APPLICATION OF ACTIVE SAMPLING AND SPTD/GC-MS ANALYSIS METHODOLOGIES FOR TERPENES AT ULUDAĞ MOUNTAIN

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YUSUF AKTAŞ

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Prof.Dr. Canan Özgen

Director

I certify this thesis satisfies all the requirements as a thesis for the degree of Master of Science

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Prof.Dr. Teoman Tinçer

Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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Prof.Dr. Semra G. Tuncel

Supervisor

Examining Committee Members:

Prof.Dr. R. Sezer AYGÜN (Chairman) _____

Prof.Dr. Mürvet VOLKAN _____

Prof.Dr. İnci GÖKMEN _____

Assoc.Prof.Dr. Nusret ERTAŞ _____

Prof.Dr. Semra G. TUNCEL _____

ABSTRACT

APPLICATION OF ACTIVE SAMPLING AND SPTD/GC-MS ANALYSIS METHODOLOGIES FOR TERPENES AT ULUDAĞ MOUNTAIN

AKTAŞ, Yusuf
M.S., Department of Chemistry
Supervisor: Prof. Dr. Semra G. Tuncel

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Measurement of monoterpenes including; alpha-pinene, camphene, beta-pinene, d-limonene, gamma-terpinene, linalool, 1-isopulegol, 1-borneol, dl-menthol, alpha-terpineol, dihydrocarveol, citronellol, pulegone, geraniol; and gas phase inorganic pollutants (O_3 and NO_x) were investigated on Uludağ Mountain, which is the highest point in North-west Turkey. The sampling site (1645 m altitude) is surrounded by mainly Uludağ fir, which is characteristic to Uludağ, while altitudes lower than 1000 m were composed of mixed deciduous trees.

Sampling apparatus and sampling strategy for collection of the studied terpenes on Uludağ Mountain were developed. Breakthrough Volume experiments were performed for accurate sampling. Samples were collected in a field campaign performed during October 2002 by means of active sampling onto glass coated stainless steel tubes containing Tenax® TA (55 mg) and Carbopack™ B (65 mg). The mass flow rate and duration of sampling were 30 mL/min and 4 hours.

Short-Path-Thermal-Desorption/Gas Chromatography-Mass Spectrometer (SPTD/GC-MS) was used for the analysis of monoterpenes during 12-14 October 2002. The cryogenic preconcentration (-40^{o}C) was maintained by liquid CO_2. HP-1-MS capillary column provided good resolution of peaks, except for pulegone and citronellol at standard operation procedure. Internal standard calibration was applied by adding carefully measured spikes of fenchone to the samples and standards. The detection limits for each terpene were found to be at sub-ppbv level. Daily intermediate standard measurements and auto-tune provided checking the instrumental capabilities. Selected Ion Monitoring mode was used for analysis of the terpenes. For quantitation of the spectra at least one qualifier ion was expected to be within 80% correlation with target ion. Alpha-pinene, camphene, beta-pinene, and d-limonene were determined. The average concentrations of monoterpenes were found to be 0.385, 0.168, 0.111, and 0.204 ppbv respectively.

Simultaneous measurements of O_3, NO_x, SO_2, and meteorological parameters were also performed. The data resolution was 15 min for each pollutant. Ozone displayed strong diurnal variation (around 5-30 ppb), while NO_x concentrations were almost constant around 15 ppb. During the sampling campaign terpenes showed similar behaviours with O_3, but not with NO_x. Terpenes had a direct relation with temperature and relative humidity.

Keywords: Thermal desorption, GC-MS, terpenes, ozone

ÖZ

AKTİF ÖRNEKLEME VE SPTD/GC-MS METODLARININ ULUDAĞ'DA TERPENLERE UYGULANMASI

AKTAŞ, Yusuf
Yüksek Lisans, Kimya Bölümü
Tez Danışmanı: Prof. Dr. Semra G. Tuncel

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Kuzey-batı Türkiye'nin en yüksek noktası olan Uludağ'da alpha-pinene, camphene, beta-pinene, d-limonene, gamma-terpinene, linalool, 1-isopulegol, 1-borneol, dl-menthol, alpha-terpineol, dihydrocarveol, citronellol, pulegone, geraniol monoterpenleri incelenmiştir. Örnekleme alanı (1645 metre yükseklikte) Uludağ'a özgü Uludağ göknarları ile kaplı olup, 1000 metrenin altındaki yüksekliklerde karışık dökülür yapraklı ağaçlar hakimdir.

Uludağ'da üzerinde çalışılan terpenlerin toplanması için örnekleme düzeneği ve stratejisi geliştirilmiştir. Hassas örnekleme için kaçış hacmi deneyleri yapılmıştır. Örnekler, Ekim 2002'de gerçekleştirilen bir kampanyada, Tenax® TA (55 mg) ve Carbopack™ B (65 mg) içeren, cam ile kaplanmış paslanmaz çelik tüplerle, aktif örnekleme yoluyla toplanmıştır. Örneklemedeki kütle akış hızı ve süresi, 30 mL/dakika ve 4 saattir.

12-14 Ekim 2002 tarihlerinde toplanan monoterpenlerin analizinde Kısa Mesafeli Isıl Çözündürme/Gaz Kromatografi-Kütle Spektrometresi (SPTD/GC-MS) kullanılmıştır. Soğuk önzenginleştirme (-40 ^{o}C) sıvı CO_2 ile sağlanmıştır. HP-1-MS kılcal kolonu standart çalışma şartlarında pulegone ve citronellol haricindeki terpenlerin iyi ayrışmasını sağlamıştır. Standartlara ve örneklere hassas olarak ölçülmüş miktarda fenchone eklenerek dahili standart kalibrasyonu uygulanmıştır. Terpenler için saptanan ölçüm limitleri sub-ppbv düzeyindedir. Günlük ara standart ölçümleri ve oto-taramalar cihazın ve metodun performans kontrolünü sağlamıştır. Örneklerin analizinde Seçili İyon Monitörleme (SIM) modu kullanılmıştır. Spektrumların kantitatif olarak kullanılabilmesi için en az bir niteleyici iyonun, hedef iyon ile %80 doğrulanması beklenmiştir. Alpha-pinene, camphene, beta-pinene, ve d-limonene tayin edilmiştir. Ortalama monoterpen derişimleri sırası ile 0.385, 0.168, 0.111 ve 0.204 ppbv'dir.

Eşzamanlı olarak O_3, NO_x, SO_2, ve meteorolojik parametre ölçümleri yapılmıştır. Data çözünürlüğü her bir kirletici için 15 dakikadır. Ozon güçlü güniçi değişimler gösterirken (5-30 ppbv dolaylarında), NO_x 15 ppbv dolaylarında hemen hemen sabit derişimler sergilemiştir. Örnekleme kampanyası sırasında terpenler ve O_3 benzer davranışlar göstermiş, fakat NO_x ile doğrulama gözlenmemiştir. Terpenler sıcaklık ve bağıl nem ile doğrudan ilişki göstermiştir.

Anahtar kelimeler: Isıl çözündürme, GC-MS, terpenler, ozon.

To My Family

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. Preview

Volatile Organic Compounds (VOCs) are emitted to the atmosphere in substantial quantities from both anthropogenic and biogenic sources. VOCs play key roles in tropospheric chemistry, but most importantly, they react with oxides of nitrogen in the presence of sunlight to produce tropospheric ozone.

Biogenic Volatile Organic Compounds (BVOC) are the highly abundant subgroup of VOC, which are emitted mainly by plants. BVOC emission data are still quite limited compared to the number of plant species, which may emit them. Isoprene and terpenes are assumed to be the dominant VOC compounds emitted from the terrestrial biosphere, representing the largest global input of non-methane hydrocarbons to the atmosphere. Other hydrocarbons make up 30 to 50 % of the biogenic emissions, and little information is available on the factors that control their emissions (Thunis P. and Cuvelier C., 2000).

Effective ozone abatement strategies cannot be developed without quantitative understanding of BVOC emissions rates. Decision-makers must know whether limiting anthropogenic VOC emissions in industrialised regions can significantly decrease photochemical ozone production. Ozone is known to be toxic to plants at concentrations that are often measured in agricultural and forested areas. There has been observed similar injury to forest ecosystem at

Uludağ National Park, which was investigated by means of O_3, NO_x, SO_2, SPM, aerosol, and trace metals by Tuncel S. G. *et al.* since 1992.

1.2. Biogenic Volatile Organic Compounds (BVOCs)

Volatile organic compounds (VOCs) cover a large number of different species belonging to the isoprenoids (isoprene and terpenoids) as well as alkanes, alkenes, carbonyls, alcohols, acids, esters, ethers etc. (Table 1.1.). The biogenic volatile organic compounds (BVOCs) consist of hydrocarbons, oxygenated hydrocarbons, and halogenated organic compounds released to the atmosphere from natural sources. Mainly vegetation have been the subject of study by atmospheric chemists for 2-3 decades, particularly isoprene (C_5H_8) and the monoterpenes ($C_{10}H_{16}$), because of their influence on the chemistry of the lower atmosphere, the troposphere (Janson R. *et al.,* 1999). Consequently, these compounds have been the focus of much of the research effort, with relatively little examination of the occurrence and release of other compounds (e.g. other VOCs among them oxygenated VOCs). For some of these compounds, initial studies have provided evidence of vegetative emissions of methanol, organic acids and aldehydes (Kesselmeier J. *et al.*, 2002).

From a biochemical perspective, isoprene and monoterpenes belong to the isoprenoids which all originate from isopentenylpyrophosphate (IPP). Flowers and fruits release VOCs, but the majority of VOCs is emitted from green leaves. The actual production of terpenes occurs within leaf plastids or cytosol (Tenhunen J. D. and Kabat P., 1999).

Several thousands of different biogenic VOCs have been identified. The most well knowns are ethene, isoprene, and the terpenes emitted by terrestrial plants. Table 1.1. lists many of the compounds that are emitted from vegetation (Alvarado A., 1998).

Table 1.1. A List of Compounds that are Emitted by Vegetation (Alvarado A., 1998).

ISOPRENE	**SESQUITERPENES**	**ACETATES**	**ALCOHOLS**
	β-Caryophyllene	Bornylacetate	*p*-Cymen-8-ol
MONOTERPENES	Cyperene	Buthylacetate	*Cis*-3-Hexen-1-ol
Camphene	α-Humulene	Cis-3-hexenylacetate	Linalool
Δ^3-Carene	**ALKENES**	**ALDEHYDES**	**AROMATICS**
d-Limonene	1-Decene	N-Hexanal	*p*-Cymene
Myrcene	1-Dodecene	*Trans*-2-Hexenal	
Cis-Ocimene	1-Hexadecene		
Trans-Ocimene	*p*-Menth-1,3,8-triene	**n-ALKANES**	**ESTERS**
α-pinene	1-Pentadecene	n-Hexane	Methylsalicylate
β-pinene	1-Tetradecene	C_{10}-C_{17}	
α-Phellandrene			
β-Phellandrene			
Sabinene	**ETHER**	**KETONES**	
α-terpinene	1,8-Cineole	2-Heptanone	
γ-terpinene	*p*-Dimenthoxybenzene	2-Methyl-6-methylene-1,7-octadien-3-one	
Terpinolene	Estragole	Pinıcarvone	
Tricyclene	*p*-Menthylanisole	Verbenone	

1.2.1. Terpenes

The most fundamental division of terpenes have structures composed of only carbon and hydrogen. They may have cyclic or acyclic skeletons and are a class of chiral hydrocarbons found in plant leaves, flowers, and fruits, the most common of which are isoprene and α-pinene (Moeder C. *et al.*, 1996).

Isoprene (2-methyl-1, 3-butadiene) is the major single, non-methane hydrocarbon (NMHC) emitted by plants. Significant amounts of larger hydrocarbons are also generated by plants and emitted to atmosphere. The larger

hydrocarbon compounds generally fall under the classification of isoprenoids, or terpenoids, consisting of group of 5-carbon isoprene type units (although they are not formed from isoprene). The monoterpenes are the C_{10} compounds (Figure 1.1.), sesquiterpenes the C_{15} compounds, diterpenes the C_{20} compounds, triterpenes the C_{30} compounds, and tetraterpenes the C_{40} compounds.

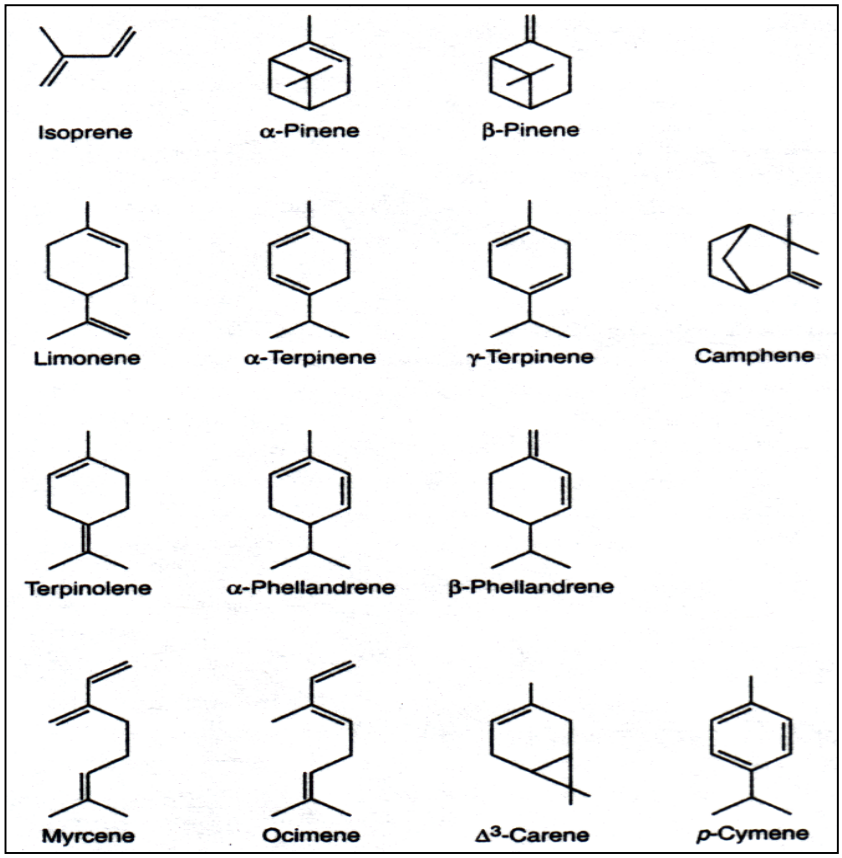

Figure 1.1. Chemical Structures of some biogenically emitted hydrocarbons (Pitts B. J. F. and Pitts J. N. Jr., 2000)

Larger hydrocarbons such as sesquiterpenes (C_{15}) also have biogenic sources such as sage. They present in many plants that synthesise other isoprenoids. But they are not detected due to sampling or analysis procedures or slower detection methods than reaction rates because of their low vapour pressure

and/or their higher reactivity toward ozone. Sesquiterpene emissions have been documented in plants growing in southern California and central Europe. Other identified plant species that emit sesquiterpenes include orange and tobacco (Fuentes J. D. *et al.*, 2000).

1.2.2. Oxygen-Containing Biogenic Organics

It has been increasingly recognised that there are oxygen-containing organics (OVOCs), from small alcohols such as methanol to larger aldehydes, ketones, and alcohols.

The tropospheric abundance of the OVOCs, methanol and acetone has received special attention due to their atmospheric chemistry, summarised in equations (1) and (2), producing two new molecules of odd hydrogen (HO_x) each after photolysis.

$$CH_3OH + OH + O_2 \rightarrow CH_2O + H_2O + HO_2$$

$$CH_2O + h\nu + 2\,O_2 \rightarrow \rightarrow CO + 2\,HO_2 \qquad (1)$$

$$CH_3C(O)CH_3 + h\nu + 2\,O_2 \rightarrow CH_3O_2 + CH_3C(O)O_2 \rightarrow PAN \qquad (2)$$

In both cases, formaldehyde (methanal, CH_2O), is produced. Formaldehyde photolysis and its oxidation by OH radicals generate HO_2 radicals (en. 1), which chiefly react with NO to produce NO_2, the precursor to ozone. Peroxyacetyl nitrate (PAN) is also formed during the course of reaction of acetone with oxygen. Formaldehyde is involved in acidification of rain and it is considered as a precursor of hydrogen peroxide (http://www.iup.physik.uni-bremen.de/~gws/introduction. html). Figure 1.2. gives the structures of some oxygen containing compounds for which there is evidence of direct biogenic emissions.

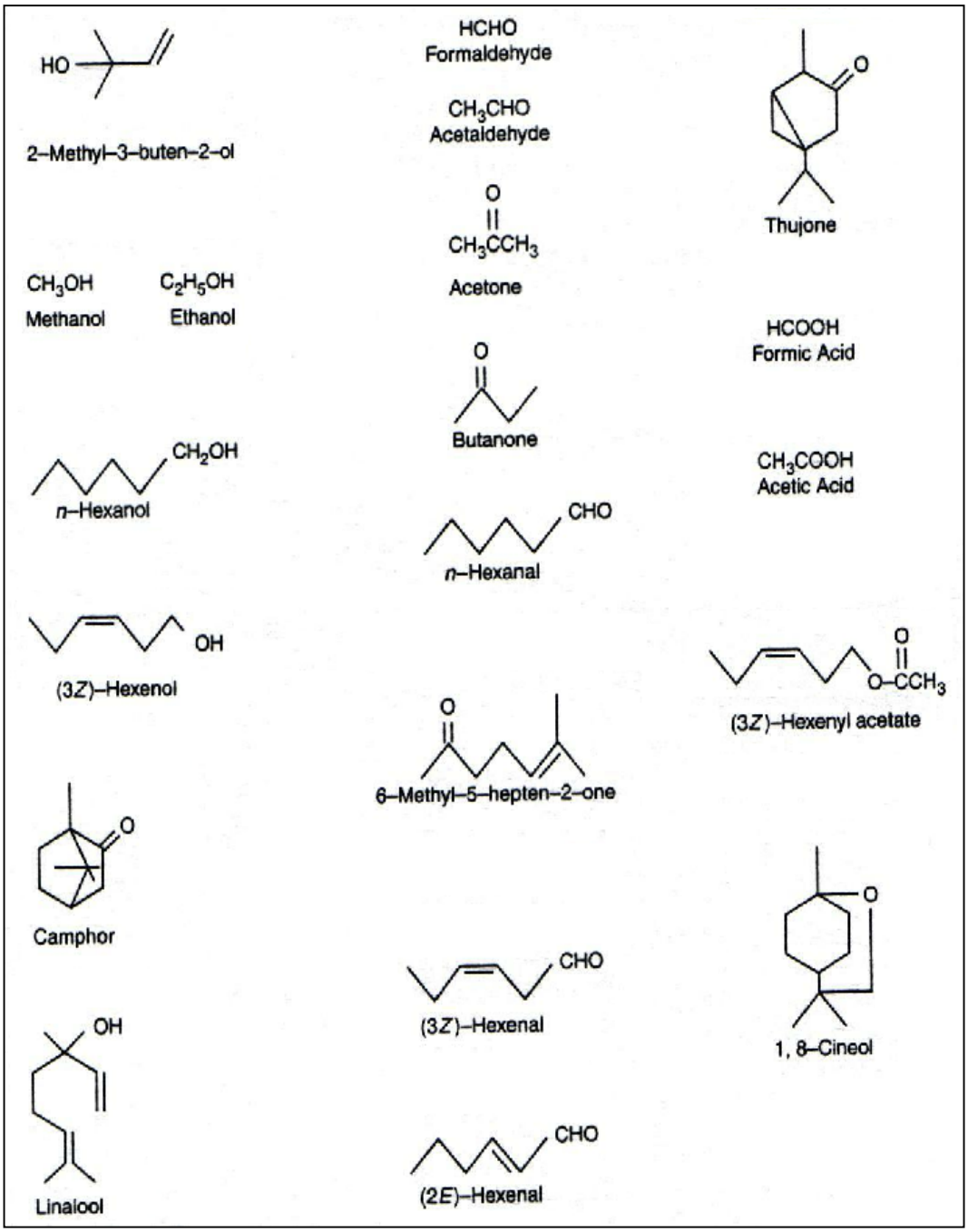

Figure 1.2. Structures of some oxygen-containing organics with biogenic emission sources (Pitts B. J. F. and Pitts J. N. Jr., 2000).

