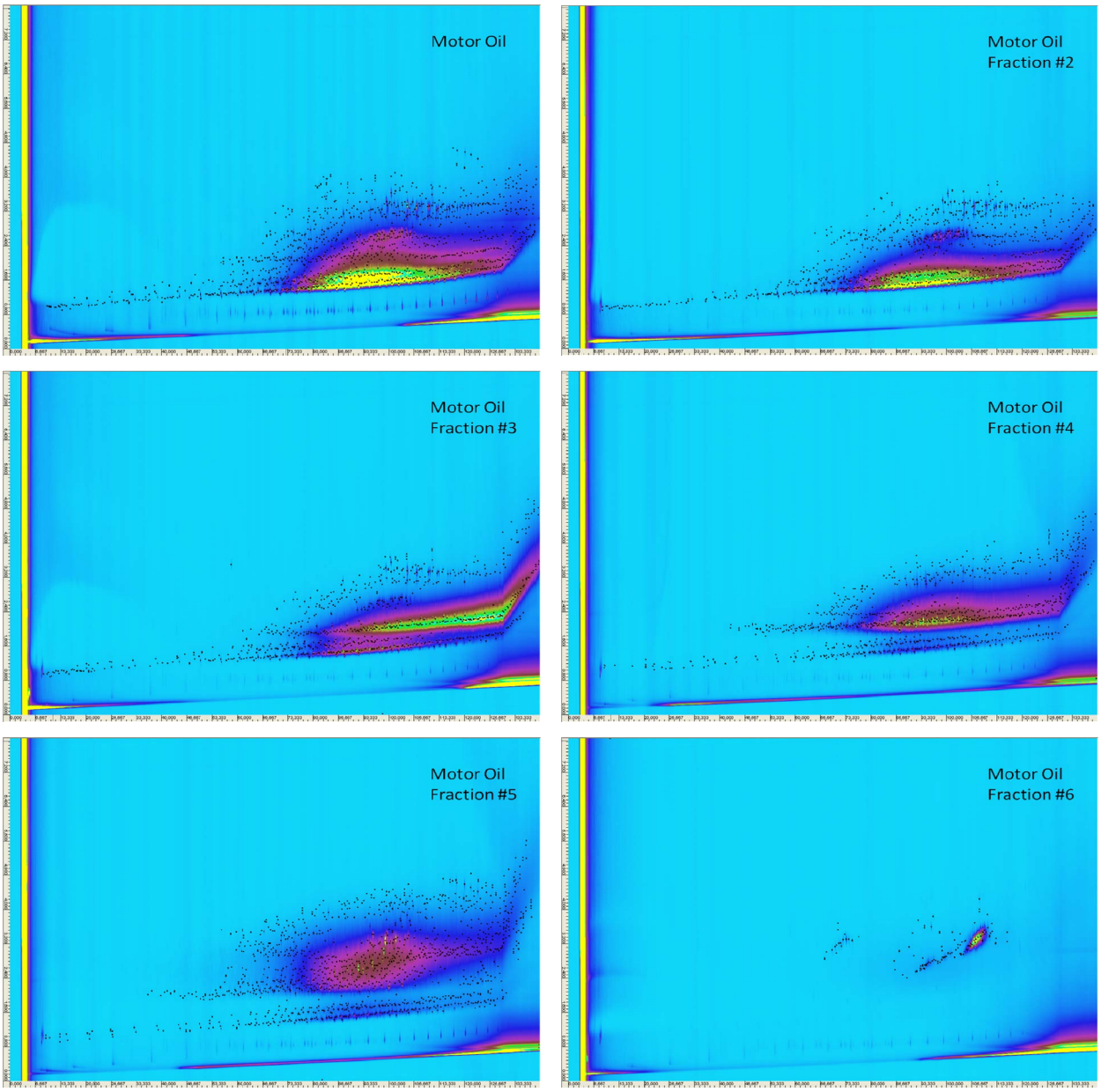


Figure 4.15 GC×GC contour plots of motor oil fractions after fractionation on silicagel column. Fraction #2 – *iso*-alkanes, cyclic-polyciclic alkanes; Fraction#3 – cyclic-polycyclic alkanes; Fraction#4 – cyclic-polycyclic alkanes; Fraction#5 – cyclic-polycyclic alkanes; Fraction#6 – cyclic-polyciclic alkanes (time windows of x-axis: 0-140 min, y-axis: 0-8 sec).