1.2.3. Volatile Halogenated Organic Compounds (VHOCs)

Volatile halogenated organic compounds, such as dibromochloromethane, tetrachloroethane, 1,3-dichloro-1-propene, trichloroethane, 1,2-dichloro ethane, tetrachloromethane, dichloromethane, trichlorofluoromethane, and bromomethane, also contribute to biogenic emissions. Recent investigations suggest the ocean as a main source of biogenic volatile halogenated organic compounds. The origin of these compounds in the oceans can be traced to release by marine algae, phytoplankton, and giant kelp. Another source to be considered is terrestrial production in sediments, air, and natural estuaries. The biogenic input in the tropical marine environments has not been fully explored because most of the VHOC's studies were focused on polar regions. VHOC concentrations measured at Atlantic ocean and Caribbean Sea using Solid Phase Microextraction GC-MS ranged from 0.9-28 ng/L (Rios B. C., 1997).

1.3. Sources of BVOC

There are many studies relating the isoprene, terpenoid and many other types of emissions to biogenic sources. In the order of decreasing emission budgets, Canopy foliage; terrestrial ground cover and soils; ocean and freshwater; Animals, humans, and insects; and anthropogenic activities, including biomass burning, account for the total biogenic emissions (Table 1.2.). The terrestrial ground cover has the second highest contribution to BVOC. However, the few measurements to date are not conclusive enough for the significance of even these fluxes.

Recent model calculations of regional and global BVOC fluxes by Simpson et al., (1995) and Guenter et al., (1995) have estimated the oxygenated species, primarily aldehydes, ketones, organic acids, and alcohols to be a considerable part of the total flux, although there exist very little experimental data. The work of

Kesselmeier et al. (1997) and Steinbrecher et al. (1993) have also shown that carbonyl compounds such as organic acids and aldehydes are emitted at significant rates by some conifers.

Researches upto now suggest; conifers emit mainly monoterpenes while deciduous species emit mainly isoprene. A number of recent works have demonstrated, however, that some conifers, in particular Norway spruce and Sikta spruce, emit significant amounts of isoprene (George M. *et al.*, 1995). Hardwood species such as oaks, poplars, aspen, and ironwood are generally isoprene emitters. However, even within plant families not all species are isoprene emitters. For example, while North American oaks emit isoprene, many European oak species do not. Kesselmeier *et al.* (1998) measured emissions of isoprene and monoterpenes from a Holm oak and a white oak growing side by side; the white oak was a strong isoprene emitter, where as the Holm oak was a strong monoterpene emitter.

1.3.1. Global Budget of BVOC

On a global basis, the total biogenic emissions may exceed antropogenic emissions by as much as an order of magnitude. Plants emit 400-800 Tg C/yr (1 Tg = 10^{12} g) as hydrocarbons, an amount equivalent to the sum of biogenic and antropogenic methane emissions. Unlike methane, which is well mixed in the atmosphere because of its long atmospheric lifetime (8-11 years), plant produced VOCs are extremely reactive in troposphere, with life-times ranging from minutes to hours (Lerdau M. *et al.*, 1997)

Table 1.2. Estimated Global Annual Biogenic VOC Emissions (Tg yr^{-1}) (Pitts B.J.F. and Pitts J. N. Jr., 2000).

Source	Isoprene	Monoterpenes	Other VOCs
Canopy foliage	460	115	500
Terrestrial ground cover and soils	40	13	50
Flowers	0	2	2
Ocean and freshwater	1	<0.001	10
Animals, humans, and insects	0.003	<0.001	0.003
Anthropogenic (include biomass burning)	0.01	1	93
Total	~500	~130	~650

As it is seen in Table 1.2., BVOCs constitute a huge amount in global carbon budget. The negligence of this background is obviously a crucial mistake, and many scientists are still paying effort to customise BVOC emissions.

Table 1.3. below gives the emission inventories of Non-methane Organic Carbon (NMOC) in USA and worldwide. It suggests a ten-fold higher estimation of annual global emissions of isoprene, other monoterpenes with respect to other VOCs as well as methane (Alvarado A., 1998).

Table 1.3. Estimated emission inventories of NMOC in US and worldwide in TgC y^{-1} (Alvarado A., 1998).

	Anthropogenic NMOC	Biogenic NMOC	Isoprene	Terpenes
USA	19.5	29.1	5.9	23.2
Worldwide	110	1150	503	127

Ten-fold higher emission of VOCs is demonstrated when the worldwide emissions are considered.

1.3.2. Regional View of BVOC

In urban areas BVOC contributions to total VOC are relatively less important. For example, Benjamin et.al. (1997) have reported that biogenic hydrocarbons in the South Coast Air Basin of Southern California are ~ 10 % of the total VOC emission inventory on a typical summer day (Figure 1.3). The contributions of biogenic organics to urban O_3 formation is variable and depend on the particular locations.

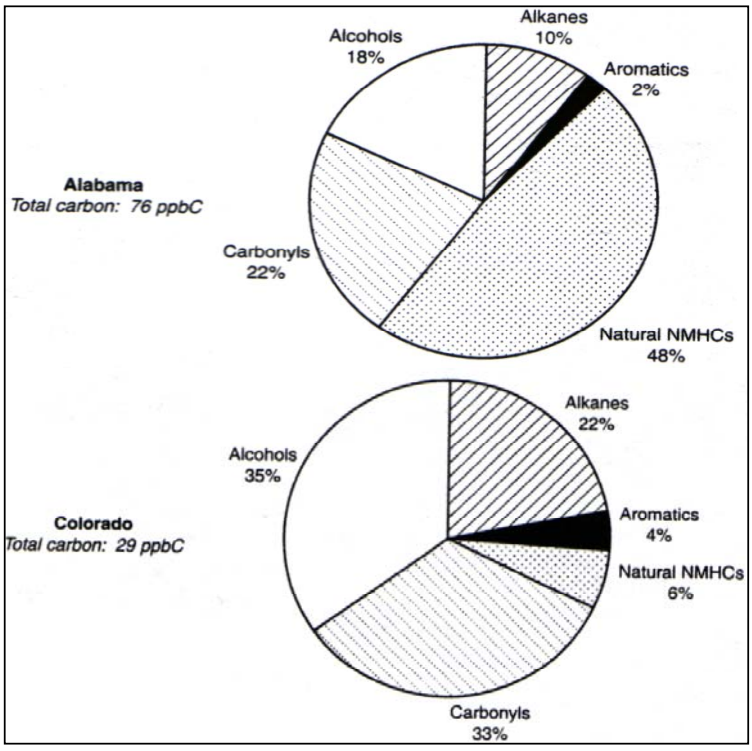

Figure 1.3. Distribution of organics observed in rural areas in Alabama and Colorado, respectively (Pitts B. J. F. and Pitts J. N. Jr., 2000).

Even within rural sites, which display higher contributions of BVOC, extreme variations can be observed when the emissions are compared. For example, Fehsenfeld *et al.* (1992) measured the composition of VOC at two rural locations in the United States, summarised in Figure 1.3.

Alcohols and carbonyls comprise 40-70% of the total. A significant portion of these alcohols and carbonyls appears to be direct emissions, with methanol being a major contributor to the oxygen-containing portion.

1.3.3. Factors Affecting Emissions of VOCs

Studies upto now displayed correlation of BVOCs with some environmental and phytological factors (Fuentes J. D. and Wang D., 1999; Kesselmeier J. *et al.*, 1998; Yokouchi Y. and Ambe Y., 1984; Tingey D. T. *et al.*, 1980). These factors (including meteorological parameters) should be further studied for the accordance of the measurements (especially for ambient air) done in uncontrollable conditions.

1.3.3.1. Effect of Light and Temperature on Biogenic Emissions

Since photosynthetical activities dominate during the production of BVOC, emissions are consequently light and temperature dependent. Figure 1.4. illustrates the production of isoprene from an aspen leaf when light in photosynthetic active range (Photosynthetic Active Radiation, PAR, 400-700 nm) is turned on and off. When the light is switched on, isoprene emissions rise and when it is turned off, fall even more rapidly.

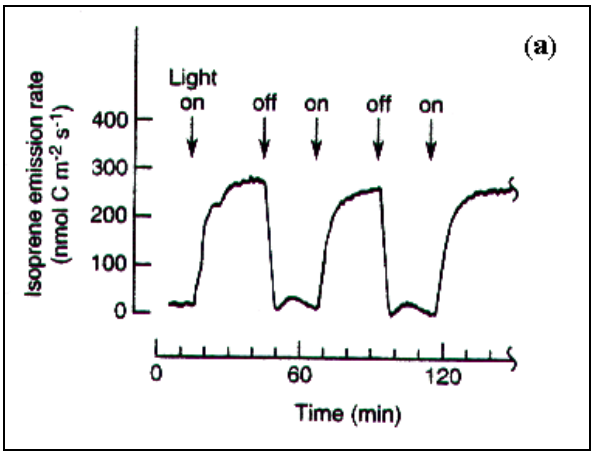

Figure 1.4. Effect of light on isoprene emission from aspen leaf (Pitts B. J. F.and Pitts J. N. Jr., 2000).

Figure 1.5. illustrates the emissions as a function of PAR intensity; the emissions rise rapidly and approach (but do not reach) a plateau.

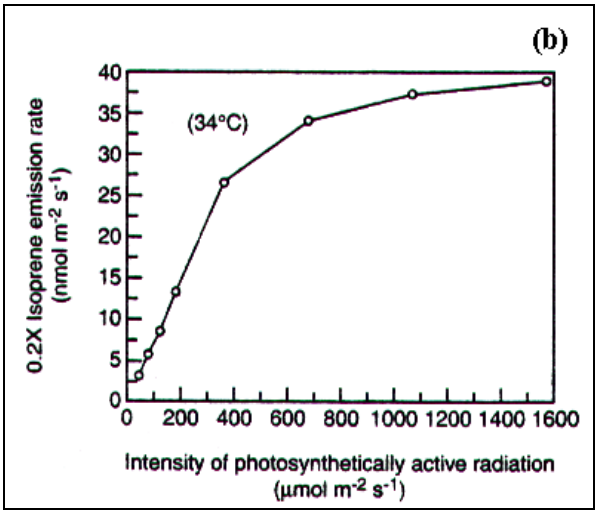

Figure 1.5. Effect of light on isoprene emission from velvet bean leaf (Pitts B. J. F. and Pitts J. N. Jr., 2000).

Figure 1.6. illustrates the effect of temperature on isoprene emission rate; emissions rise to a temperature of ~40-45 ^{o}C and then rapidly fall.

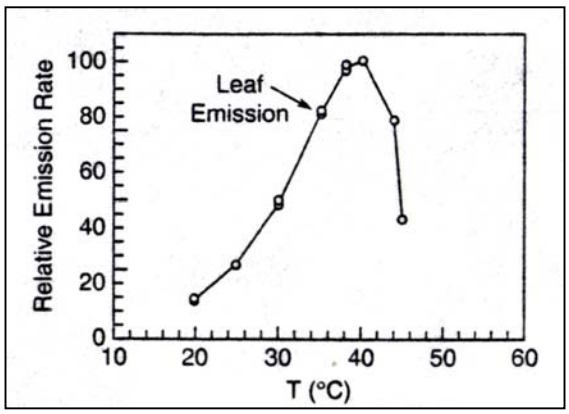

Figure 1.6. Effect of willow leaf temperature on isoprene emission rate (Pitts B. J. F. and Pitts J. N. Jr., 2000).

These influences of light and temperature are believed to be major factors in the variable emissions of isoprene measured in field studies. As for isoprene, emission rates of monoterpenes increase with temperature although different plant species exhibit different temperature sensitivities and different compounds can also show different dependencies on temperature. The temperature dependency of monoterpene emissions is often taken into account by multiplying the base emission rate at a reference temperature T_s by the factor $e^{[\beta(T-Ts)]}$, where T is the leaf temperature and β is coefficient that reflects the temperature sensitivity of emissions. Light also affects monoterpene emissions but does not appear to be as significant as for isoprene. Owen S. M. *et al.* have studied the light and temperature dependency of ten plant species commonly found in the Mediterranean region using a fully controlled cuvette in the laboratory (Owen S. M. *et al*., 2002). VOC emissions from all of the plant species investigated showed some degree of light dependency, which was distinguishable from temperature dependency.

Wiedinmyer C. *et al.* has investigated isoprene fluxes at Central Texas (Figure 1.7.), where the sampling station (tower) was surrounded by pine and hardwood forests and a post oak woods and forests (Wiedinmyer C. *et al.*, 2002). The isoprene concentrations were also both light and PAR dependent in that study.

1.3.3.2. Effect of Leaf Development to Biogenic Emissions

Another important factor that influences the isoprene rate is leaf development. Jennifer Kuzma and Ray Fall have reported that the isoprene emission rate for velvet bean (*Mucuna* sp.*)* increased as much as 125-fold as leaves developed, and then declined in older leaves. In addition, in these leaves, photosynthetic capability developed several days before significant isoprene emission occurred (Kuzma J. and Fall R., 1993).

1.4. Interaction of BVOCs with Other Atmospheric Chemicals

BVOCs interact with many chemicals in the atmosphere like NO_x, OH radical, and O_3. Several undesired atmospheric events are brought about as a result of these interactions:

1) As the natural VOC are biogenic, there is an obvious relation and possible feedback between climate change and both the composition and magnitude of BVOC fluxes (George M. *et al.*, 1995).

2) The conversion of NO to NO_2 is enhanced in the presence BVOCs that can be oxidised in the troposphere to produce peroxy radicals (Hollender J. *et al.*, 2002).

3) BVOCs are oxidized by the OH radical and some by ozone, thus influencing the oxidative capacity of the troposphere and affecting the lifetimes of other gases (George M. *et al.*, 1995).

4) They act as a sink for ozone or may contribute to the photochemical production of ozone in some rural and urban areas depending on the availabilty of NO/NO_x (Morales-Morales R., 1998, Thunis P. and Cuvelier C., 2000).

5) They contribute to the carbon monoxide budget as well as produce organic acids which in turn can acidify the atmospheric aerosol and precipitation (Müller K. *et al.*, 2002, Chebbi A. and Carlier P., 1996).

6) They contribute to aerosol formation with possible implication for climate (Dekermenjian M., 1998, Hoffmann E. D. *et al.,* 1996).

7) They contribute to production of peroxyacetyl nitrate (Pippin M. R., 1999).

1.4.1. Global Climatic Change: Climatically Important Biogenic Gases and Feedbacks

In the next century the earth will almost certainly experience climatic change. In the normal course of events, the planet would enter another glacial maximum about 23,000 years from now (Figure 1.7.). However, the flywheels of population growth and fossil fuel burning are turning rapidly and will be difficult to slow within a century. The global population is growing at a rate of 1.6% per year, representing a doubling time of 44 years; the rate implies a population of at least nine billion people by 2040. All these people will require energy to sustain themselves; most scenarios of future global energy use project a continuous heavy reliance on fossil fuel into the 21^{st} century. Such reliance will result in continuous emissions of greenhouse trace gases CO_2, CH_4 (indirectly stratospheric H_2O, a

greenhouse gas), and N_2O, along with trace metals, nonmethanehydrocarbons (NMHCs), SO_x, and NO_x. These latter three substances react with other chemical components of the climatic system, particularly the OH radicals, and have various effects on the radiative properties of the atmosphere. SO_x and NO_x are the principal constituents in acid deposition; NO_x and NMHCs are involved in formation of tropospheric ozone, another greenhouse gas. All of these greenhouse gases are biogenic in origin. The continuous accumulation of these gases in the atmosphere could lead to a "super interglaciation" (Figure 1.7).

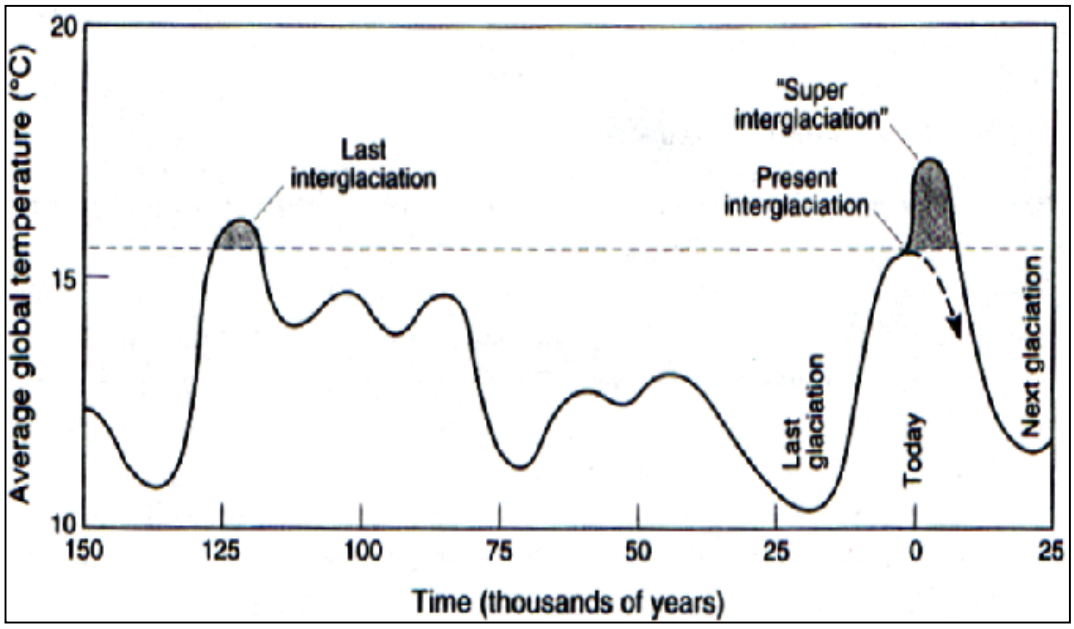

Figure 1.7. The earth's climate during the last 150.000 years and an interpretation of its future (George M. *et al.*, 1995).