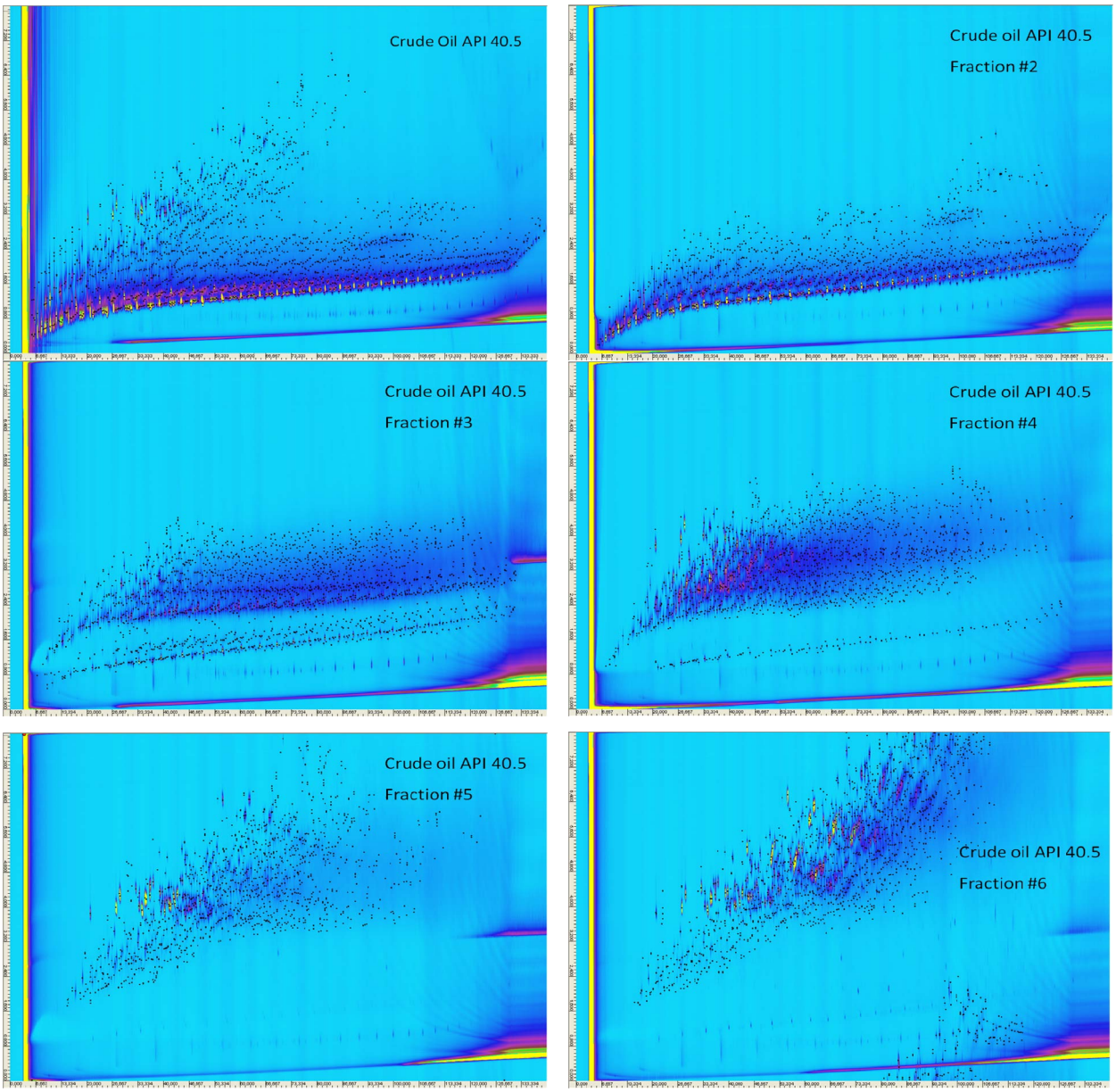


Figure 4.16 GC×GC/FID contour plots of crude oil (API 40.5) fractions after fractionation on silicagel column. Fraction #2 – alkanes, *iso*-alkanes, cyclic-polyciylic alkanes; Fraction#3 – monoaromatic; Fraction#4 – monoaromatic, di-aromatic; Fraction#5 – polyaromatic; Fraction#6 – polyaromatic (time windows of x-axis: 0-140 min, y-axis: 0-8 sec).

Table 4.10 Experimentally obtained compound-class specific correction factors for each fraction after oil fractionation over silica gel. Response expressed relative to *n*-alkanes (C10-C40, total concentration 0.8 mg/mL)

Heavy Heating Oil

	Weight after fractionation, mg	Prepared concentration c, mg/mL	Relative response (relative to *n*-alkanes), %	Correction Factor	Group-types of hydrocarbons
					alkanes + *iso*-alkanes + cyclic-/polycyclic
Fraction #2	44.8	1.12	40.5	**2.5**	alkanes
Fraction #3	36.4	0.91	31.0	**3.2**	mono-aromatics + di-aromatics
Fraction #4	59	1.18	18.6	**5.4**	polyaromatics
Fraction #5	23.2	1.16	4.1	**24.4**	
total weight, mg	*164.3 mg*	*from 209.5 mg*			

Motor Oil

	Weight after fractionation, mg	Prepared concentration c, mg/mL	Relative response (relative to *n*-alkanes), %	Correction Factor	Group-types of hydrocarbons
Fraction #2	121.3	1.04	87.7	**1.1**	iso-alkanes + cyclic-/polycyclic alkanes
Fraction #3	2.4	1.70	66.3	**1.5**	cyclic-/polycyclic alkanes
Fraction #4	21.6	1.08	71.1	**1.4**	cyclic/polycyclic alkanes
Fraction #5	22.1	1.38	64.8	**1.5**	cyclic/polycyclic alkanes
Fraction #6	0.5	0.08	58.8	**1.7**	cyclic/polycyclic alkanes
total weight, mg	*167.9 mg*	*from 200.0 mg*			

Crude Oil API 40.5

	Weight after fractionation, mg	Prepared concentration c, mg/mL	Relative response (relative to *n*-alkanes), %	Correction Factor	Group-types of hydrocarbons
Fraction #2	47.7	0.95	83.8	**1.2**	alkanes + *iso*-alkanes + cycloalkanes
Fraction #3	5.3	1.06	49.2	**2.0**	mono-aromatics
Fraction #4	4.4	1.00	54.2	**1.8**	mono-aromatics + di-aromatics
Fraction #5	2.1	1.05	32.0	**3.1**	polyaromatics
Fraction #6					polyaromatics

Table 4.11 Correction factors of compound-classes applied as a correction factor of compound-class aresponses for quantification petroleum hydrocarbons

Compound-class	Response Factor
n-Alkanes	**1**
iso-Alkanes	**1**
Cyclic-/polycyclic alkanes	**1.5**
Monoaromatic hydrocarbons	**2**
Polyaromatic hydrocarbons (PAH)	**3**
Othrers	**1**

4.6.4. Quantitative determination of petroleum hydrocarbons by one- and two-dimensional gas chromatography

We propose a model that aims at providing a more uniform GC×GC/FID response in the analysis of petroleum hydrocarbons of largely varying composition. The model is based on the correction of the response of five different petroleum hydrocarbon compound-classes with empirical correction factors, **Table 4.11**. With this refinement, and the unique capability of GC×GC to provide compound-class specific separation in the 2D separation space, the model can be used to address the problems observed in the analysis of petroleum hydrocarbons by one-dimensional GC (*Chapter 2*), namely to find an explanation for the very different quantitative results for different types of oil and to investigate whether the losses during sample preparation and clean-up of petroleum hydrocarbon samples are compound-class specific.