1.4.2. NO_x and Nonmethane Hydrocarbons (NMHCs)

Nitrogen oxides (NO_x) and Non-Methane Hydrocarbons (NMHCs) are both biologically produced; NO_x from the bacterial decomposition of organic matter in soils and NMHCs as by-products of plant productivity in terrestial and

marine environments. Increasing temperature alone would probably increase the flux of NO_x from soils, leading to potential OH radical depletion and enhanced formation of tropospheric ozone

However, the situation is much more complex with respect to tropospheric ozone. The concentration of ozone depends in a very non-linear way on the atmospheric concentrations of other reduced biogenic gases, including CH_4, CO, NMHCs, and NO_x. In general, under atmospheric conditions in which NO_x concentrations are low 5-30 parts per trillion by volume [pptv], increases in the concentrations of the trace gases CH_4, CO, and NMHCs lead to a decrease in the concentration of tropospheric ozone.

The concentration of the OH radical, which is the main determinant of the oxidative capacity of the atmosphere, depends on concentrations of various trace gases, including tropospheric ozone and water vapor. Elevated concentrations of O_3, NO_x, and H_2O will result in increases in OH radical levels, whereas increases in the trace gases CH_4, CO, and NMHCs will lead to lower levels. One critical positive climatic feedback is that increases in CO concentrations in the atmosphere could lead to reduction in OH radical levels because NO_x has too short lifetime to counteract that effect on a global scale. Decreased concentrations of the OH radical lead to an increase in the lifetime of CH_4, which is a positive feedback.

The production of excited atomic oxygen $O(^1D)$ by the photodissociation of tropospheric ozone ($O_3 + h\nu \rightarrow O(^1D) + O_2$ for $\lambda \leq 330$ nm) is of great importance, because the $O(^1D)$ reaction with water vapor is the major source of OH radical, the dominant tropospheric oxidizing agent. In the troposphere, O_3 photochemical production is driven by the photolysis of nitrogen dioxide ($NO_2 + h\nu \rightarrow NO + O(^3P)$), which provides the required atomic oxygen $O(^3P)$ for O_3 production via the combination of $O_3(P)$ with molecular oxygen. As nitrogen oxides (NO_x = nitrogen dioxide, NO_2, and nitric oxide, NO) are mainly emitted as NO into the troposphere, NO_2 is formed by NO reaction with hydroperoxy radical

(HO_2) or with higher peroxy radicals (RO_2), produced from hydrocarbon oxidation.

$$NO + HO_2 \rightarrow NO_2 + OH \qquad (1)$$

$$NO + RO_2 \rightarrow NO_2 + RO \qquad (2)$$

Reactions (1) and (2) produce NO_2 without consumption of O_3. Subsequently, NO_2 is photodissociated to $O(^3P)$, which recombines with molecular oxygen to form O_3. The overall effect of the above-mentioned reactions is a NO_x catalytic cycle leading to O_3 formation.

The conversion of NO to NO_2 is enhanced in the presence of non-methane hydrocarbons and other volatile organic compounds (VOCs) that can be oxidised in the troposphere to produce peroxy radicals (RO_2 = HO_2 + organic R'O_2). The chemical behaviour of VOCs is complex. Their tropospheric degradation is initiated by reaction with OH radicals, O_3 and to a lesser extent nitrate (NO_3) radicals and is consequently a sink for these oxidants. These cycles may lead to a net chemical production or destruction of O_3 and a depletion or an enhancement of OH radicals in the troposphere depending on the levels of NO_x.

Anthropogenic NO_x and hydrocarbon emissions from biomass burning affect the tropospheric budgets of O_3, OH and NO_3 radicals. Over forested areas and additional effect is due to the hydrocarbon emissions from vegetation, mainly isoprene – C_5H_8 – and terpenes: α-pinene, β-pinene and homolog, but also carbonyl compounds such as acetone (Hollender J. *et al.*, 2002).

1.4.3. Carboxylic Acid Formation

Carboxylic acids has an important role in tropospheric chemistryin terms of their contribution to the acidity of precipitation. To illustrate, Keene and Galloway (1984) estimated that carboxylic acids, particularly formic and acetic acids, may contribute between 16 and 35 % of the free acidity of precipitation in U.S.A.. Gallowa *et al.* (1982) have mentioned that the contribution of these acids to the free acidity of precipitation may be as high as 65 % in some remote areas.

They have been found in fog water, rain water, cloud water, snow and ice water, gas phase, and aerosol. Potential sources are:

1. Primary emissions from anthropogenic sources: Formaldehyde, acetaldehyde, acetone, butanone and other carbonyl compounds, which are important components in solvents and disinfectants. Fossil fuel burning processes and wood burning are other anthropogenic sources for carbonyl compounds, such as formaldehyde, acetaldehyde, acrolein, crotonaldehyde and benzaldehyde.

2. Primary emission from biogenic sources: Formaldehyde, acetaldehyde, acetone, pentanal, hexanal, trans-2-hexenal, and others have been detected from plant emissions.

3. Secondary formation from photochemically formed radicals derived from VOC: e.g. formaldehyde, methyl vinyl ketone, and methacrolein are typical products of the isoprene decay.

Plant physiological stress and rapid increase of BVOC emissions as a result of high ozone levels in the surrounding has been reported by Wildt *et al.* (2001). The emission of carbonyl compounds increased enormously during ozone episodes with 100 $\mu g\ m^{-3}$ of ozone (Müller K. *et al.*, 2002).

Ozonolysis of anthropogenic or natural unsaturated hydrocarbons should lead to the formation of carboxylic acids. The reaction between ozone and olefins seems to be an effective pathway for producing acetic acid, but the amount of acetic acid formed is believed to be smaller than that of formic acid. The photochemical oxidation of isoprene and terpenes emitted from plants produces formic acid, and in less significance acetic acid (Chebbi A. and Carlier P., 1996).

1.4.4. Ozone Formation by BVOC

In the past, the impacts of biogenic VOC were not considered when ozone control strategies to limit emissions of either NO_x or VOC were developed. However, the importance of biogenic VOC emissions in an ozone inventory became apparent in some regions when the biogenic VOC emission estimates were compared to the anthropogenic VOC emission estimates.

The chemistry involved in the formation of ozone can be represented by the reactions below:

$$RH + OH \longrightarrow R + H_2O \quad (1)$$

$$R + O_2 + M \longrightarrow RO_2 + M \quad (2)$$

$$RO_2 + NO \longrightarrow NO_2 + RO \quad (3)$$

$$NO_2 + h\nu \longrightarrow NO + O \quad (4)$$

$$O + O_2 + M \longrightarrow O_3 + M \quad (5)$$

Here, VOCs (denoted above as RH) react with hydroxyl radicals (OH) to form organic radicals. Peroxy radicals (RO_2) are produced by the reaction of organic radicals with oxygen molecules in the presence of a third body (M). A

nitric oxide molecule (NO) is oxidised to NO_2 and then photolyzed to generate an oxygen atom, which combines with on oxygen molecule to produce ozone. Since tropospheric ozone production depends on VOC and NO_x mixing ratios, variable O_3 concentrations in different areas can be observed (Morales-Morales R., 1998).

Biogenic emission estimates for the United States have been reported at 30,860,000 tons of VOC per year and 346,000 tons of NO_x per year (Radian Corporation, 1996). This is in comparison to estimates of 21,090,000 tons of anthropogenic VOC and 23,550,000 tons of anthropogenic NO_x, estimated for 1990. Because of the interaction between NO_x and VOC in terms of atmospheric ozone levels, biogenic emissions should be included in any inventory which will be used to predict or to monitor atmospheric ozone levels. Inclusion of biogenic emissions is essential for photochemical air quality modelling.

For example, in the study of P. Thunis and C. Cuvelier in a Mediterranean area, the impact of biogenic emissions on peak ozone values by performing simulations with and without biogenic emissions, while keeping anthropogenic emissions constant (Thunis P. and Cuvelier C., 2000). The impact on ozone formation was also studied in combination with some anthropogenic emissions reduction strategies, i.e. when anthropogenic VOC emissions and/or NO_x emissions are reduced. The factor analyses technique was applied to isolate the impact due to biogenic emissions from the overall impact due to biogenic and anthropogenic emissions together. Amongst the BVOCs; d-limonene, sabinene, isoprene, α-pinene, β-pinene, HCHO, nonanal, acetaldehyde, MHO, and NO, the major impact on ozone formation [in percentages of the sum of the individual impacts, $\Sigma(S_{AB} - S_A)$], came from the carbonyls formaldehyde (~27%), MHO (13%), acetaldehyde (~11%) and nonanal (6%), where as it was in the order of 20% for limonene, 14% for isoprene, 4% for sabinene and 3% for NO. The larger emitted amounts for carbonyls could of course explain these numbers, but it clearly indicates that these species must be considered if a correct estimate of the BVOC contribution to ozone formation has to be established.

1.4.5. Secondary Aerosol Formation Potential of BVOCs

Carbonaceous aerosols can be classified as primary or secondary. Primary aerosol is particles that have been forced airborne such as soil dust, diesel exhaust, emissions from burning, etc.. Because chemical processes are not involved, the composition of an ambient primary aerosol reflects its origin and source directly. On the other hand, secondary aerosol is particles formed by gas to particle conversion processes in the air following the emissions from source. Odum *et al* (1996) has stated that, under peak photochemical conditions, secondary aerosol can constitute as much as 70% of the organic aerosol. The relationship between the composition of the formed aerosol and its source often includes a complex network of chemical transformations (Dekermenjian M., 1998).

Oxidation of BVOC leads to the formation of condensable products that can undergo gas-to-particle conversion. Although the aerosol formation potential of the BVOCs has received attention since at least 1960, the magnitude of the natural contribution to the particulate burden in the atmosphere is still not well characterised. Recent studies have focused on isoprene and a few monoterpenes as precursors for organic aerosol but much less is known about the aerosol formation potential of other important biogenic emissions, such as sesquiterpenes and oxygenated compounds.

Because of the enormous complexity of the chemical matrix of organic aerosol and the lack of direct chemical analysis methods for most of the compounds covering the organic aerosol fraction, estimation of secondary organic aerosol (SOA) contributions have been restricted to indirect methods of determination. One possibility is to run smog chamber experiments and to determine the degradation of the primary hydrocarbon (ΔHC) and the evaluation of the aerosol volume (ΔV) in order to obtain the fractional aerosol yield ($Y = \Delta V/\Delta HC$).

In a study conducted at CalTech. the aerosol formation from several monoterpenes, sesquiterpenes as well as oxygenated biogenic compounds in a series of outdoor smog chamber experiments was studied (Hoffmann E. D. *et al.,* 1996). Two sets of experiments were carried out: daylight experiments to investigate the photochemical aerosol formation in the presence of NO_x and dark reactions with elevated ozone concentrations to assess the potential of ozonolysis reactions of the natural alkenes to form condensable species. Table 1.4. summarises the experimentally determined fractional aerosol yields of the biogenic hydrocarbons investigated. Usually, the aerosol formation of each hydrocarbon was investigated at different initial concentrations and consequently a range for the aerosol yields was given in the table. Overall, the yields increased with increasing initial HC concentration. These results could be explained to a large extent in the framework of a gas/particle-partitioning model, showing the yield to be a function of the organic mass concentration. However, the comparison of the fractional aerosol yields observed during daylight experiments, where the NMHC degradation was induced simultaneously by ozone, OH- as well as NO_3-radicals, and the dark experiments, where oxidation occurs almost exclusively by ozone, clearly showed that the primary oxidation step also influenced the amount of aerosol formed in the chamber. In all experiments the yield was remarkably higher if only O_3 was responsible for the oxidation, indicating the importance of ozonolysis reactions as a direct pathway to produce nonvolatile products. Furthermore, a close evaluation of the daylight experiments implied also a high potential of NO_3/biogenic alkene reactions to form secondary organic aerosol.

Table. 1.4. Fractional aerosol yields for a series of biogenic hydrocarbons (outdoor smog chamber experiments summer/fall 1995, Caltech, Pasadena) (Hoffmann E. D. *et al.,* 1996)

	Daylight Experiments (Photochemical Oxidation)		**Dark Experiments (Ozone Organic Reaction)**	
	Δ HC [ppb]	Y [%]	Δ HC [ppb]	Y [%]
α-pinene	20 - 144	1.2 – 12.5	41 - 170	14.3 – 67.6
d-3-carene	112 - 117	22.0 – 25.9	90	78.2
β-pinene	95	30.0	53	32.7
d-limonene	90 - 159	34.2 – 38.9	-	-
Ocimene	104	5.3	85	17.9
Linalool	76 - 230	4.0 – 9.4	78	8.1
Terpinene-4-ol	71 - 229	2.9 – 5.8	90	35.1
Caryophyllene	100 - 102	99 - 120	-	-

Some additional conclusions from the investigations should be mentioned here. In general, the potential to form SOA is higher for cyclic hydrocarbons, like α-pinene and d-3-carene than for open chain compounds such as ocimene, most likely due to the formation of multifunctional oxidation products without breaking the molecules apart. Polyunsaturated biogenic alkenes, such as limonene, show an even higher aerosol yield. Probably secondary oxidation reactions of the primary formed degradation products are responsible for this observation. For caryophyllene, a naturally emitted sesquiterpene, a fractional aerosol yield of close to 100% was found. Consequently, in respect to recent findings that sesquiterpenes can be emitted in large quantities from vegetation, future estimations of the SOA formation from natural precursors should also consider this group of biogenic compounds (Hoffmann E. D. *et al.,* 1996).

1.4.6. Contribution of BVOCs to the Production of PANs

Peroxyacetyl nitrates (PANs) are produced as secondary organic pollutants in the atmosphere from the photooxidation of hydrocarbons in the presence of NO_x, and are considered excellent indicators of photochemical air pollution. Since PAN, the most abundant member of homologous series, was first discovered, it has been found to be ubiquitous throughout the troposphere. PAN is a major component of total oxidised odd nitrogen (NO_y) and can be transported over long distances before thermally decomposing. Thus, PAN can act as a reservoir for NO_x and free radicals, serving as a non-photolytic source of NO_x and free radicals in regions far from sources. PAN has long been known to be a potent lachrymator and a major phytotoxic component of photochemical smog. The second most abundant member in the series of PAN-type compounds is PPN (peroxypropionyl nitrate), which is commonly measured in urban areas at concentrations of approximately 10% that of PAN. The compound MPAN (peroxymethacrolyl nitrate) has been recently synthesised and characterised by Bertman and Roberts and has been observed in areas where isoprene emissions contribute to ambient hydrocarbons.

A major assumption employed in the PAN-MPAN-PPN statistical correlation is that MPAN is produced uniquely from biogenic sources, specifically methacrolein, and that PPN is produced solely from anthropogenic precursors. However, there is evidence of biogenic emissions from grasses and other plants that contribute to PPN formation and anthropogenic emissions from auto exhaust that can contribute to the formation of MPAN (Pippin M. R., 1999).

1.4.7. Oxidation Products of some BVOCs

As is expected, there is also a variety of oxygen containing organics found in rural and remote areas, which are the oxidation products of the directly emitted biogenics (Pitts B. J. F. and Pitts J. N. Jr., 2000). For example, in areas with

significant isoprene emissions, the oxidation products methyl vinyl ketone (MVK), methacrolein (MACR), and 3-methyl-furan are also typically present. Bisenthal and Shepson (1997) suggest that MVK and MACR may also be generated by automobile exhaust, based on the correlation of these compounds with CO in an urban area.

Indeed, separating out direct emissions and the formation by oxidation in air of other biogenics is not straightforward. For example, 6-methyl-5-heptene-2-one has been reported in air in different locations by a number of groups. However, the reaction of O_3 with organics containing the structural group $(CH_3)_2C=CHCH_2CH_2C(CH_3)=C-$ also gives this compound as is expected from he mechanism of ozonolysis and studies of structurally similar compounds such as linalool, $(CH_3)_2C=CHCH_2CH_2C(CH_3)(OH)CH=CH_2$. For example, ozonolysis of squalene was demonstrated to form gaseous 6-methyl-5-hepten-2-one, acetone, and geranyl acetone, respectively. 4-Oxopentanal was also formed from the further oxidation of 6-methyl-5-hepten-2-one. These compounds were also observed when leaves of common vegetation found in the Mediterranean were exposed to O_3. Furthermore, these products can be formed from the reaction of glass wool that had been in contact with human skin, which also contains squalene as a lipid; such observations suggest the importance of avoiding contamination of samples during measurements of biogenic organics.

In short, while a variety of oxygen containing biogenic organics have been observed to be generated from plants and most are likely direct emissions, care must be taken to distinguish such direct emissions from possible formation from larger biogenic hydrocarbons and/or, in some cases, contamination during sample handling.

1.5. Overview of Commonly Used Techniques for Measurement of BVOC

Sampling biogenic compounds onto porous polymeric adsorbents followed by thermal desorption under an inert gas stream to GC-FID or GC/MS is a technique used worlwide and this technique is preferred because of the relatively less contamination possibility when compared to the technique including collection into canisters. The method provides sub-ppb detection limits for various VOC and the equipment is less expensive than that for canister sampling. The tubes have small sizes adequate for sampling and the apparatus used to perform the conditioning occupies less space at laboratory and less complex. Problems that may arise with solid adsorbents include the potential for unwanted surface reactions between analyte and oxidizing species such as ozone (Colorado *et al.*, 1998). Because of very strong retention of adsorbent materials, undesired effects such as incomplete desorption and artifact formation are observed. Polar solutes are easily adsorbed but readily undergo surface-catalyzed reactions and on desorption yield compounds different than those originally sampled. High-molecular weight compounds can not be desorbed because of extremely strong interactions with the adsorbent and their low volatility (Baltussen *et al.* 2002). Another sources of error which can complicate adsorptive enrichment starategies are the formation of artifacts caused by degradation of both the adsorbed analytes and the adsorbent itself. Fortunately, to overcome these problems many adsorbents based on carbon and polymer resin are developed and are investigated for proper usage; moreover for ozone rich enviroments usage of ozone scrubbers are employed to remove ozone before entering the sorbent trap. Especially Tenax promises bright enhancement with highly reproducible recoveries. As hydrophobic sorbents like Tenax are commercially available the method promises to overcome interferances (especially at MS) caused by water (U.S.-EPA, 1997a). Thermal desorption can be coupled rather conveniently to a gas chromatograph and the (heated) carrier gas is used for thermal desorption. When, cryogenic focusing is employed, quantitative transfer of the analytes trapped on the adsorbent material to

the chromatographic column is possible; this results in considerable increase in sensitivity (Dettmer K. *et al.*, 2000; Yassaa N. *et al.,* 2000).

Another common method used for the determination of atmospheric VOCs involves sampling into pressurized stainless steel canisters followed by cryogenic focusing and chromatographic analysis (Colorado A. *et al.,* 1998). The method was originally based on collection of whole air samples in SUMMA® passivated stainless steel canisters, but has now been generalized to other specially prepared canisters. The VOCs are separated by gas chromatography and measured by a mass spectrometer or by multidetector techniques. Method TO-15 documents sampling and analytical procedures for the measurement of subsets of the 97 VOCs that are included in the 189 hazardous air pollutants (HAPs). However, this method is applicable to specific VOCs that have been tested and determined to be stable when stored in pressurized and sub-atmospheric pressure canisters. Minimal documentation is currently available demonstrating stability of VOCs in subatmospheric pressure canisters (U.S.-EPA, 1997b). Moreover, recent evidence indicates that this technique is not very reproducible for lower volatility non-methane hydro-carbons (NMHCs) and more polar oxygenated VOCs, which adsorb to and react on the walls of the canisters, or for light alkenes such as ethane that may be generated on the canister walls.

The most significant limitations of the above two techniques is that they are quite tedious and the cycle time usually exceeds the time scale of the chemistry. For, example, for a day time maximum concentration of OH of 1×10^7 molecules/cm^3, the atmospheric lifetime of isoprene is 17 min, given the rate constant for its reaction with OH of 1.0×10^{-10} cm^3 molecule^{-1} s^{-1}. Thus, chromatographic analytical detection schemes normally produce data sets that have insufficient time resolution to capture the temporal behaviour of this and other reactive VOCs, such as terpenes.

1.6. Previous Studies

Lewis *et al.* introduced a programmed vaporization injector two-dimensional gas chromatographic method for the measurement of isoprene and other hydrocarbons at a sampling rate of 12 min/sample; however, their sampling system consisted of a charcoal-packed, solid adsorbent, preconcentration trap (Colorado A. *et al.*, 1998).

In another study, Greenberg et al. Utilized a reduction gas detector (RGA) interfaced with a gas chromatograph for the determination of isoprene in a variety of environments. The analysis time from sample injection to detection was ~4 min; however, the air sampling system was not automated. Instead, stainless steel canisters were utilized for collecting air samples, which is tedious and severely limits the effective long-term sample time resolution. In addition, RGA detectors are known to have shortened lifetimes in the presence of aromatic and halogenated compounds.

As an alternative to electron ionisation (EI), mass spectrometric methods involving chemical ionisation (CI) in conjunction with tandem mass spectrometry (MS/MS), employing collision-induced dissociation (CID), have been used to solve a variety of analytically challenging problems by Armando et al. Although there has recently been a great deal of activity in applications of CI-MS/MS to studies of compounds of biological importance, this powerful technique can also be successfully applied to smaller molecules important to environmental and atmospheric chemistry. With the combined selectivity of CI and MS/MS, analysis of complex environmental mixtures may be possible without chromatographic separation, enabling the development of fast time response techniques. For example, in a recent study, Dearth et al. Developed a fast time response method that used nitric oxide CI with CID for the nonchromatographic analysis of automotive exhaust gases in a quadrupole ion trap equipped with a special Townsend glow discharge external source. However, a potential concern for the direct inlet used may be the effect of ambient (highly variable) water vapor on

sensitivity. Ambient humidity levels in the sample can cause unwanted ion-molecule reactions in the high-pressure source resulting in lower and variable sensitivity for both polar and nonpolar VOCs (Callahan G. S. M. *et al.*, 1996). This compromise of the CI chemistry makes effective removal of water vapor from air-sampling systems essential.

The utility of membrane inlet mass spectrometry (MIMS) for the effective reduction of water from the sample stream has been well documented. Although MIMS can provide good sensitivity for aqueous samples, relatively few studies have shown detection limits that are adequate for measurement of reactive atmospheric VOCs. To attain sufficient limits of detection, preconcentration is often required for the determination of many atmospheric analytes that are present in the atmosphere at concentrations reaching to very low ppt levels.

Colorado *et al.* have developed a novel membrane sampling cryogenic focusing system for the determination of isoprene in a quadrupole ion trap utilizing vinyl methyl ether (VME, Matheson) as a chemical ioniser (Colorado A. *et al.*, 1998). Here, VME was introduced into the vacuum chamber with a Varian leak valve and was allowed to react with the analyte for a period of 100 ms. To achieve lower detection limits, preconcentration of the sample components was conducted by drawing the sample air through a 30-in. Silcosteel (Alltech) sample tube containing a small diameter tubular silicone membrane that was continuously purged with helium at 30 cm^3/min. The analytes were then transferred to a cryogenic sample preconcentration trap (SPT) packed with glass beads that ballistically heated and injected the analytes into 30 m (0.25-mm i.d.) Supelco DB-1 column held isothermally at 150 ^oC. At this temperature there is very little analyte retention, and the column thus acts largely as a heated sample transfer-line. Ionised VME most likely reacts with dienes via a [2 + 4] Diels-Alder cycloaddition process that forms a highly unstable methoxycyclohexene radical cation which further fragments to lose methanol as shown in Figure 1.8.

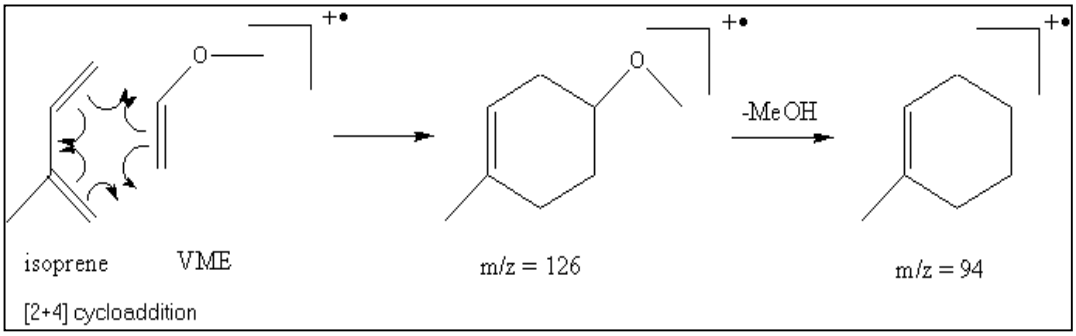

Figure 1.8. Proposed reaction for the formation of $[M + VME - MeOH]^{+\bullet}$ adduct ion (Colorado *et al.*, 1998).

Geiger J. *et al*. have used a commercially available GC/cryocondensation-FT-IR instrument with a home-built thermodesorption injector to complement GC/MS investigations (Geiger J. *et al*., 1998). The method was used to determine the compositions of biogenic hydrocarbons emitted from plants. Beer's law was used for quantitation of the peaks. To obtain quantitative data with the GC/cryocondensation- FT-IR method, a calibration procedure has been carried out by using the areas of the CH-stretching bands in the respective spectra. They have noted that; each terpene peak can be identified through the IR spectrum and corresponds to and amount of about 800 pg. Correlation factors (R^2) ranging from 0.9974 to 1 were obtained for terpenes including tricyclene, α-pinene, camphene, β-pinene, myrcene, Δ^3-carene. The detected concentrations were as 1 ng, 60 ng, 8 ng, 6 ng, 0.8 ng and 125 ng respectively. They have shown that GC/cryocondensation-FT-IR allows the sensitive determination of biogenic hydrocarbons in complex emissions of plants. Although GC/MS is a routine method for this application and GC/IR requires still more time and skill in practical use, IR-detection can supply additional information of the structure of the analytes – hence complementing GC/MS measurements. Finally, it can be concluded that the chromatographic and spectroscopic data obtained are reproducible enough for qualitative and quantitative analysis for the investigations of biogenic VOC emissions.

Biogenic Nonmethane Volatile Organic Compounds (NMOCs) inventories are required to assess the impact of hydrocarbons on regional chemistry. To derive such inventories, several mesurement techniques exist to identify chemical species and determine hydrocabon emission rates from vegetation. Biogenic VOC emission from plants has been measured in natural environments using branch or leaf enclosure ((Fuentes J. D. *et al.*, 2000, Komenda M. *et al.*, 2001, Janson R. *et al.,* 1999, Pier P. A., 1995), tracer release (Lamb B. *et al.*, 1986), and micrometeorological techniques (Fuentes J. D. *et al.*, 1996) and in controlled laboratory settings (Goldstein A. H. et al., 2001)

Hollender J. *et al.* has investigated the indoor air monitoring of terpenes using different adsorbents (Tenax GR, Tenax TA, Carbosieve SIII, and Chromosorb 106) and thermal desorption gas chromatography with mass selective detection (Hollender J. *et al.*, 2002). Monoterpenes such as α-pinene, β-pinene, camphene, Δ^3-carene, α-terpinene, and limonene are released into indoor air mainly from building materials of wood, paints and varnishes, cleaning agents and cosmetics. Because of increasing use of natural products and furniture made of wood, the indoor air terpene concentrations are reported to be higher. Some authors stated that, in new dwelling houses, maximal terpene concentrations of 797 $\mu g/m^3$ were measured. Therefore the question arises whether these concentrations lead to health effects and sensitive, simple and valid methods for indoor air monitoring are necessary. They have set up a method including 11 terpenes, namely; α-pinene, camphene, β-pinene, Δ^3-Carene, α-terpinene, Limonene, 1,8-Cineol, γ-Terpinene, Linalool, Fenchyl alcohol, and Camphor. For calibration, they have used and artificial air generator which employs introduction of 1 μL methanol and mixing chambers. Tenax GR followed by Tenax TA established the highest yield. Sampling flow of 10 mL/min was established and total volume was 600 mL. Five terpenes, α-pinene (26.4 $\mu g\ m^{-3}$), β-pinene (13.0 $\mu g\ m^{-3}$), Δ^3-carene (14.2 $\mu g\ m^{-3}$), camphene (9.4 $\mu g\ m^{-3}$) and limonene (18.2 $\mu g\ m^{-3}$), could be identified clearly in addition to other volatile organic compounds.

Yassaa N. *et al.* performed the sampling of monoterpenes at room temperature through two-stage traps consisting of 90 x 6 mm ID borosilicate glass tube filled with 100 and 50 mg of 20/40 mesh charcoal coconut shells (Supelco). Using a high precision portable air sampling pump (Buck I.H. pump, Orlando, USA), operating at a flow rate of 200 mL min^{-1} during 8 hours (Yassaa N. *et al.*, 2000). The ambient air sampling was carried out in June 1997 where the temperature ranged from 25 to 28 ^{o}C. The both charcoal layers were transferred to a 2 mL vial containing 1 mL carbon disulfide (Puriss Grade, Flucka, Switzerland) and the vial was immediately sealed with a septum cap. The samples were allowed to stand at room temperature for a period of 30 min under slight shaking, and the solutions were subjected to analysis. A few microliters of the biogenic extract of each tree were analysed with a HP 5708 A gas chromatograph equipped with a flame ionisation detector, and a CP Sil 8 CB fused silica capillary column (25 m x 0.2 mm, 0.25 μm film, Chrompack). The stationary phase was a poly (dimethylphenylsiloxane). The programmed temperature used was 8 min at 40 ^{o}C, then 2 ^{o}C min^{-1} to 200 ^{o}C. Injector and detector temperatures were 250 ^{o}C. The use of charcoal tubes with two beds (100 and 50 mg as respectively first and second layers) in the sampling conditions provided a good information about the breakthrough volumes since the absence of the compounds in the second layer confirm the fact that the sampling was done without breakthrough and hence the compounds were mostly adsorbed in the first layer. The desorption coefficient was found up to 98% in previous studies of Yassaa *et al.*. Nevertheless, the charcoal adsorbents were found not suitable for quantitative sampling of isoprene. For this reason, they limit their investigations to the monoterpenic BVOCs that were appropriately collected with this sampling technique.

H. Hakola *et al.* have evaluated the monthly average concentration profiles of monoterpenes, 1,8-cineol and isoprene at Ilomantsi, 1997-1998 (Hakola H. *et al.*, 2000). Due to their different sources, the relative abundances of the terpenoid compounds vary throughout the growing season (Fig. 1.9). In May, almost 70% of the terpenoid compounds were α-pinene, but in June isoprene concentrations

increase, and in July isoprene comprises half of the total terpenoid amount. α-Pinene and isoprene were the most abundant compounds throughout the growing season, but the share of the other monoterpenes was substantial, the other important monoterpenes being β-pinene/myrcene, Δ3-carene and camphene. The amount of sabinene was significant in July and August only.

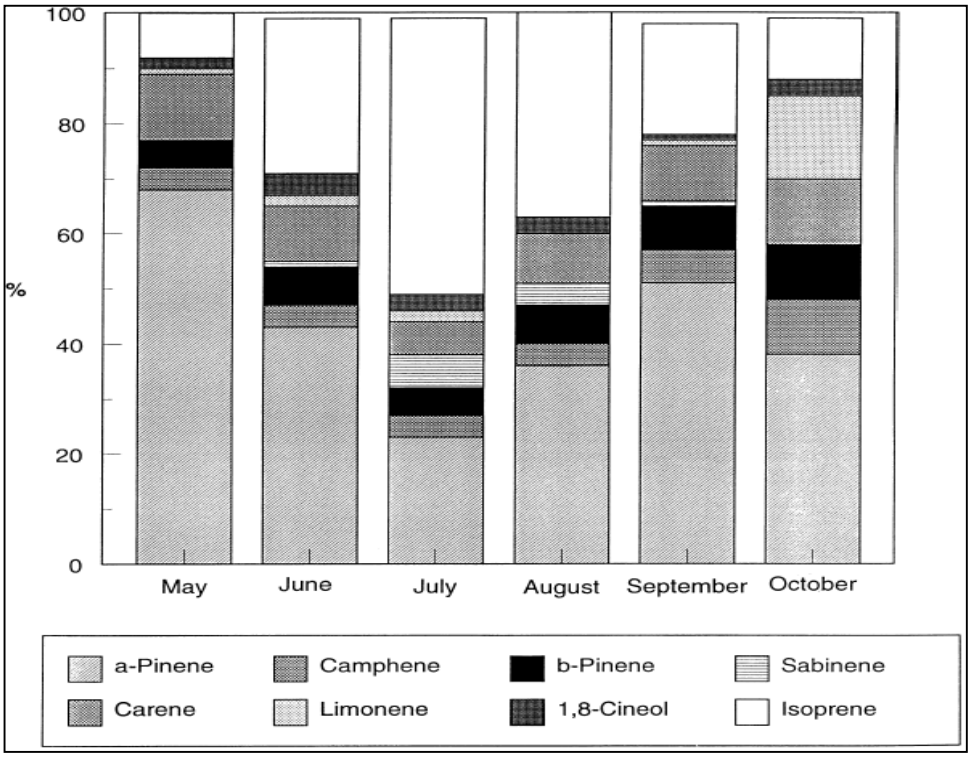

Figure 1.9. Monthly average concentration profiles of monoterpenes, 1,8-cineol and isoprene at Ilomantsi 1997-1998 (Hakola H. *et al.*, 2000).

Roberts *et al.* (1985) have measured the daytime and nighttime concentrations of monoterpenes in a forest environment (Alvarado A., 1998). They have measured 2-3 fold higher concentration for α-pinene, β-pinene, camphene, Δ^3-carene, and d-limonene at night despite the evidence of increasing concentration for these species with temperature (Figure 1.10).

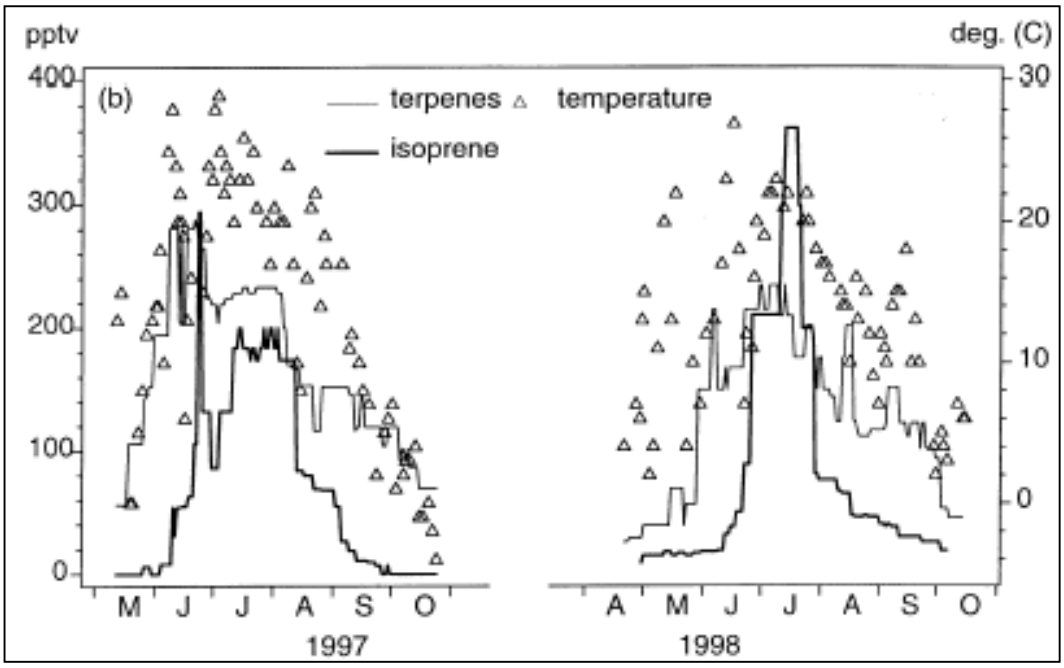

Figure 1.10. Temperature and 30-day running medians of isoprene and the sum of monoterpenes and 1,8-cineol (Alvarado A., 1998).

They have suggested that, lower concentrations of OH radicals and O_3 at night increase the lifetime of the monoterpenes contributing to their higher observed concentrations. In addition, they mentioned those meteorological factors such as reduced wind speed and a lower inversion height (a lower mixing height at night means a smaller mixing volume) could be responsible for these observations.

1.7. Aim of the Study

Our group has studied the tropospheric ozone in terms of its relation with NO_x, SO_2, suspended particulate matter (SPM), and aerosol composition. As mentioned before, the complete understanding of tropospheric ozone is not possible without the complimentary data based on VOCs. BVOCs constitute a background for total organic emissions and has to be determined for the estimation of a control strategy for both anthropogenic VOC and inorganic pollutants. Therefore, O_3 inventories should also include BVOC contributions.