First of all, the proposed correction factors for correction of compound-class responses were applied for nine different oil types investigated. The quantitative results obtained for relative response according to the BAM standard and the data on compound-class percentage composition for the same oils (obtained by GC×GC) were compared with the corresponding quantitative results before applying the correction of response. The peak volume found for each compound-class was multiplied with the corresponding correction factor, the corrected total peak volume was determined and the relative composition (in %) was recalculated, **Figure 4.17**. Accepted standard procedures suggest the use of the BAM standard for quantitative analysis which was also used here for normalization taking in account the corrected responses for all oil types including the BAM standard. Our earlier experiments with one-dimensional GC showed that different types of oil lead to qualitatively and quantitatively strongly differing results even for equal mass fractions of oil, which is one of the reasons why quantitative results obtained according to the procedure of hydrocarbon index must be interpreted with care. The quantitative GC×GC/FID results presented earlier compare favorably with results obtained by one-dimensional GC. The specific responses of the samples analysed appeared to be always lower than the specific response of the BAM standard, **Table 4.8**. The introduction of compound-class specific correction factors (from the **Table 4.11**) to correct the largely variable composition of different oils improves the situation in most cases, **Table 4.12**, although it does not completely eliminate the sample-specific bias. The correction for all oil types has a positive effect, from 4.6% to 28.0% depending on oil type, except for the crude oil API 40.5, where the effect is negative: -1.4%. This negative effect is considered as statistically not significant, because this value most likely is within the method's uncertainty (which can only be estimated as it has not been studied in detail). The correction for the mineral oil mixture brings its response almost to 100%. For some oils, like light heating oil, heavy heating oil, A-/P-Oil, and crude oil API 18.7 the recovery results increased for 10 – 28%. It is obvious that the proposed correction model in principle works for the quantification of petroleum hydrocarbon and represents a big leap forward in the efforts of achieving a universal calibration for petroleum hydrocarbon analysis which is typically based on the use of the BAM

calibration standard. Corrected quantitative results are in most, although not all cases (e.g. diesel and Crude Oil API 40.5) closer to the real amounts, even when the routine calibration with the BAM standard is performed.

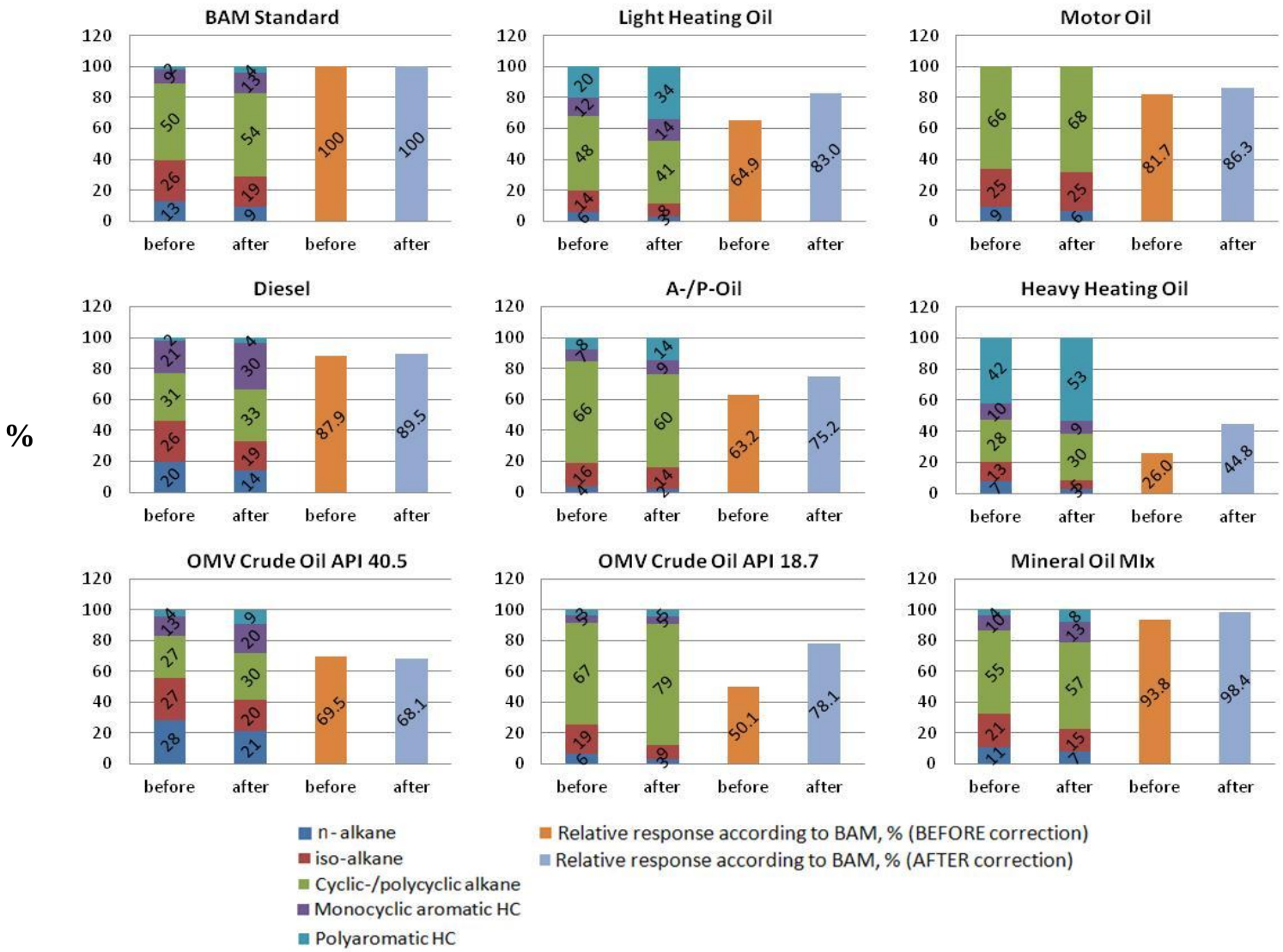


Figure 4.17 Compound-class percentage composition and relative response according to BAM standard for different oils analysed by GC×GC/FID BEFORE and AFTER correction of compound-class responses (before: results before correction, after: results after correction).