The aim of this study is twofold. First aim is to investigate applicability of methodologies for sampling and analysis of selected terpenes (isoprene, α-pinene, camphene, beta-pinene, d-limonene, γ-terpinene, linalool, 1-isopulegol, 1-borneol, dl-menthol, alpha-terpineol, dihydrocarveol, citronellol, pulegone, and geraniol) by short path thermal desorption gas chromatography mass spectrometer (SPTD/GC-MS) at Sarıalan region of Uludağ mountain. Second aim is to characterise ambient concentrations of selected terpenes and their relations with other major pollutants, O_3 and NO_x, and meteorological parameters.

CHAPTER 2

EXPERIMENTAL

2.1. Sampling Site

In this study, ambient air samples were collected on adsorbent tubes by active sampling for analyses of selected terpenes at Sarıalan region in Uludağ National Park, Bursa (Figure 2.1.).

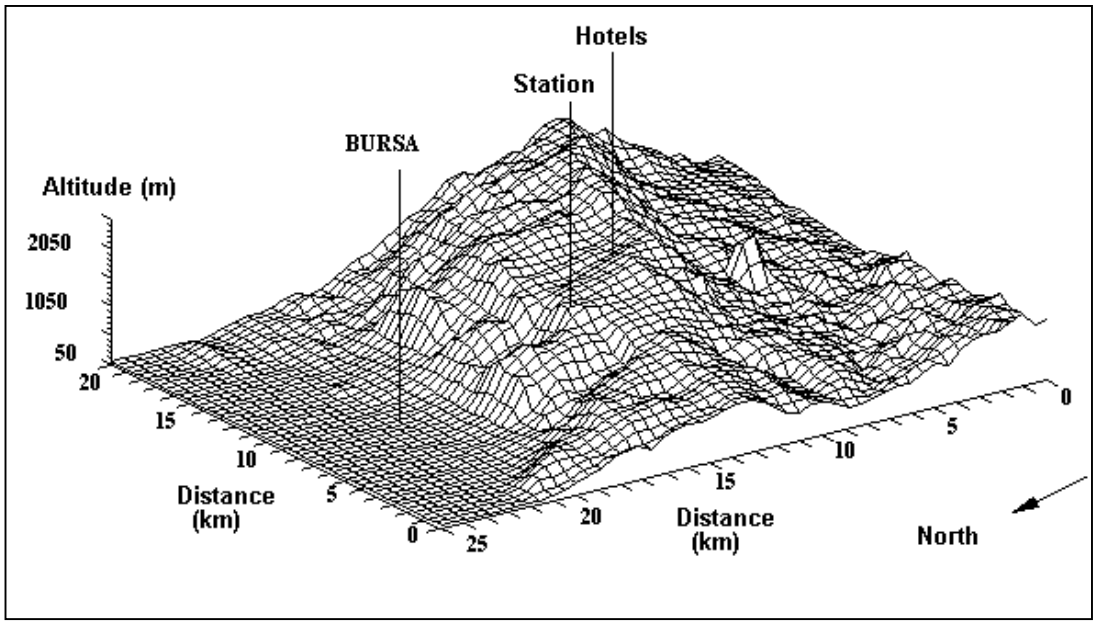

Figure 2.1. Location of the sampling site (Tuncel *et al.*, 1996).

Ozone, NO_x (NO and NO_2), and meteorological parameters were measured simultaneously. The site had an altitude of 1645 m and was located at 20 km south of the city of Bursa. The 40^o 08' 076" latitude and 29^o 06' 018" longitude specified the location. The station was 7 km far from the hotels.

2.2. Sampling of Terpenes

The ambient air samples were collected by means of active sampling on glass coated stainless steel tubes filled with Tenax® TA and Carbopack™ B sorbents for analyses of selected terpenes. The ambient air samples were collected for 4 hours at 30 mL/min flow, generated by low flow pump and controlled on-line by Mass Flow Controller and off-line by digital flow-meter.

2.2.1. Sampling Apparatus

The samples were collected using a special pump coupled to mass flow controller (MFC, Aalborg Inc., USA, 0-500mL/min)). The sampling pump (SKC Inc., USA, 5-500mL/min) was capable of low flow sampling . MFC prevents fluctuation of the flow (Figure 2.2.).

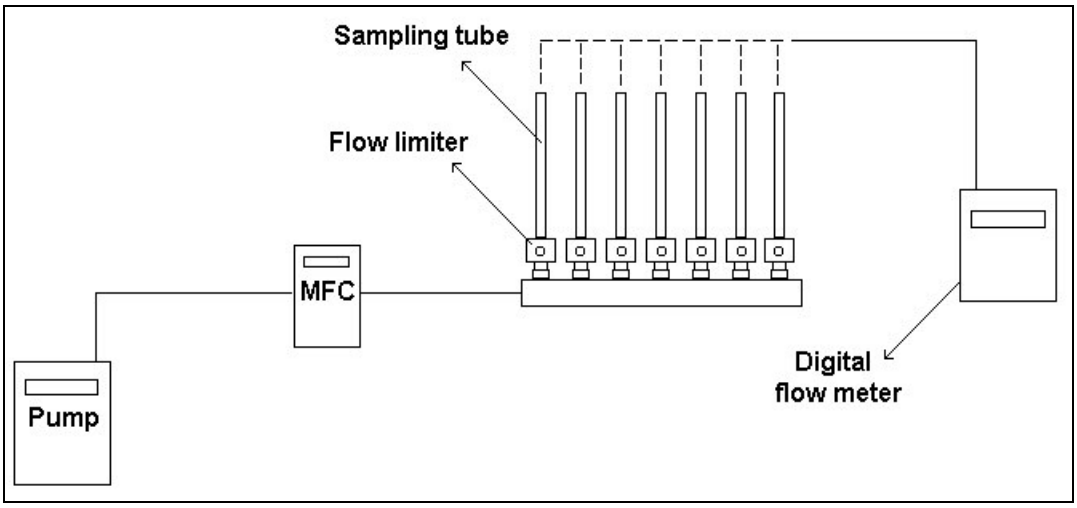

Figure 2.2. Sampling apparatus used during biogenic emission study.

The flow restrictors (cleaned with methanol, Merck) were used to limit the flow to desired value. The flow was set by measuring the flow at the sample entrance end of the tube, and adjusting the MFC to the set point. The flow was checked off-line by digital flow meter every 30 min and the small variations were considered when calculating the total collected air.

2.2.2. Sorbent Tubes

Sorbent tubes constitute the most important part of the sampling system and therefore special care has to be taken to ensure their preparation and cleanliness. The stainless steel tubes were homemade at Ostim, Ankara, and the glass coatings were done at METU Chemistry Department by glazier. The dimensions of the tube are illustrated in Figure 2.3. below.

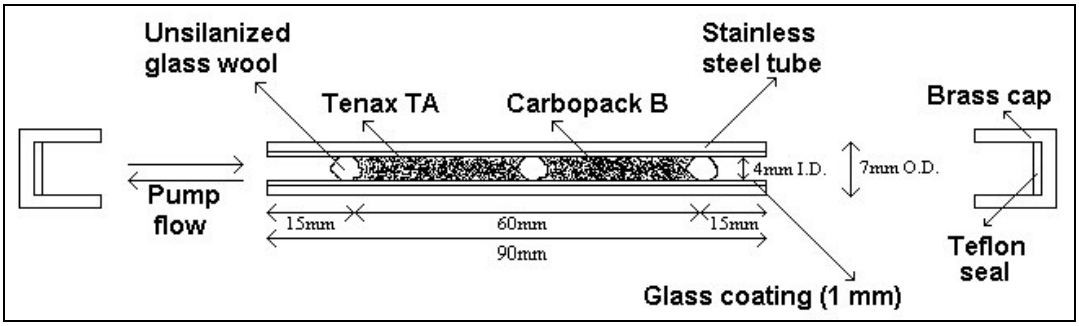

Figure 2.3. Sorbent tube filled with Tenax® TA and Carbopack™ B.

Brass caps fit to tubes with screw threads and they were teflon-sealed to ensure air-tightness.

2.2.2.1. Adsorbent Selection for BVOC

There are number of different adsorbents commercially available, and the user is very often faced with the difficulty of selecting an appropriate adsorbent from the great variety of materials on the market. The adsorbent used for adsorptive enrichment in combination with thermal desorption should generally meet the following criteria to guarantee an accurate determination of VOCs:

- Complete enrichment of the analytes of interest
  The specific surface area and the porous structure gives a rough indication of the adsorption strength of a material. The specific breakthrough volume (specific BTV) of the compounds gives a closer characterisation of the adsorption strength.

- Complete and fast desorption of the analytes.

- Homogenous and inert surface to avoid artifact formation, irreversible adsorption, and catalytic effects during sampling, storage of the loaded adsorbent tubes, and desorption.

- Low affinity to water to avoid displacement and hydrolysis reactions and to minimise disturbances of the gas chromatographic analysis, for example, damage of the stationary phase or retention time shift.

- Low adsorption capacity for other inorganic constituents of air, for example, nitrogen oxide, sulfur dioxide, carbon dioxide or ozone.

- High inertness against reactive species such as ozone. High mechanical and thermal stability.

- Multiple useability. (Dettmer K. and Engewald W., 2002).

In this study, Table.1 and Table-2 was referred (US-EPA, 1997c), for the adsorbent selection.

Tenax® TA (60/80 mesh, SUPELCO), and Carbopack™ B (60/80 mesh, SUPELCO) were chosen as sorbents since they cover the suitable range for isoprene and terpenes, hydrophobic and durable at temperatures of our applications.

Tenax, a porous polymer of 2,6-diphenyl-*p*-phenylene oxide, is by far the most popular material as trap adsorbent, because of its high reproducibility (Calogirou A. *et al.,* 1996). Selection of the flow direction during sampling and standard injection depends on the sorbent strength. Tenax TA, a weak sorbent, was placed at first order and Carbopack B was placed later since it is medium sorbent. Ease of desorption is provided with that configuration.

Table 2.1. Guidelines for Sorbent Selection (US-EPA, 1997c).

Sample Tube Sorbent	Approx. Analyte Volatility Range	Max. Temp., (°C)	Specific Surface Area, (m^2/g)	Example Analytes
CarbotrapC®, CarbopackC® Anasorb® GCB2	n-C_8 to n-C_{20}	>400	12	Alkyl benzenes and aliphatics ranging in volatility from n-C to n-C.
Tenax® TA	bp 100°C to 400°C n-C_7 to n-C_{26}	350	35	Aromatics except benzene, Apolar components (bp>100°C) and less volatile polar components (bp>150°C).
Tenax GR	bp 100°C to 450°C n-C_7 to n-C_{30}	350	35	Alkyl benzenes, vapor phae PAHs and PCBs and as above for Tenax TA.
Carbotrap®, CarbopackB® Anasorb® GCB1	(n-C_4) n-C_5 to n-C_{14}	>400	100	Wide range of VOCs inc., ketones, alcohols and aldehydes (bp>75°C) and all apolar compounds within the volatility range specified. Plus perfluorocarbon tracer gases.
Chromosorb® 102	bp 50°C to 200°C	250	350	Suits a wide range of VOCs incl. Oxygenated compounds and haloforms less volatile than methylene chloride.
Chromosorb 106	bp 50°C to 200°C	250	750	Suits a wide range of VOCs incl. hydrocarbons. Also good for volatile oxygenated compounds.
Porapak Q	bp 50°C to 200°C n-C_5 to n-C_{12}	250	550	Suits a wide range of VOCs incl. oxygenated compounds.
Porapack N	bp 50°C to 150°C n-C_5 to n-C_8	180	300	Specially selected for volatile nitriles; acrilonitrile andacetonitrile and propionitrile. Also good for pyridine, volatile alcohols from ethanol, MEK, etc.
Spherocarb*	bp -30°C to 150°C C_3 to n-C_8	>400	1200	Good for very volatile compounds such as VCM, ethylene oxide, CS and CH_3Cl. Also good for volatile polars e.g. MeOH, EtOH and acetone.
Carbosieve SIII*® Carboxen 1000*®	bp -60°C to 80°C	400	800	Good for ultravolatile compounds such as C C hydrocarbons, volatile haloforms and freons.
Zeolite Molecular Sieve 13X**	bp -60°C to 80°C	350		Used specially for 1,3-butadiene and nitrous oxide.
Coconut Charcoal* (rarely used)	bp -80°C to 50°C	>400	>1000	Rarely used for thermal desorption because metal content may catalyse analyte degradation. Petroleum charcoal and Anasorb®747 are used with thermaldesorption in the EPA's volatile organic sampling

* These sorbents exhibit some water retention. Safe sampling volumes should be reduced by a factor of 10 if high (>90%) relative humidity.

** Significantly hydrophilic. Do not use in high humidity atmospheres unless silicone membrane caps can be fitted for diffusive monitoring purposes.

CarbotrapC™, CarbopackC™, CarbopackB™, Carboxen™ and Carbosieve SIII™ are all trademarks of Supelco, Inc., USA; Tenax® is a trademark of Enka Research Institute; Chromosorb® is a trademark of Manville Corp.; Anasorb® is a trademark of SKC, Inc.; Porapak® is a trademark of Waters Corporation.

Table 2.2. Guidelines for Sorbent Use

Sample Tube Sorbent	Max. T., (°C)	Hydrophobic (?)	Temp. and Gas Flow for conditioning	Temp. and Min. Gas Flow for Desorption	Recommended Focussing Trap Packing
CarbotrapC® CarbopackC® Anasorb® GCB2	>400	Yes	350°C and 100 mL/min	325 °C and 30 mL/min	Tenax® or CarbopackC®
Tenax® TA	350	Yes	330°C and 100 mL/min	300 °C and 30 mL/min	Tenax®
Tenax GR	350	Yes	330 °C and 100 mL/min	300 °C and 30 mL/min	Tenax®
Carbotrap® CarbopackB® Anasorb® GCB1	>400	Yes	350 °C and 100 mL/min	325 °C and 30 mL/min	Tenax® or CarbopackB®
Chromosorb® 102	250	Yes	250 °C and 100 mL/min	225 °C and 30 mL/min	Dual-bed CB plus CMS trap or Chrom. 102
Chromosorb 106	250	Yes	250 °C and 100 mL/min	250 °C and 30 mL/min	Dual-bed CB plus CMS trap or Chrom. 106
Porapak Q	250	Yes	250 °C and 100 mL/min	225 °C and 30 mL/min	Dual-bed CB plus CMS trap or Porapak Q
Porapack N	180	Yes	180 °C and 100 mL/min	180 °C and 30 mL/min	Dual-bed CB plus CMS trap or Porapak N
Spherocarb*	>400	No	400 °C and 100 mL/min	390 °C and 30 mL/min	Dual-bed CB plus CMS trap or Spherocarb
CMS such as CSIII*® Carboxen 1000*®	400	No	350 °C and 100 mL/min	325 °C and 30 mL/min	Dual-bed CB plus CMS trap or CMS alone
Zeolite Molecular Sieve 13X**	350	No	330 °C and 100 mL/min	300 °C and 30 mL/min	Dual-bed CB plus CMS trap or CMS alone
Tenax/CB Comb. Tube Type 1	350	Yes	330 °C and 100 mL/min	300 °C and 30 mL/min	Tenax
Carb B/ CMS* Comb. Tube Type 2	400	No	350 °C and 100 mL/min	325 °C and 30 mL/min	Dual-bed CB plus CMS trap
Carb.300 type*, Comb. Tube Type 3	400	No	350 °C and 100 mL/min	325 °C and 30 mL/min	Dual-bed CB plus CMS trap

* These sorbents exhibit some water retention. Safe sampling volumes should be reduced by a factor of 10 if high (>90%) relative humidity.

** Significantly hydrophilic. Do not use in high humidity atmospheres unless silicone membrane caps can be fitted for diffusive monitoring purposes.

CB is short for Carbopack B and CMS is short for carbonized molecular sieve.

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2.2.2.2. Conditioning of the Tubes

The tubes were conditioned when they were empty and after they were filled by using the home-made conditioning oven (Figure 2.4.). Initial conditioning step just after the tubes were filled was done by conditioning oven. The final step before first usage were done by the thermal desorption system for 30 min, at 100 mL/min. He flow, since some of the impurities still could not be eliminated by using the conditioning oven.

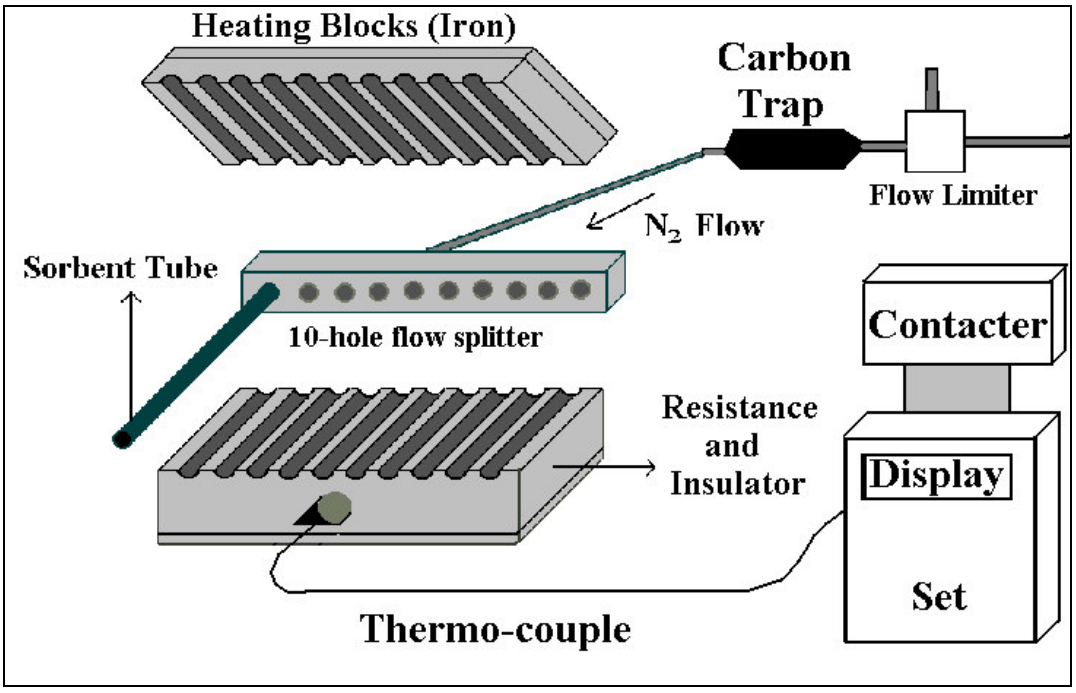


Figure 2.4. Home-made tube conditioning oven.

The oven can be operated at temperatures covering highest maxima of our sorbents, which are 350 ^{o}C for Tenax TA and 400 ^{o}C for Carbopack B, with a tolerance of ± 5 ^{o}C. The heating blocks were made of iron, while the 10-hole flow splitter that distributes the N_2 flow to the sorbent tubes was made of brass (brass is more ductile than iron). Thermocouple (Gürbüzoğlu Ltd., Ankara) and contacter

(Siemens, Germany) provided the temperature control. The system was mounted on a piece of heat insulating material and the whole system was covered with a plexiglass box. A fan ventilated the box by taking out the air inside the plexiglass box when the system was operating in order not to be exposed to the chemicals emitted. Carbon trap (HP, Hydrocarbon removal trap) was used to improve purity of the N_2.

2.2.2.3. Handling the Conditioned Adsorbent Tubes

The sampling tubes were kept in test tubes cleaned with ethanol and closed with caps wrapped with teflon tape (Figure 2.5.). The bottom of the tubes were filled with anhydrous calcium sulfate (8 mesh, W.A. Hammond Drierite Company, USA) for uptake of humidity and activated charcoal (8 mesh) to prevent contamination.

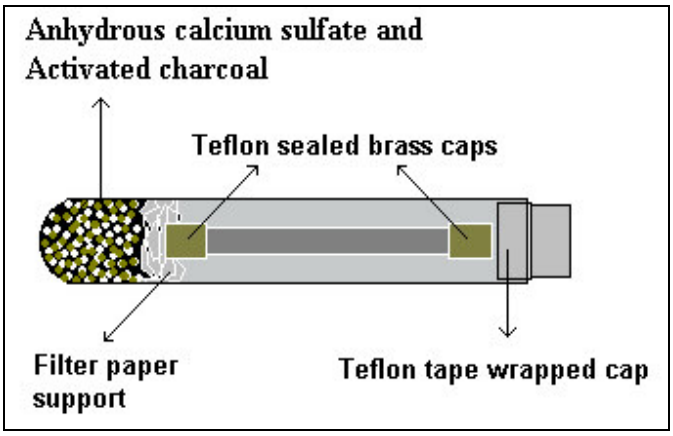


Figure 2.5. Tube storage in solvent-washed glass tubes.

Filter paper was used to support the drier and charcoal in the test tube. The tubes were taken to the sampling site in these tubes and after sampling they were

kept at ice bags during transportation and refrigerator for storage. The samples were analysed within 1 week after their collection.

2.3. Handling and Preparation of Terpene Standards

The terpene standards preparation and handling requires special care since the solvent (ethanol) and the standards are volatile and after their mixing the reactivity of the constituents limits the continuous usage of the standards for long periods. Especially the accuracy during addition of fenchone (ISTD) is extremely important since it is the exact amount of the ISTD which provides the control of detector response and eliminates the errors that can be brought about during sample introduction.

2.3.1. Preparation of Terpene Standards

The terpene standards (ULTRAkit™ WRK-105 Terpenes) were acquired from (Ultra Scientific, North Kingstown). The complete set of terpene standards included, alpha-pinene, camphene, beta-pinene, d-limonene, gamma-terpinene, fenchone **(ISTD**), linalool, 1-isopulegol, 1-borneol, dl-menthol, alpha-terpineol, dihydrocarveol, citronellol, pulegone, geraniol, myrecene, and citral (Table 2.3. and Table 2.4.). Except for myrcene (2% w/v myrcene dissolved in chloroform) the terpene standards were prepared in ethanol (2% w/v). In order to prevent the error caused by different solvent usage, myrcene was eliminated from the analyte list. Citral was also eliminated since it was degragated. Isoprene standard (99.5%) was obtained from Dr. Ehrestorfer, Institute for Reference Materials and Measurements, Belgium.

Table 2.3. Structures of isoprene and selected terpenes.

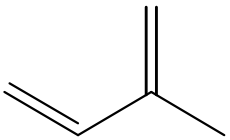
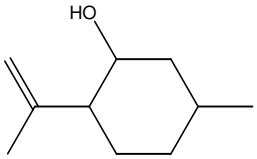
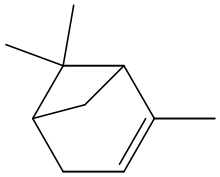
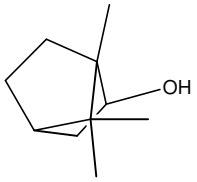
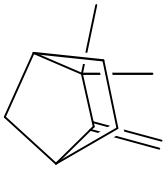
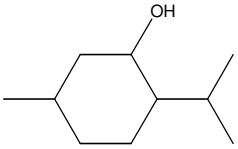
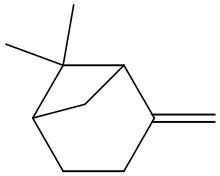
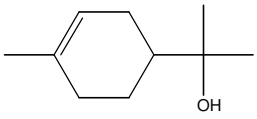
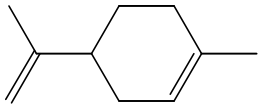
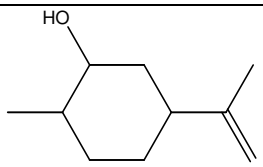
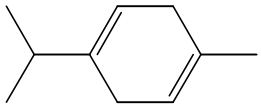
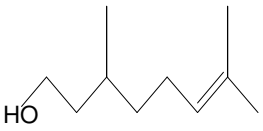
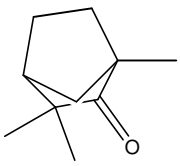
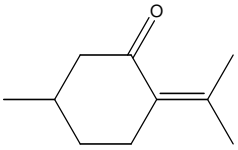
Isoprene C_5H_8		Isopulegol $C_{10}H_{18}O$	
Alpha-pinene $C_{10}H_{16}$		1-borneol $C_{10}H_{19}O$	
Camphene $C_{10}H_{16}$		Menthol $C_{10}H_{20}O$	
Beta-pinene $C_{10}H_{16}$		α-terpineol $C_{10}H_{18}O$	
d-limonene $C_{10}H_{16}$		Dihydrocarveol $C_{10}H_{18}O$	
γ-terpinene $C_{10}H_{16}$		Citronellol $C_{10}H_{20}O$	
Fenchone $C_{10}H_{16}O$		Pulegone $C_{10}H_{16}O$	
Linalool $C_{10}H_{18}O$		Geraniol $C_{10}H_{18}O$	

Table 2.4. Nomenclature and properties of selected terpenes.

Terpene	M.W. (g/mol)	B.p. (^{o}C)	CAS#	IUPAC Names
Isoprene	68.12	34	78-79-5	2-methyl-1,3-butadiene
Alpha-pinene	136.23	155-156	80-56-8	2,6,6-Trimethyl-bicyclo[3.1.1]-2-heptene
Camphene	136.23	159-160	79-92-5	2,2-Dimethyl-3-methylene-bicyclo[2.2.1]heptane
Beta-pinene	136.23	164-167	99-86-5	6,6-Dimethyl-2-methylene-bicyclo[3.1.1]heptane
d-limonene	136.23	175-176	5989-27-5	4-Isoprophenyl-1-methyl-cyclohexene
γ-terpinene	136.23	182	99-85-4	1-Isopropyl-4-methyl-1,4-cyclohexadiene
Fenchone	152.23	192-194	4695-62-9	1,3,3-Trimethyl-bicyclo[2.2.1]-2-heptanone
Linalool	154.25	194-197	78-70-6	3,7-Dimethyl-1,6-octadien-3-ol
1-isopulegol	154.25	224	59905-53-2	2-Isopropenyl-5-methyl-cyclohexanol
1-borneol	155.26	206-208	507-70-0	1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-ol
Menthol	56.27	212	1490-04-6	2-Isopropyl-5-methyl-cyclohexanol
α-terpineol	154.25	217-218	10482-56-1	2-(4-Methyl-3-cyclohexenyl)-propan-2-ol
Dihydrocarveol	154.25	224-225	619-01-2	5-Isopropenyl-2-methyl-cyclohexanol
Citronellol	156.27	222	106-22-9	3,7-Dimethyl-6-octen-1-ol
Pulegone	152.23	224	89-82-7	2-Isopropylidene-5-methyl-cyclohexanone
Geraniol	154.25	229-230	106-24-1	3,7-Dimethyl-2,6-octadiene-1-ol

The standards were prepared in 2 mL and 4 mL amber vials (screw top with polypropylene hole cap, PTFE/Silicone Septa, SUPELCO) using gastight syringes (Agilent Technologies and Hamilton Company) including 10 μL, 50 μL, 100 μL, 500 μL and 5 mL volumes. The additions of both standard and solvent were controlled by weighing the empty and full syringe with analytical balance (Mettler AJ100) in order to ensure accurate addition.

In Table 2.4. CAS# is the number given by Chemical Abstracts Service, which is a division of American Chemical Society, that provides a definite citation of the compounds in mass library database. Ethanol (99.9 % JT Baker and 99.8 % Riedel de Haen) was used for dilutions. Cleaning of the preparation and storage apparatus was done with solvent prior to use.

2.3.1. Internal Standard Calibration

Six point internal standard calibration plot was prepared for determination of terpenes. To prepare the 6 calibration standards, a stock intermediate was prepared and the successive dilutions were done (Table 2.5.). The standards were prepared fresh before calibration and they were stored at refrigerator, under 4^{o}C, when not in use. A carefully measured internal standard, fenchone, was spiked to the samples prior to analyses making up an amount that was equal to the portion added to the real samples. To decide about a level to be contained in linear regression; the ion ratios were taken into account. Target and qualifier ion ratios (see Chapter 3), which supply 80% correlation with library database, NBS75K.L and WILEY275.L, were accepted. The ratio of analyte to internal standard peak area served as analytical parameter to obtain calibration plot. Table 2.5. shows the concentration of standards and the corresponding correlation coefficients.

Table 2.5. Concentrations of the standard levels and correlation coefficients.

Terpene/Level (ng)	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	R^2
alpha-pinene	0.967	1.934	5.802	9.670	19.34	30.94	0.990
Camphene	0.967	1.934	5.802	9.670	19.34	30.94	0.996
beta-pinene	0.961	1.922	5.766	9.610	19.22	30.75	1.000
d-limonene	0.967	1.934	5.802	9.670	19.34	30.94	0.998
γ-terpinene	0.991	1.982	5.946	9.910	19.82	31.71	0.997
Linalool	0.973	1.946	5.838	9.730	19.46	31.14	0.997
1-isopulegol	1.003	2.006	6.018	10.03	20.06	32.10	0.997
1-borneol	1.020	2.040	6.120	10.20	20.40	32.64	0.999
dl-menthol	1.040	2.080	6.240	10.40	20.80	33.28	0.996
alpha-terpineol	0.973	1.946	5.838	9.730	19.46	31.13	0.996
Dihydrocarveol	0.923	1.846	5.538	9.230	18.46	29.53	0.995
Citronellol + pulegone	1.980	3.960	11.89	19.81	39.62	63.39	0.991
Geraniol	0.991	1.982	5.946	9.910	19.82	31.71	0.989

2.3.2. Introduction of the Terpene Standards and Ambient Air Samples

10 µL syringe was used to inject 2 µL standard to the adsorbent tube from tenax site and the tube was immediately fitted to the thermal desorption port. The purging proceeded for 20 min at 30 mL/min helium flow aimed to get rid of excess solvent Theoretically ethanol is not adsorbed on neither Tenax® TA nor Carbopack™ B, however complete removal could not be acquired. Furthermore the purging aims to expel the humidity of real samples. During that process the flow direction was from Tenax® TA to Carbopack™ B. For ease of desorption the anlytes should interact first weak and then medium sorbent. After 20 min purge with 30 mL/min Helium at Short Path Thermal Desorption Unit (SPTD), the tube was fit to SPTD so that the flow direction pointed from Carbopack B to Tenax TA site, and the SPTD program was started (Table 2.8.).

The standards contained the internal standard, fenchone, which was added just before the analyses. On the other hand, the ISTD was added to the sample prior to the analysis. 2µL, corresponding to 24 ng of fenchone, which is the same amount as standards were added to sample and the tube was purged as for the case of standards. For the samples, after collection of the desired volume of ambient air, the same procedure as the standards was applied, except for the substitution of the standard injection with a 2 µL solution that contained only fenchone in ethanol.

2.4. Analytical Method Used for Terpenes, Short Path Thermal Desorption Gas Chromatography Mass Spectrometer (SPTD-GC/MS)

The combination of gas chromatography (GC) for separation and mass spectrometer (MS) for detection and identification of the components of a mixture of compounds has been used commonly as an analytical tool in the research and commercial analytical laboratories. Use of short path thermal desorption for introduction of the samples collected on adsorbent beds is gaining popularity as the manufacturers are offering selective adsorbents with enhanced thermal properties , i.e. more resistant to high temperatures and providing complete desorption of the analytes at reasonable temperatures.

In this study the terpenoids in ambient air of Sarýdan Region in Uludað were collected on stainless steel tubes covered with tempered glass inside and containing Tenax TA® and Carbopack™ B. The samples were introduced to GC by means of short path thermal desorption unit (SPTD, Model TD-4, S.I.S., USA). GC (HP-6890+) coupled to MS (HP-5973 Mass Selective Detector) was used for separation and identification by full-scan mode, 35-550 amu, and quantification of the terpenoids by Selected Ion Monitoring, SIM.

The simplified view of SPTD/GC-MS is given in Figure 2.6..

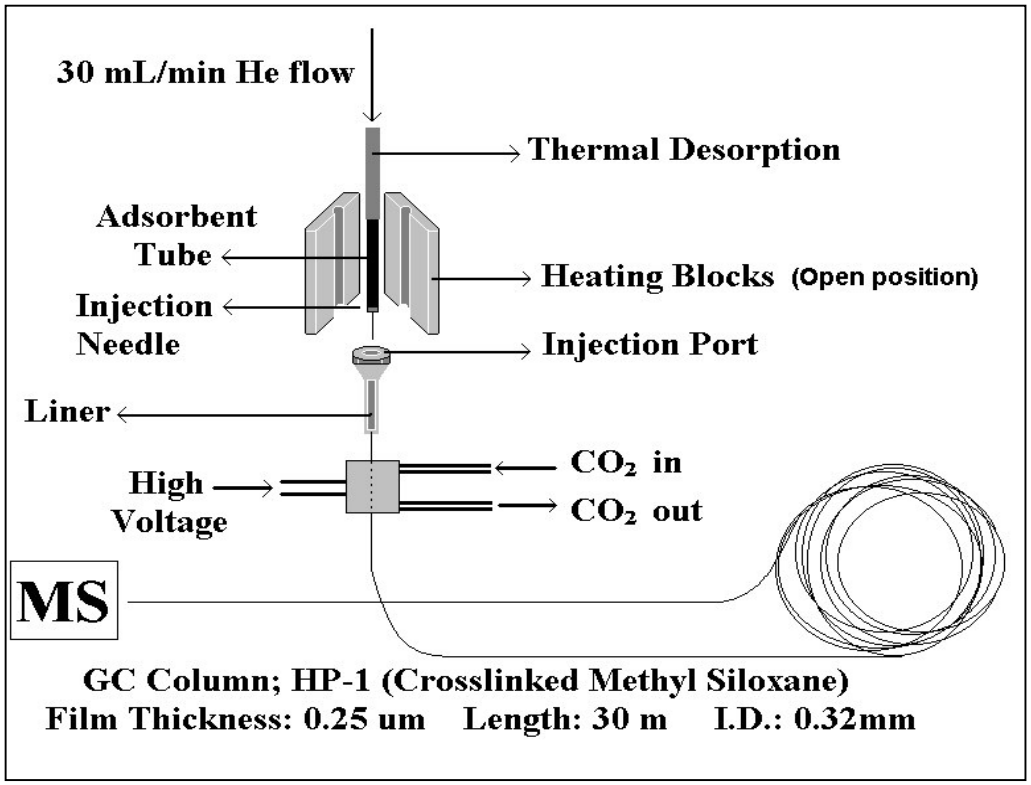


Figure 2.6. Simplified view of SPTD/GC-MS.

CO_2 served as coolant while the high voltage provided the flash heating of the cryotrap.

2.4.1. Gas Chromatography Mass Spectrometry

Gas Chromatography is a popular, powerful, reasonably inexpensive, and easy to use analytical tool. Mixtures to be analysed are injected to an inert gas stream and swept into a tube packed with a solid support coated with a resolving phase. Interaction between the components in the gas stream and the coating leads to a differential separation of the components of the mixture, which are then swept through the detector interface.

The mass spectrometer takes injected material, ionises it in a high vacuum, propels and focuses these ions and their fragmentation products through a magnetic mass analyser, and then collects and measures the amount of selected ions in a detector (Smith and Busch, 1999). Figure 2.7. illustrates a typical GC-MS system equipped with quadrupole analyser. The theory and instrumental features of Mass Spectrometer will be given in detail in the following sections.

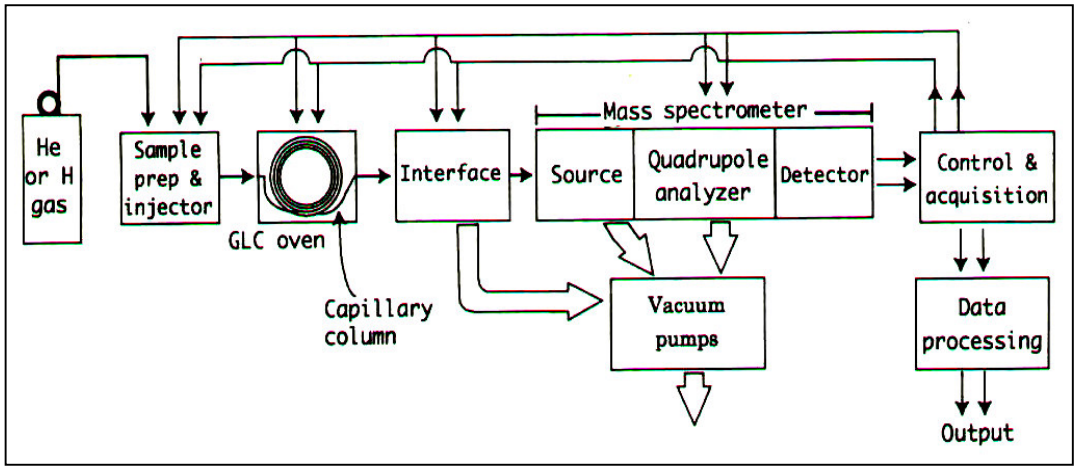


Figure 2.7. A typical GC/MS system diagram (Smith and Busch, 1999).