Table 4.12 Normalised relative responses for different types after clean-up of oil obtained by GC×GC/FID, GC×GC/FID with correction based on RF, ISO 9377-2 and OSPAR methods.

	Normalised relative response according to BAM, %			
	GC×GC-FID	GC×GC-FID corrected with RF	ISO 9377-2	OSPAR
BAM	100	**100**	99.7	98.0
Light Heating Oil	64.9	**83.0**	68.4	70.0
Motor Oil	81.7	**86.3**	83.3	81.5
Diesel	87.9	**89.5**	81.0	92.7
A-/P-Oil	63.2	**75.2**	67.5	73.3
Heavy Heating Oil	26.0	**44.8**	33.9	37.0
OMV Crude Oil API 40.5	69.5	**68.1**	62.0	81.7
OMV Crude Oil API 18.7	50.1	**78.1**	55.0	54.7
Mineral Oil Mix	93.8	**98.4**	86.0	98.5

**: All peak integration values have been normalised to the same reference concentration*

It should also be mentioned that the GC × GC-FID method, if considering the injection technique (which is split/splitless injection) and integration borders, is closer to the DIN ISO 9377-2:2000 method. Peaks in the chromatograms are integrated starting approximately from C10 and until the last peak which can be detected. The use of lower boiling solvent (e.g. *n*-pentane) and the programmed temperature vaporizer large volume injection (PTV LVI), as it is proposed by OSPAR method, for GC × GC method, would allow detection of more volatile hydrocarbons in the range C7-C10 and thus increasing the total response of the analysed oil, e.g. in case of diesel and A/P-oil samples (which contain a significant fraction of highly volatile constituents).

4.6.5. GC×GC evaluation of losses in the clean-up step in the quantitative analysis of petroleum hydrocarbons

Another important aspect which might bring the relative responses of different oils related to the BAM standard closer to 100 % and which must be considered in quantitative analyses of petroleum hydrocarbons is the potential loss of substance in the clean-up step. There are hydrocarbon losses (up to 16%) for a variety of oils in the clean-up step using Florisil, and the subsequent analysis of oils according to DIN ISO 9377-2:2000 standard and OSPAR method (**Figure 2.5**, *Section 2*). This can be explained by the different composition of light, medium and heavy oils which also contain different fractions of oxygenated and polar compounds that are removed by the Florisil clean-up step.

GC×GC/MS allows to specifically trace the compounds that are removed by the clean-up step, although complete removal is observed in only few cases (**Figures 4.18**). The more polar the compounds become, the higher is the tendency to be lost, due to the higher interaction with the polar Florisil. In the example of diesel the fatty acid methyl esters (FAME) are removed during the clean-up procedure, what is actually the aim of this procedure – to remove polar and non petroleum hydrocarbons. The same is observed for motor oil, where some amino compounds are removed.

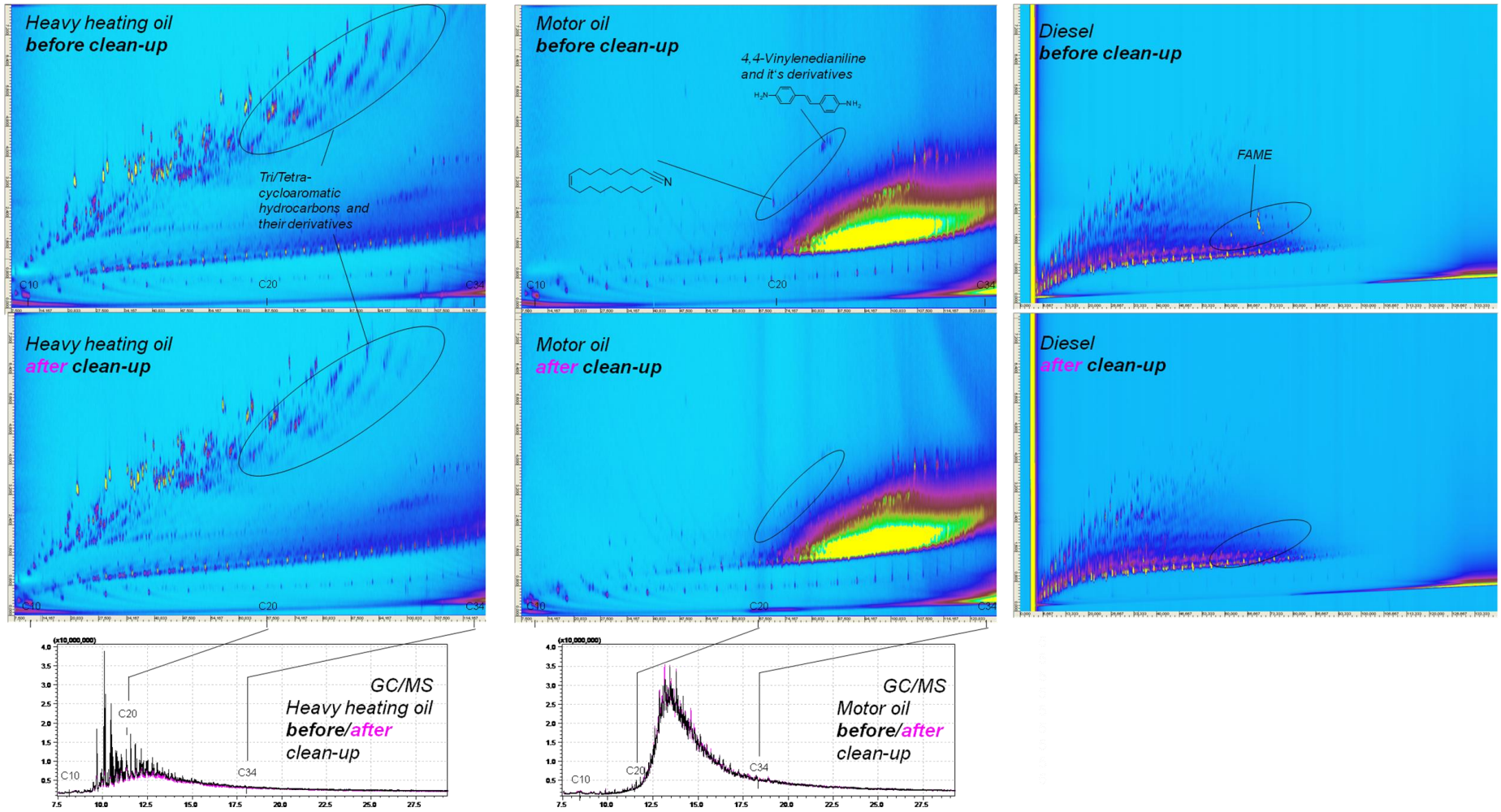


Figure 4.18 GC×GC/MS and GC-MS chromatograms obtained for heavy heating oil, motor oil and diesel sample before and after clean-up step (time windows of x-axis: 7-110 min, y-axis: 0-8 sec).