In this study, for the separation of the terpenoids HP-6890 Gas Chromatography Mass Spectrometer was used with HP-1 high performance capillary column (Crosslinked Methyl Siloxane, Film thickness: 0.25µm, Length: 30m, I.D.: 0.32mm and Phase Ratio: 320, Hewlett Packard). The Hewlett Packard 6890 GC/MSD is a benchtop GC/MS interfaced to a HP 5973 Mass Selective Detector Quadrupole Mass Spectrometer. The system uses electron impact (EI) ionisation and is capable of performing full mass scans or selective ion monitoring (SIM). The GC was set up for capillary columns and is capable of split (splits the sample for dilution purpose) or splitless injections. HP-1 column was applicable to amines, hydrocarbons, pesticides, PCBs, phenols, and sulfur compounds, and the temperature range is –60 to 350 ^{o}C, which was suitable for our experimental conditions. Helium (min. 99.999%, B.O.S.) was used as carrier gas and it was

passed through hydrocarbon trap (HP, hydrocarbon removal trap) for further purification.

Since this is a community instrument, used for a wide range of applications, sample cleanliness is essential. Any nonvolatile compounds that you inject onto the column will be left behind and will cause future column and/or instrument problems for you and all other users. All metals must be removed from your samples and no acids or bases should be injected (http://www.chem.nwu.edu/~asl/GC.html). The identity of each sample was stored in the instrument logbook so that, instrument problems could be traced to the injected compounds.

To ensure the system cleanliness, daily blank measurements were done. A clean blank was always preferred rather than subtraction of blank from the analyte peaks, since the column conditions may change after each run and the contamination peaks that the analyst observed for the blank may have been eliminated during that individual run. The accessed blank consisted of the 2µL ethanol injections by thermal desorption. When a contamination was noticed; column, tube, and solvent are examined separately and the problem is solved by conditioning the column for sufficiently long time under column maximum, which was 300^{o}C for HP-1-MS, or conditioning the tube few times at 300^{o}C, if necessary.

The system was operated using the instrumental parameters summarised in Tables 2.6. and 2.7.:

Table 2.6. GC-MS operation parameters.

INLET	Mode	Splitless
	Gas	He
	Heater	260 ^{o}C
	Pressure	10.12 psi
	Total flow	14.3 mL/min
COLUMN	Mode	Constant flow
	Pressure	10.12 psi
	Flow	1.3 mL/min
	Avg. velocity	41 cm/sec
	Column max.	300 ^{o}C
MS	Foreline	≅ 70mtorr
	Heater Aux.	260^{o}C
	$E_{ionisation}$	70 eV

In Table 2.6, foreline indicates the low-pressure inside the mass detector. All mass spectrometers must be operated under high vacuum. Otherwise, collisions would produce a deviation in the path that ions follow (trajectory) and the ion would lose its charge against the walls of the instrument. On the other hand, ion-molecule collisions could produce uncontrolled reactions and hence increase the complexity of the spectrum. Theoretically the maximum pressure should be 66 nbar to keep the mean free path above 1 m (Hoffmann E. D. *et al.*, 1996). 70 mtorr (equal to 49.51 nbar) was maintained for this particular study using two-stage pump system. HP G1099A model turbomolecular (turbo) pump and a HP G1098A model high vacuum vapour diffusion pump were used. The carrier gas (He) flow maxima of the later pump was 2 mL/min.

The oven program (Table 2.7) was decided by altering the temperatures, ramps, and durations so that the best resolution of the peaks could be observed.

Table 2.7. GC oven temperature program.

Ramp (^{o}C/min)	Initial Temp. (^{o}C)	Hold (min)	Total Time (min)
	40	0	0
12	60	0	1.67
8	180	0	16.67
20	280	10	31.67

The final step ensures the column cleanliness (baking step). Optimisation of oven parameters is discussed in Chapter 3.

2.4.2. Mass Spectrometer

Mass spectrometry (MS) is an analytical technique in which atoms or molecules from a sample are ionised usually positively, separated according to their *mass-to-charge ratio* (*m/z*), and then recorded. Since it forms and utilises ions rather than molecules, which are concerned in techniques such as infrared, ultraviolet/visible and nuclear magnetic resonance spectroscopies, it presents destructive analyses. The system that was used in this study (HP-5973 Mass Selective Detector) contained electron ionisation (EI) to ionise the terpenes, quadrupole mass analyser to separate the ions, and continuous dynode electron multiplier (CEM) to detect the molecular ions and their fragments.

2.4.2.1. Ionisation of Molecules

In a mass spectrometer used for organic analysis, the analyst usually deals with the positive ions derived from molecules. When electrons pass through or very close to a molecule, they can bring about ionisation. Usually this process results in formation of positive ions, by simply removing one electron from the

original molecule, but electron attachment and hence negative ion formation is also possible. The probability of electron capture is about 100 times less than that of electron removal and, furthermore, when a molecule takes up a moving electron, the translational energy of the electron must be taken up by the molecular ion (M^-). This translational energy is usually converted into vibrational energy.

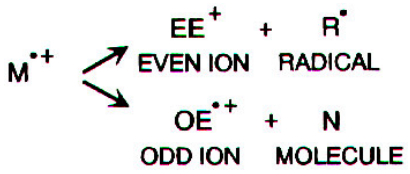
Virtually, all stable organic molecules have an even number of electrons. These electrons occupy either bonding or non-bonding orbitals. Therefore, if one electron is removed during ionisation, the resulting ion will have an odd electron configuration – it will be left with one unpaired electron. Molecular ions ($M^{+\cdot}$) always have an odd electron configuration. They are radical cations (Davis R. and Frearson, 1987).

$$M: \rightarrow M^{+\cdot} + e^-$$

The ion's *mass (m)* and *charge (z)* are measured by the mass spectrometer and what is used in overall is their ratio. Often ions with only a single positive charge are formed, and therefore the mass to charge ratio is equal to the mass of the ion itself.

2.4.2.2. Fragmentation

The process of ionisation (e.g. electron impact ionisation) often uses an energy which is far greater than that needed to simply ionise the molecules. This excess energy can be transferred to the molecular ion as it is created, in the form of rotational, translational, vibrational or electronic energy. Owing to the latter two categories, the molecular ion normally undergoes fragmentations. As it is a radical cation, with an odd number of electrons, it can fragment to give either a radical or an ion with an even number of electrons, or a molecule and a new radical cation (Hoffmann E. D. *et al.,*1996).



The fragmentation pathway and the corresponding products are influenced by; the strengths of the bonds which are to be broken; the stability of the products of fragmentation (both ions and neutral species); the internal energies of the fragmenting ions; and the time interval between ion formation and ion detection.

2.4.2.3. Electron Impact Ionisation

There are several ionisation methods including ionisation by electron impact, chemical ionisation (methane, isobutane, ammonia etc.), fast ion or atom bombardment ionisation (FAB), field desorption, laser desorption etc. depending on the nature of the sample to be ionised and the experimental conditions each may provide some advantageous features. Among these methods electron ionisation (EI) is the most popular method because of its wide-applicability to volatile and semi-volatile organic compounds and relative simplicity. Chemical ionisation (CI) has an advantage of producing highly abundant molecular ions, and other methods like FAB are especially useful when high molecular weight species are studied.

Electron ionisation (EI) is the most commonly used ionisation method. This is because for most molecules it produces both molecular (necessary for molecular weight determination) and fragment ions (to specify molecular structure). After the separation of the species is completed the sample material, which is in the form of gas or vapour, is passed through an inlet into an ionisation chamber, where it is bombarded by the beam of electrons (Figure 2.8), whose energy may be varied to produce maximum ionisation efficiency.

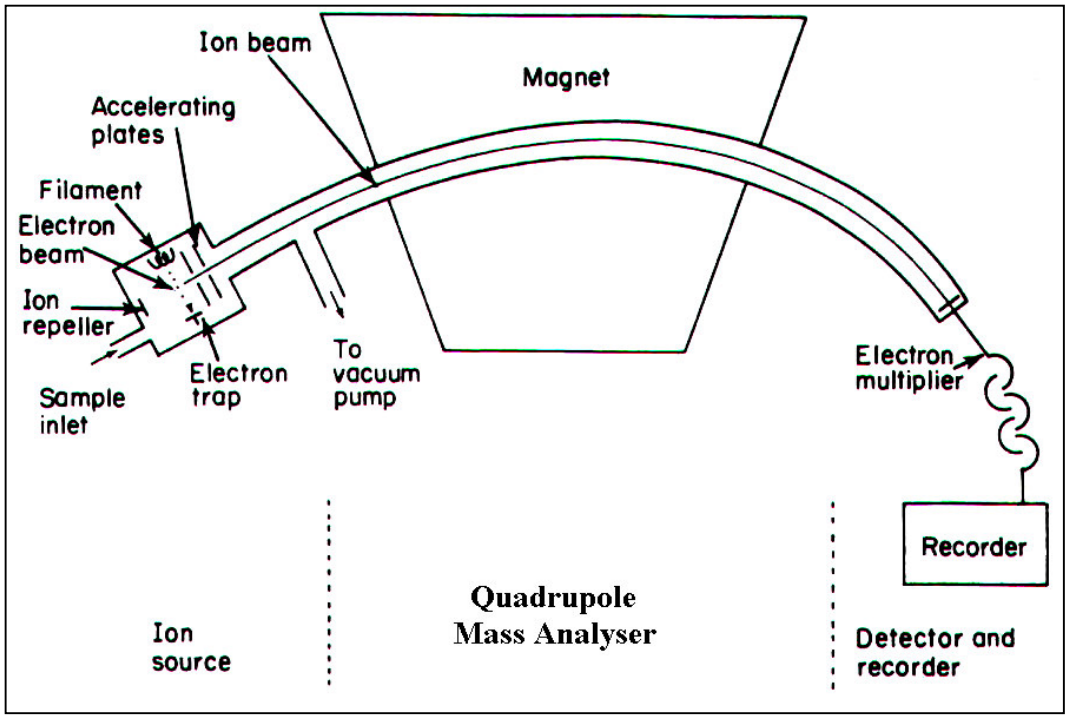


Figure 2.8. Schematic Diagram of a magnetic sector analyser mass spectrometer with an electron impact ion source. (Barker, 1999).

At low potentials the energy is lower than the molecule's ionisation energy. At high potentials, the wavelength becomes too small and molecule becomes "transparent" to these electrons. For the case of organic molecules, a wide maximum appears around 70 eV.

2.4.2.4. Quadrupole Mass Analyser

Once the ions have been produced, they need to be separated according to their masses. There are many analysers that are generally scanning devices such as quadrupole mass analyser (single or several quadrupoles, tandem mass spectrometer, MS/MS), quadrupole ion trap (Quistor), and magnetic sector analyser, or discrete multiple ion monitoring devices such as time of flight (TOF), and fourier transform (FT) instruments.

The mass spectrometer used in this study contained a quadrupole mass analyser (Figure 2.9) for separation of the ions before detection by continuous dynode electron multiplier CEM. Since the quadrupole is relatively cheap to build, smaller and lighter, more robust and has a fast scan rate (the time it takes to record the spectrum is <100ms) it became by far the most popular mass analyser in use today.

The analyser consists of two pairs of precisely parallel rods (Figure 2.9), which are located between an ion source (EI) and a detector (CEM). By varying the electrical signals to a quadrupole it is possible to vary the range of *m/z* values transmitted, thus making spectral scanning possible.

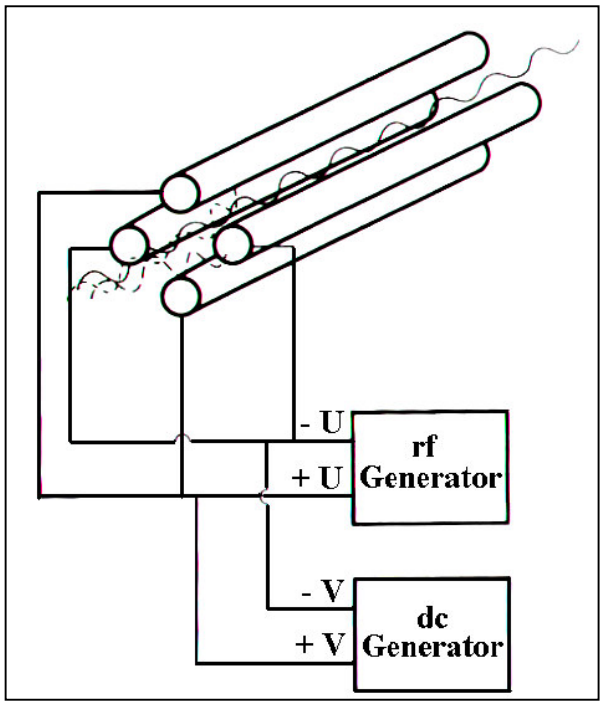


Figure 2.9. Quadrupole Mass Analyser (Smith and Busch, 1999).

2.4.2.5. Continuous Dynode Electron Multiplier (CEM)

Electron multipliers (discrete or continuous), array detectors, and photon multipliers can be used alternatively in detection of ions produced and separated in mass spectrometry. The high amplification power and robust structure of continuous dynode electron multiplier (CEM) detectors (upto 10^7) makes them most sensitive among the others (10^4-10^5 for photon multipliers, and 10^5 for diode arrays)

In this study, the detector used in the MSD analyzer was a high energy conversion dynode (HED) coupled to an electron multiplier (EM, Figure 2.10.). The detector was located at the exit end of the quadrupole mass filter. It received the ions that have passed through the mass filter. The detector generated an electronic signal proportional to the number of ions striking it. The detector had three main components: the detector focus lens (directed the ion beam into the HED, which was located off axis), the high energy dynode (operated at -10,000 volts, attracting the positive sample ions exiting the quadrupole), and the electron multiplier horn.

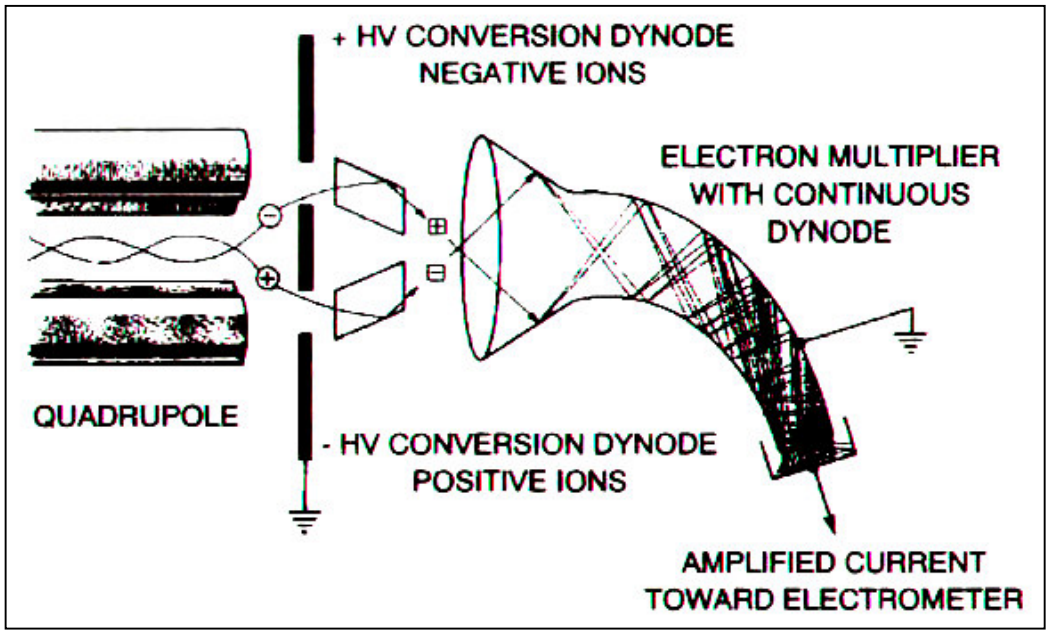


Figure 2.10. Continuous Dynode Electron Multiplier (CEM). | = Incident ions; ⟨ = Secondary particles (Hoffmann E. D. *et al.,* 1996).

The electron multiplier horn carries a voltage of up to -3000 volts at its opening and 0 volts at the other end. The electrons emitted by the HED strike the EM horn and go through the horn, liberating more electrons as they go. At the far end of the horn, the current generated by the electrons is carried to the electrometer.

2.4.3. Advantages of Mass Spectrometer as a GC Detector

Gas chromatography used to suffer from a few weaknesses, such as its requirement for volatile organic compounds, but its major problem was the lack of definitive proof of the nature of the detected compounds as they were separated. For most GC detectors, identification is based solely on retention time on the column, like the most popularly used Flame Ionisation Detector (FID), Electron Capture Detector (ECD), and Thermal Conductivity Detector (TCD). Since many

compounds may posses the same retention time, the analysts are left in doubt as to the nature and purity of the compound(s) in the separated peak.

From the point of view of the chromatographer the gas chromatography/mass spectrometer is simply a gas chromatograph with a very large and very expensive detector but one can give a definitive identification of the separated compounds by characteristic mass spectra with high sensitivity of 10^{-12} g (Karasek and Clement, 1988).

2.4.4. Short Path Thermal Desorption System

SPTD was mounted directly over the GC injection port. It was automated by desorption unit electronics console, so that it could work on-line with GC-MS system, i.e. could control the cryotrap and GC start.

Individual flow path for each sample prevents contamination of transfer lines, therefore, no memory effect was expected. Glass lined stainless steel sample tubes were both inert to samples and strong for sample handling and transporting.

Both calibration standards and the samples were introduced to GC/MS by means of short path thermal desorption system (Figure 2.11.). The maximum desorption temperature permissible with the system was 350 ^{o}C and the heater blocks could be ballistically heated or temperature programmed at ramp rates up to 40 ^{o}C/min.. Carrier gas flow through the desorption tube could be accurately adjusted from 1.0 mL/min to 100 mL/min using the two-ball rotameter and flow controller. However one should carefully check the flow-rate since throughout the desorption flow changes, mainly due to back-pressure from the inlet, may occur. This was the only drawback observed about the system throughout this study.