For the other oils, a general loss is associated with the polarity of their constituents. The case of heavy heating oil is a good example of this situation. The loss of some polyaromatic hydrocarbons in heavy heating oil contributes with 17.3% to the total loss of 19.6% (**Table 4.13,** data are presented after correction model is applied) and can be observed just by comparing two GC × GC contour plots of this oil obtained before and after the clean-up step, **Figure 4.18**, A similarly high loss of polyaromatic hydrocarbons was also observed for light heating oil where the loss of PAHs contributed with 10.4% of the total 12.4% reduction in response . When these two oil samples are compared with other types of oil, their characteristics are that these two contain larger fraction of polyaromatic hydrocarbons with 4, 5 or 6 aromatic rings (notably seen in the late eluting fraction). This fraction is more polar and interacts with Florisil to a stronger extent than other hydrocarbons, and thus is largely lost in the clean-up step. Other, although by far smaller deviations are listed in the **Tables 4.13 and 4.14**. However, as the associated uncertainty of the results was not determined explicitly, differences below 2% were considered to be within method uncertainty and thus insignificant.

Table 4.13 Contribution of the individual compound classes to the total loss in the clean-up step for different oil types, determined from GC×GC results. To account for different compound class-specific response, the data from which the compound-class specific losses were determined have been corrected by the application of the empirical correction factors (repeatability is not studied).

	n-Alkanes, %	**iso-Alkane, %**	**Cyclic-/polycyclic alkane, %**	**Monocyclic aromatic HC, %**	**Polyaromatic HC, %**	**Others, %**	**Total loss, %**
BAM	0.0	0.0	0.3	-0.1	0.1		**0.2**
Light heating oil	0.2	-0.1	0.2	1.8	10.4	0	**12.4**
Motor oil	0.1	0.0	4.4	0.0	0.0		**4.5**
Diesel	0.0	0.1	1.4	1.6	0.9	3.3	**7.2**
A-/P-Oil	0.1	0.7	2.9	2.7	6.5		**13.0**
Heavy heating oil	0.2	0.4	0.7	1.0	17.3		**19.6**
Crude Oil API 40.5	0.0	0.1	1.1	2.6	4.0		**7.8**
Crude Oil API 18.7	0.0	0.1	1.1	0.6	2.0		**3.8**
Mineral oil mix	0.0	1.1	1.3	1.3	3.7		**7.6**

* HC - hydrocarbons

In the **Table 4.14** the relative losses in the Florisil clean-up step for individual compound-classes in different types of oil are reported. All oil samples – with the exception of the BAM standard –show a similar trend in this type of evaluation, exhibiting a loss of between 20-30% of polyaromatic hydrocarbons (from all petroleum hydrocarbons present in the sample before clean-up step). The relative loss of monoaromatic hydrocarbons varies from 5.4 to 25.3 % depending on oil type. Interesting is that the loss of *n*-alkanes and *iso*-alkanes is observed for some oil types: light heating oil – absolute loss 7.7%, heavy heating oil – 7.2% and A-/P-oil – 6.3%. These oils have similar constitution, they are very dark, viscous and in general their content of *n*-alkanes is low (up to ~3% after the correction is applied, see

Figure 4.17). So the effect of this *n*-alkane losses are negligible comparing to the total losses, which are, as an interesting observation, found to be high in these three oils.

Table 4.14 Relative losses for each individual compound-class in the clean-up step for different oil types, determined from GC × GC results (repeatability was not studied).

	n-Alkanes, %	iso-Alkane, %	Cyclic-/polycyclic alkane, %	Monocyclic aromatic HC, %	Polyaromatic HC, %	Others, %	Total loss BEFORE correction, %	Total loss AFTER correction, %
BAM	0.0	0.1	0.5	-1.0	1.5		0.2	0.2
Light heating oil	7.7	-1.9	0.5	12.7	25.9		8.4	12.4
Motor oil	1.8	-2.6	6.2				3.8	4.5
Diesel	-0.7	0.8	4.2	5.4	20.1	100.0	7.5	7.2
A-/P-Oil	6.3	5.4	5.3	25.3	34.3		10.3	13.0
Heavy heating oil	7.2	7.8	2.9	12.9	28.7		17.3	19.6
Crude Oil API 40.5	0.2	0.8	3.7	12.6	32.5		4.9	7.8
Crude Oil API 18.7	1.2	0.9	1.4	12.9	30.6		3.3	3.8
Mineral oil mix	0.0	7.6	2.5	9.9	34.1		5.8	7.6

* HC - hydrocarbons

The actual type of oil has a significant influence on the quantitative result in the determination of petroleum hydrocarbon concentrations. Also the recovery of sample from the clean-up step clearly depends on the actual type of oil sample. It is demonstrated here that the more polar the compounds in the analysed oil are, the higher the losses that are observed in the clean up step.

If the response of each oil type is corrected according to the proposed model using the empirical correction factor and if the losses in the clean-up step are considered, then the corrected response of individual oil types comes closer to the response of the BAM standard, **Figure 4.19**. This correction procedure does not work equally well for all oils, e.g for heavy heating oil and for crude oil API 40.5 it brings some, but not sufficient improvement. It is very difficult to explain why after the considering the loss and after the applying the correction model the relative response of the heavy heating oil is still low, at 55.6 %. It is possible that very heavy and low volatility compounds of this oil are not properly dissolved in the solvent, or stick to the wall of glassware used for sample preparation, or just remain on the top of the gas chromatographic column after injection and do not elute.

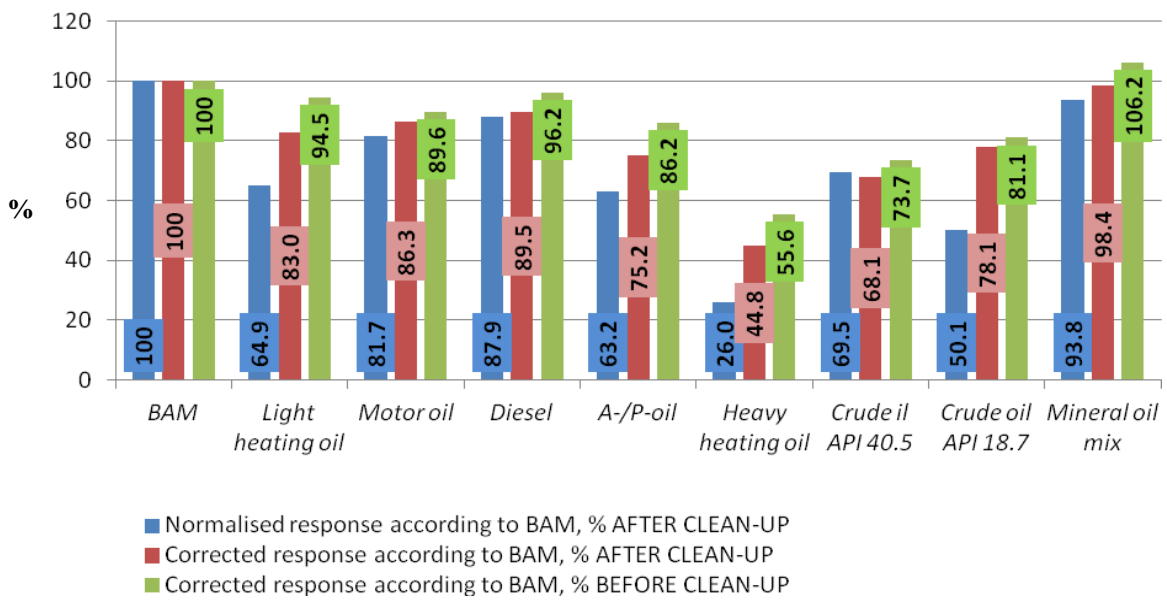


Figure 4.19 Normalised response according to the BAM standard for different oil types after the clean-up procedure and before clean-up.

4.7. Conclusions

When performing quantitative analysis of petroleum hydrocarbon contaminations, one must have in mind that the results – typically obtained by following one of the standardized procedures and by using the BAM standard as a reference – are procedurally defined. They do not represent true values as the quantitative results of petroleum hydrocarbon analysis strongly depend on the composition of the oil present in the sample. The following factors contribute to a larger or lesser extent to the observed differences in relative response of different petroleum hydrocarbon contaminations:

- Choice of integration borders (particularly critical for oils which have significant fraction outside the interval C7-C40 or C10-C40, respectively,
- losses in the clean up-proceudre, which are considered to be compound-class specific,
- losses in the chromatographic system, which are also depending on the compound-classes as well as on volatility,

To improve the quantitative determination of total petroleum hydrocarbons and to achieve a more uniform response when calibrating with a 'well-behaved' standard such as the BAM standard it is proposed that:

- losses in the chromatographic system could be reduced by using more inert components (column, liner) and higher oven temperatures,
- GC × GC can be used to avoid the clean-up step, as this is known to introduce a sample-specific negative bias (= loss of certain compounds). Elimination of the clean-up step makes the use of

GC×GC mandatory, as non-hydrocarbon interferences have then to be separates chromatographically.

The experimental approach and model that was followed in this case was to introduce empirical response correction factors for each specific compound-class, based on the compound class-specific chromatographic separation provided by GC×GC. Although this model in many cases improved the quantitative response, there still are, however, significant deviations between the expected response and the modeled values. This indicates that the model in principle is useful, but requires further refinement (especially for the range of heavier hydrocarbons).

Summary and conclusion

Due to the importance and widespread use of petroleum hydrocarbons for energy production, transport and as raw material in the chemical industries, there are many routes for their inadvertent or accidental release into the environment. They do thus represent one of the most important sources of large-scale environmental pollution. Oil spills, especially large-scale oil spills always have caused severe damage to the environment as the affected ecosystems recover only slowly (*see Chapter 1, Section 1.1*). Analytical methods are available for the qualitative and quantitative determination of the composition of oil samples, and the assessment of pollution levels in various environmental compartments. Gas chromatographic techniques mostly have supplanted the former analytical standard method based on Freon extraction and mid-IR determination, but there is further research and development going on to develop either alternative detection methods such as the ones based on MID-IR lasers as light sources – or more powerful analytical methods – such as two-dimensional GC (*see Chapter 1, Section 1.2*).

Two standard methods based on GC, namely the DIN ISO 9377-2:2000 standard and the OSPAR method are currently the preferred methods for petroleum hydrocarbon measurement. These methods are based on quantitative analysis of the total extractable amount of hydrocarbons with low-molecular weight alkanes as extracting solvent, followed by column chromatographic clean-up (with Florisil) and GC determination with flame ionization detection (GC-FID).

The OSPAR method is inherently more sensitive due to the preconcentration of a larger volume of sample (up to 50 µL) directly in the liner, while with the DIN ISO 9377-2:2000 method, this preconcentration must be done prior to analysis, if higher sensitivity is required. The OSPAR method is thus not only more efficient as it is faster, but also as it avoids potential losses of analyte as the preconcentration step is done automated and in-situ using the programmed-temperature vaporizer (PTV) for large volume injection (LVI) (*see Chapter 3*). The most critical components in petroleum hydrocarbon analysis are the more volatile solutes (hydrocarbons starting from C7), which elute immediately after the solvent peak. Initial column temperature, initial inlet temperature, inlet initial time, vent pressure and vent flow, purge time, liner design, and injection speed are important parameters for GC with PTV-LVI for the analysis of (total) petroleum hydrocarbons and must be carefully adjusted. Once PTV-LVI parameters have been optimised for one solvent, they then can be easily transferred to other solvents on the base of the particular solvent's vapor pressure at the conditions of sample introduction, while maintaining the method's performance and without the need of tedious re-optimisation.

In *Chapter 2* the two standard methods for petroleum hydrocarbon determination were compared, and their particular challenges and limitations were discussed. The quantitative results of the two methods correlate well for individual types of oil, although there is a significant method-and oil-type specific bias.

This is actually explained by the fact that the OSPAR method has a wider time window for integration than the DIN ISO 9377-2:2000 method. Both methods lose those parts of the sample which are too volatile or too involatile at the beginning and at the end of the chromatogram and thus are outside of the integration borders set by the individual standard. For this reason, the results obtained with these two methods will hardly ever be identical, and even rarely be comparable in terms of absolute concentrations (*see Chapter 2, Section 2.3.3*). Another observed limitation of the two methods are losses resulting from the clean-up procedure, which are depending on the oil type present in the sample (*see Chapter 2, Section 2.3.6*). This can be explained by the different composition of light, medium and heavy oils which also contain different fractions of oxygenated and polar compounds that are removed by the Florisil clean-up step. Moreover, it was also found that the specific responses of the analysed samples are always lower than the specific response of the BAM standard and that they differ depending on the oil type (*see Chapter 2, Section 2.3.4*). Even for equal amounts of oil (on the basis of their gravimetric preparation) qualitatively and quantitatively strongly differing results are usually obtained for the different types of oil.

The concentrations of petroleum hydrocarbons provided by one of the standard methods will never represent the true value of the actual concentration of total petroleum hydrocarbons in the water or soil samples. The found value is just a good indicator, called "hydrocarbon index", of the relative contamination of oil.

To improve the quantitative determination of total petroleum hydrocarbons and to obtain a more uniform response for the different types of oil samples, which translates to obtaining more accurate results when performing external calibration with the BAM standard, two approaches could be considered. In the first approach losses in the chromatographic system could be reduced by using more inert components (column, liner) and higher oven temperatures, and also to broaden the integration interval. However, this would just include some hydrocarbons, which currently are outside of the integration borders set by the individual standard. Comprehensive two-dimensional gas chromatography (GC×GC) is proposed as the second approach, which can provide a deeper insight in the qualitative and quantitative composition of petroleum hydrocarbon samples.

The presence of an ordered structure in the GC×GC plot allows the classification of chemical compounds and their assignment to classes e.g. linear and branched aliphatics, mono-/poylaromatics, alkyl-substituted aromatics, etc., through visual inspection of the contour plot. This provides the maximum information on the compounds being present in the different oil samples, and on the differences of oil composition between the different sources. It was shown here (*see Chapter 4, Section 4.4*) that the structural and compositional difference in the oils of different origin are the key to understanding the large difference in specific response observed in one-dimensional chromatography for different oil samples, which appeared to be comparable with the differences also registered by the GC×GC method (*see Chapter 4, Section 4.6.2)*. This difference, however, is not associated with (or has only a small contribution to) the GC/FID response factor for different classes of compounds.

The approach of this thesis was to introduce an empirical correction factor (found experimentally) of the specific compound-class response, which accounts for the various known or even unknown factors that affect response as a function of the particular compound class. In many cases, the use of these correction factors improved and made the quantitative response more uniform. The proposed correction model in principle works for the quantification of petroleum hydrocarbon and represents a big leap forward in the efforts of achieving a universal calibration for petroleum hydrocarbon analysis which is typically based on the use of the BAM calibration standard (*see Chapter 4, Section 4.6.3*). However there are significant deviations between expected/theoretical and modeled values for some oils (*see Chapter 4, Section 4.6.4*). This indicates that the model needs further refinement (especially in the heavier fraction of the samples).

The calibration of the GC×GC method for petroleum hydrocarbon determination (for which, similar to one-dimensional GC methods, the BAM standard is used for calibration) showed excellent linearity over the investigated range (*see Chapter 4, Section 4.6.1*). The limit of quantitation (LOQ) and limit of detection (LOD) values are comparable, or actually are the same, as the ones obtained by DIN ISO 9377-2:2000 without preconcentration. Although GC×GC provides improved selectivity over one-dimensional GC, it cannot offer a higher sensitivity in the determination of total petroleum hydrocarbons if no preconcentration or large volume injection (LVI) is applied. This finding is surprising and complementary to the observation in the quantitative analysis of individual components (e.g., FAMEs, *see Chapter 4, Section 4.5*) by one- and two-dimensional GC, where the GC×GC method offers a higher sensitivity for individual components. The detection of small total petroleum hydrocarbon concentrations in GC×GC is more difficult, because, unlike in 1D-GC, where the limit of detection is depending on the ability to reproducibly integrate small signal in a wide retention time window, in GC×GC many individual compounds have to be detected, which may or may not be above the LOD.

GC×GC/MS allows to specifically trace the compounds that are removed by the clean-up step. The loss in the clean-up step is associated with the polarity of compounds present in a sample. The more polar the compounds become, the higher is their tendency to be lost, due to a stronger interaction with the polar Florisil. Oxygenates and other polar heteroatom compounds present in the oil samples are completely removed after clean-up, which in fact is also the aim of this procedure. Besides this the unwanted removal of some hydrocarbons also occurs. Through GC×GC/MS it was observed that the clean-up step introduces losses to the sample which are the highest for monoaromatic and polyaromatic hydrocarbons (*see Chapter 4, Section 4.6.5*).

An evident solution to avoiding the losses from the clean-up step is to omit the clean-up step as such. In that case, the use of GC×GC for the direct analysis of extracted oil samples becomes mandatory. It provides the chromatographic separation of petroleum hydrocarbons from non-hydrocarbons and polar compounds, and can thus circumvent the separation of petroleum hydrocarbons from the interfering non-hydrocarbon matrix by column chromatographic clean-up over Florisil and the associated problems of reduced recovery from this clean-up step.

Given the fact that clean-up of environmental samples is not only required to remove interferences, but also to protect the analytical system, it appears unlikely that – although a highly attractive concept – clean-up would be completely eliminated by the use of GC×GC. More practical appears the approach of empirical correction factors to obtain a universal and improved calibration. In that case, however, the currently very simple model using compound-class specific correction factors must be further refined to better respond to the class of higher polyaromatic compounds, particularly with five to seven aromatic rings. The great challenge not only is to experimentally determine the corresponding correction factors, but rather to better understand the determining factors in this model. A better understanding of the proposed model would allow deciding whether the correction factors have a defined physico-chemical reason, or whether they are merely introduced to correct for the inadequacy of our currently available instrumentation.

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WORK EXPERIENCE

10.2008 – 10.2012 **Institute of Chemical Technologies and Analytics, Vienna University of Technology**, Austria

- Research assistant, under supervision of Ao.Prof.Dr. Egon Erwin Rosenberg
- Focus: Environmental and trace analysis by chromatography and masspectrometry, one-dimensional (GC) and two-dimensional gas chromatography (GC×GC)

06.2006 – 09.2008 **JSC "Grindex"**, Riga, Latvia

- Pharmaceutical industry, Chemist-analyst in the chromatography laboratory of quality control department
- Focus: Qualitative and quantitative analysis using HPLC, UPLS with UV, PDA and ELSD

07.2003 – 10.2005 **The Institute of Organic Synthesis**, Riga, Latvia

Part-time, 20 h/week

- Laboratory assistant, under supervision of Dr. A. Amolins
- Focus: Organic synthesis

EDUCATION AND TRAINING

03.2009 – 11.2012 **Doctoral Program in Technical Sciences – Chemical Technologies and Analytics**

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- Dissertation thesis about „Qualitative and quantitative analysis of petroleum hydrocarbons by one- and two-dimensional gas chromatography" under supervision of Ao.Prof.Dr. Egon Erwin Rosenberg in cooperation with QuantaRed Technologies (in English)
- 06.2011 – 07.2011 Scientific work abroad in the Department of Pharmaceutical Chemistry, University of Messina, Italy, under the supervision of Prof. L. Mondello and Prof. P. Tranchida (Focus: Multidimensional gas chromatography – MDGC)

09.2006 – 06.2008 **Master of Natural Sciences – Chemistry**, University of Latvia, Latvia

- Focus: Organic Chemistry, Organic Synthesis, Modern Analytical Methods, Chromatography
- Master thesis: „Alkylation of ambident indole anion in ionic liquids" under the supervision of Prof. Andris Zicmanis (in Latvian)
- 08.2007-10.2007 Scientific training abroad in the Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria, under the supervision of Prof. I.Marini (Focus: Glass fibre technology)

09.2002 – 06.2006 **Bachelor of Natural Sciences – Chemistry**, University of Latvia, Latvia

- Master thesis: „Alkylation of indole anion in ionic liquids" under the supervision of Prof. Andris Zicmanis

1991 – 2002 **Secondary Education**, Daugavpils Secondary School No 4, Daugavpils, Latvia

General high and primary school education

PERSONAL SKILLS AND COMPETENCES

LANGUAGES	Russian : mother tongue Latvian: fluent (spoken and written) English: fluent (spoken) and good (written) German: good spoken and satisfactory written Türkisch: Basiskenntnisse
COURSES	▪ Theoretical and practical course on comprehensive two-dimensional gas chromatography (GC×GC) in the Analytical Chemistry laboratory of the Department of Pharmaceutical Chemistry in the University of Messina, Italy, July, 2011. Certificate from Prof. L. Mondello ▪ Deutschkurse – Innovationszentrum Universität Wien, 2008-2011, Certificate C1/1 ▪ Short Course from HTC-11 & HTSP, "*Comprehensive two-dimensional separation*" 25 January, 2010, Bruges, Belgium ▪ Three day ECA GMP Education Course "*Maximising HPLC Productivity – Combining Science and Compliance in a GMP Laboratory* ", 16-18 April, 2008, Barcelona, Spain ▪ Course "*Validation of Analytical Methods*", 21 March, 2007, Riga, Latvia. Certificate LAF003-LAF-A-SK01
SCHOLARSHIPS AND AWARDS	▪ 2011 Auslandsstipendium der TU Wien, Kurzfristige wissenschaftliche Arbeit im Ausland ▪ 2007-2008 K. Morberga scholarship, from University of Latvia, Riga, Latvia ▪ 2007-2008 „Zinātnes un izglītības atbalstam" scholarship, from fund of *JSC "Grindex",* Riga,Latvia
TECHNICAL SKILLS AND COMPETENCES	Work with computer (Windows 98/2000/XP/Vista/7, MS Office, etc); Proficiency in different chromatography software (GCsolution, GCMScolution, ChemStation, Xcalibur, Empower, GC Image, ChromSquare), Chromatography and mass spectrometry equipment of different producers (Shimadzu, Agilent, Thermo, Waters)
DRIVING LICENCE(S)	Since 2002, Class B

ADDITIONAL INFORMATION

PUBLICATIONS:

1. Drozdova S., Rosenberg E. Petroleum Hydrocarbon Contaminations. In: Jorgensen SE, editor. Encyclopedia of Environmental Management, New York: Taylor & Francis, 2012, in press.
2. Drozdova S., Rosenberg E. Challenges in the determination of petroleum hydrocarbons in water by gas chromatography (Hydrocarbon Index), submitted in Fuel, July 2012.
3. Drozdova S., Rosenberg E. Parameter Optimisation for the Determination of Petroleum Hydrocarbons by Gas Chromatography Using the Large Volume Injection Technique, prepared for submission in J. Sep. Sci.
4. Вавилина Г., Зицманис А., Дроздова С., Мекш П., Клявиньш М. Алкилирование амбидентного иона индола в ионных жидкостях. Химия гетероцикл. соед, 2008, 5, 676.
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5. Drozdova S., Rosenberg E. Challenges in the Determination of Total Petroleum Hydrocarbons (Hydrocarbon Index) in Water by Gas Chromatography. 6. ASAC JunganalytikerInnen Forum, 11-12 June, Vienna, Austria, 2010, p 30.
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