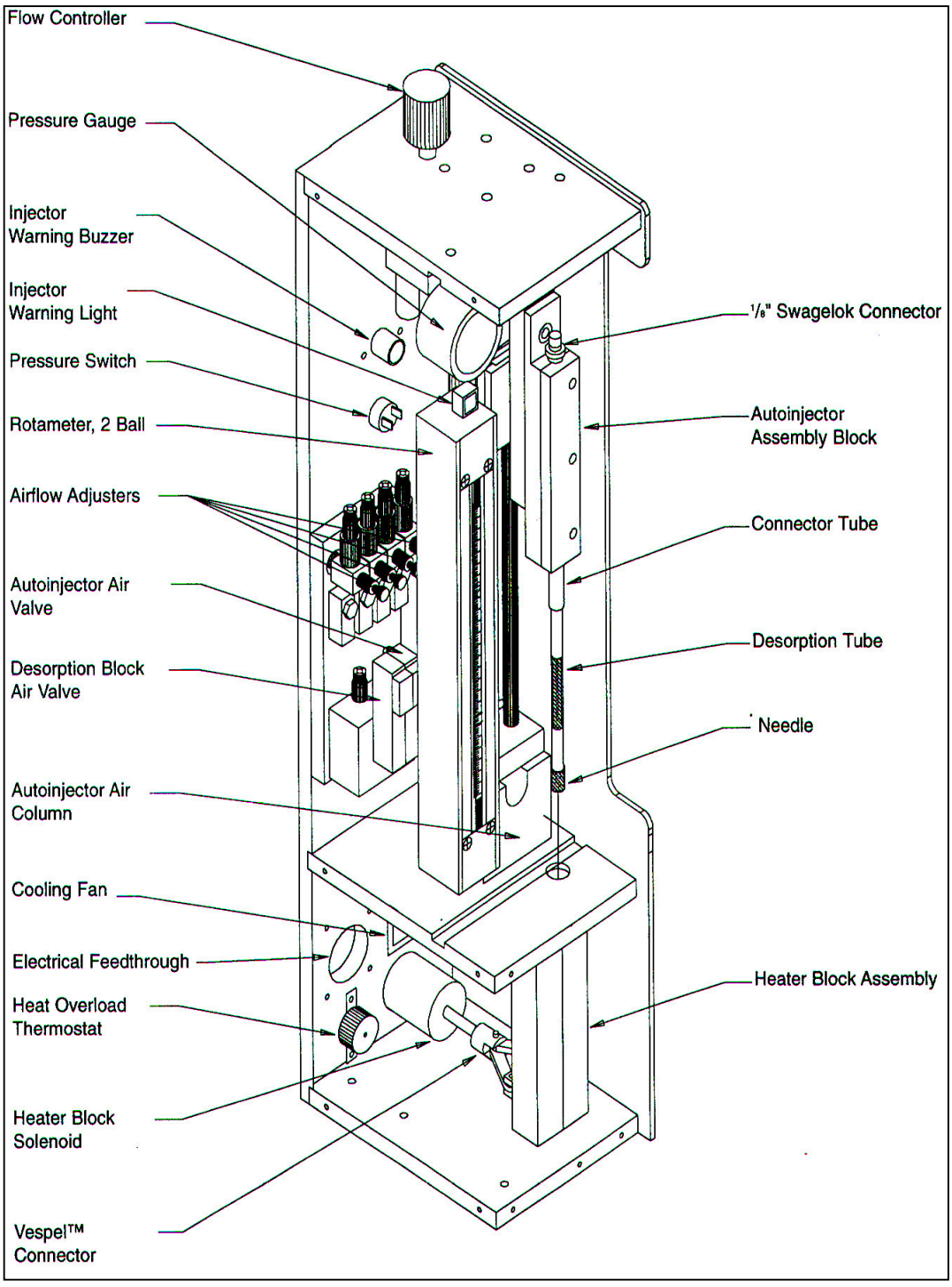


Figure 2.11. Short Path Thermal Desorption (SPTD) System (S.I.S., 1999).

Once the desorption tube was fitted to the connector tube of the SPTD, the cryotrap, which was cooled to –40 ^{o}C with liquid CO_2 for this study (see Figure 2.6.), was turned on. As soon as the desired temperatures of cryotrap (–40 ^{o}C) and thermal blocks (260^{o}C) were reached the SPTD program (Table 2.8.) was started.

Table 2.8. Thermal Desorption Unit operation parameters.

Process	**Temp.(^{o}C)**	**Time (min:sec)**
Purge	25	01:00
Injection	25	00:30
Delay	25	00:10
Desorption	260	05:00

As the program was started, the purge step provided the elimination of the easily removable moisture. The injector needle was out of the GC-inlet during this first step and it was inserted to the GC after this step. Pressurised dry air was used to provide the mechanical movements of the thermal desorption apparatus during injection. Injection process provided the automated leak check that ascertained the complete introduction without sample loss. After a 10-seconds delay the heater blocks were closed on the tube and the sample introduction started. When desorption was completed the cryotrap was flash-heated to 260 ^{o}C (≈5sec.) and the injection of the sample to the column took place.

Off-set purge, injection, and delay times were used, yet the desorption time was selected so as to minimise the coolant (CO_2) consumption without incomplete desorption. The reanalysis of the desorbed tube did not indicate any artifact for the desorption parameters of 5 minutes and 30 mL/min Helium flow at 260 ^{o}C.

2.5. Measurements of Gas Phase Pollutants; O_3, and NO_x

The gas phase pollutants were measured using continuous analysers (Environnement S.A., France) capable of 15-min data resolution and have 0.5 ppbv detection limit for each of O_3, NO_x (NO and NO_2). These are sufficiently robust instruments that can be suitably used in field studies. They require minimum maintenance as soon as the periodical controls and replacements, such as filters, lamps, converters, etc., are performed regularly.

2.5.1. UV Photometry Ozone Analyser

The measurements of O_3 were performed using O_3-41M UV photometry ozone analyser (Environnement S.A.,France). The ozone absorption spectrum lies at a maximum of 253.7 nm, which is the main emission line of the low-pressure mercury vapour UV lamp. To correct the drifts in UV lamp a reference UV detector was employed, which was shown in Figure 2.12., optical bench. The equation below is used for calculations under measurement conditions. It is derived from Beer Lambert law.

$$C_{ppm}[O_3] = \frac{10^6}{a\,b} \times Ln\left[\frac{i_o}{i}\right] \frac{P_o}{P} \times \frac{t}{t_o}$$

Where;

a: absorption coefficient, (308 atm^{-1} at P_o =103.1 kPa and t_o = 273 ^oK)

b: optical path length (73.05 cm)

i_o: UV energy measured through measurement cell when sample does not contain ozone molecules (passage through selective filter)

i: UV energy measured on sample containing ozone to be measured (direct passage)

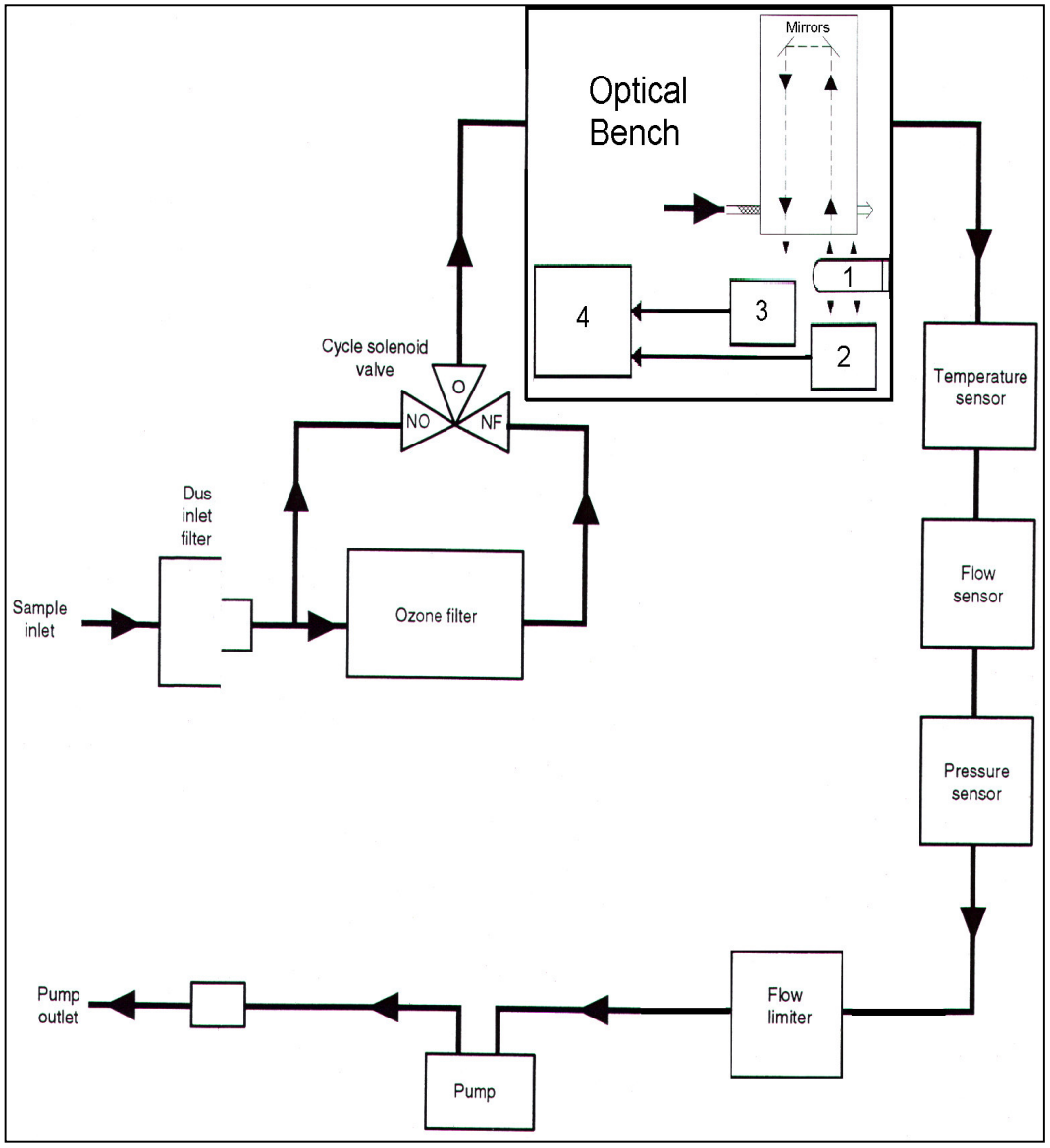


Figure 2.12. General schematic diagram of O_3-41M UV photometry ozone analyser (1: mercury vapour UV lamp, 2: UV detector reference, 3: UV sensor measurement, 4: data acquisition) (Environnement S.A., 2000a).

One complete cycle is done in 10 seconds, including the steps of; ventilation by O_3 filtered gas (4 seconds), measurement of "i_o" (reference UV), switching of solenoid valve, passage of sample gas directly into measurement chamber and ventilation (4 seconds), measurement of "i" (measurement UV).

A barometric sensor measuring the pressure "p" in the measurement chamber and a sensor measuring the temperature "t" of the gas is employed to make the pressure and temperature compensation. The corrected concentration is therefore calculated using:

$$C_c = C \times \frac{273+T}{273} \times \frac{1013}{P}$$

The calibration was done using internal O_3 generator prior to field study. The 184.9 nm wavelength UV rays emitted by the mercury lamp convert part of the oxygen contained in the zero air into O_3, through the reaction:

$$O_2 + h\nu \rightarrow O + O$$
$$O_2 + O \rightarrow O_3$$

The electrical signal were checked daily and no considerable response change were observed throughout the sampling campaign.

2.5.2. Chemiluminescent Nitrogen Oxide Analyser

The measurements of ozone (O_3) were performed using AC-31M chemiluminescent nitrogen oxide analyser (Environnement S.A., France). The monitor operates on the principle that nitrogen oxide (NO) will emit light (chemiluminescence) in the presence of high oxidising ozone molecules:

$$NO + O_3 \rightarrow NO_2^* \; O_2$$

$$NO_2^* \rightarrow NO_2 + h\nu \text{ (600-1200 nm)}$$

The relaxation of the exited NO_2 molecules results in a luminous radiation on a spectrum of 600 to 1200 nm. The wavelengths greater than 610 are selected by an optical filter (Figure 2.13.) to eliminate interferences due to hydrocarbons.

In order to be measured by chemiluminescence, the NO_2 must be first transformed into NO. A heated molybdenum oven with an internal discharge ozone generator is used to carry out the reaction:

$$3\,NO_2 + Mo \rightarrow 3\,NO + MoO_3$$

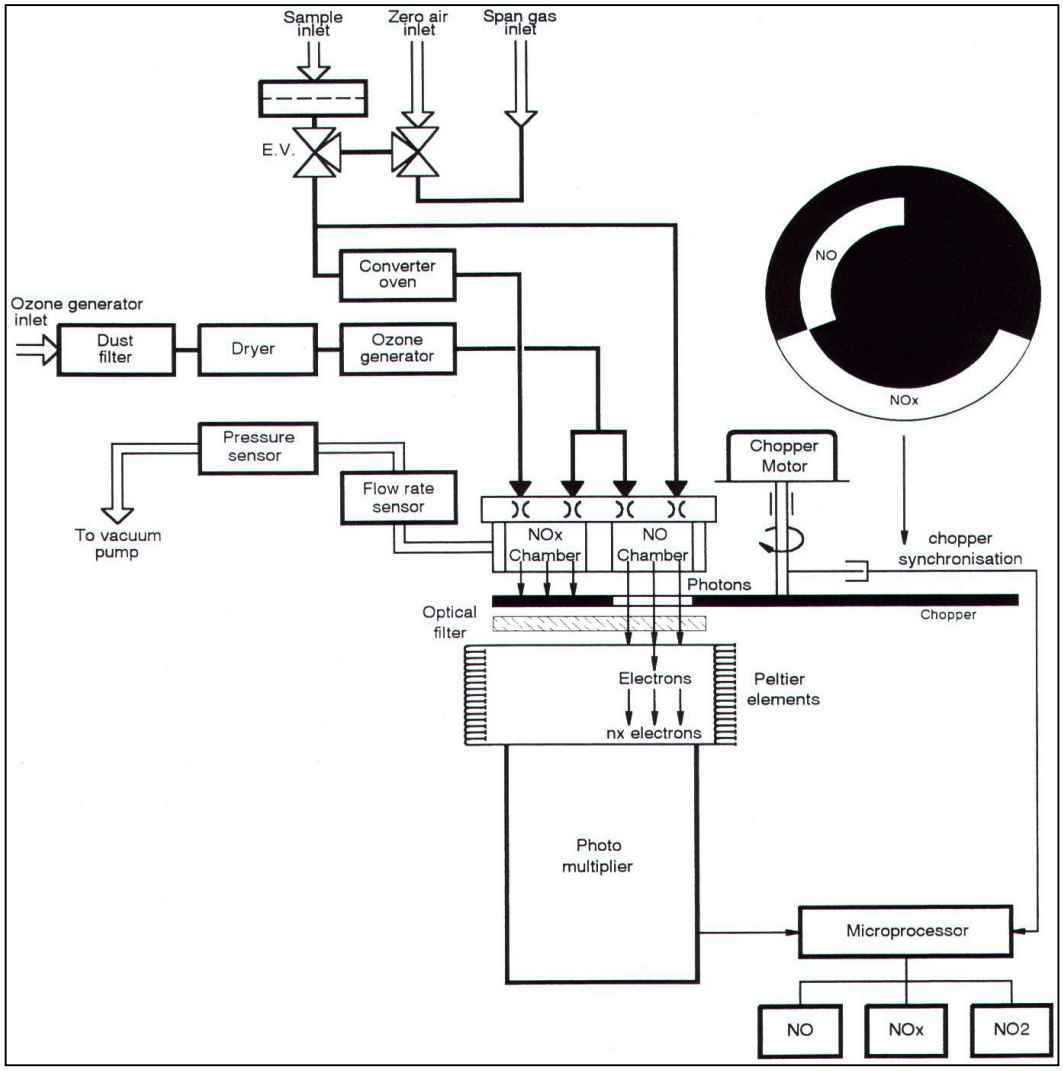


Figure 2.13. Schematic view of NO_x analyser (Environnement S.A., 2000b).

NO chamber (Figure 2.13) and NO_x chamber serve for two channel measurements of the practically the same air sample. The measurements synchronised by 3-sector chopper disk correspond to; electrical zero (black) when both chambers are blanked; NO signal when NO chamber is open; and NO_x signal when NO_x chamber is open.

The calibration of the instrument was done using nitric oxide (Messer Grilsheim, 0.746 ppm Nitric Oxide, ± 5 % Rel analytical tolerance) and the calibration was checked daily by using permeation device. The permeation device measurements and the stability of the electrical signals checks suggested no significant variation in response.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Determination of Sampling Strategies for BVOC Measurements

Because of the complexity, heterogeneity, and occurrence of many analytes at ppt(*v*)-ppb(*v*) levels, air belongs to the most complicated matrices to be analysed. The crucial point in air analysis is the sampling step. It has to fulfil the following requirements:

- The sample taken should be representative.
- Qualitative and quantitative adulterations of the sample composition should be avoided.
- The sampling procedure should be as simple as possible to enable field sampling.

3.1.1. Selection of the Sampling Site

We have mentioned before that biogenic emissions were mainly released from vegetation. The coniferous type trees mainly emit terpenes and deciduous types mainly emit isoprene with few exceptions. For a good estimation of biogenic

sources the site must be selected so that the dilution is minimised. This enables the quantitative and representative sampling, i.e. the detection limits are satisfied for the majority of the samples. For ground level biogenic emission campaigns, many scientists have preferred forest sites far away to anthropogenic sources (Peters R. J. B. *et al.,* 1994; Janson R. *et al.,* 1999; Fuentes J. D. *et al.,* 1996). Since BVOCs are very reactive, elevated levels of anthropogenic sources lead to interferences.

Atmospheric research station at Uludağ was selected as a suitable site for BVOC investigations since:

- The site is a strategically important point for workshops with European Community research groups. These measurements produced data for EUROTRAC-2 environmental research project.
- It is far away from anthropogenic activities like settlements, factories and highways.
- The site consists of mainly coniferous trees as plant cover. From top to 1000 m altitude, Uludağ Fir, which is characteristic to Uludağ, dominates. Mixed deciduous trees cover the altitudes under 1000 m.
- Our Environmental Chemistry Research Group has ten-year experience about the behaviour of gas phase pollutants, O_3, NO_x, SO_2, and SPM, and aerosols. This experience provides estimation of interferences during sampling due to oxidants, especially ozone.
- Having 1645 m altitude, it is one of the very few mountainous research site around the world.

The sampling campaign covered, 7 days, however the initially collected samples did not gave quantitative amounts of terpenes, so they were eliminated. This may be attributed to the low temperature of the days during the sampling campaign. The meaningful data were obtained on 12-13-14 October.

3.1.2. Selection of Flow-rate and Sampling Duration

Other important parameters of sampling strategy are flow-rate and sampling duration. These two parameters are closely related and were determined together.

For example, insufficient sampling will result in indeterminate amount of analyte or lead to poor accuracy since the measured values will be close to LOD. But, too long sampling period may lead to saturation of the adsorbent with the analytes, and therefore lead to breakthrough , which is the loss of the unretained analytes in the sample air. Furthermore, together with the analyte some other constituents of the atmosphere which are generally included in the range of the adsorbent are also retained during sampling. So, enormous increase in the concentration of these concomitants leads to some drawbacks. First, the coeluting species with similar *m/z* may lead to increase in background and even distortion in ion ratios of the analytes. Increasing background leads to decrease in sensitivity and the distortion in ion ratios brings about unacceptable results. Second, the elevated concentrations of the species, which do not interfere on the chromatogram, may still affect the measurement and results by affecting the cryotrap efficiency and the retention times of the analytes. Finally, during sampling storage, the risk of analyte-loss by reactions on adsorbent increases by interaction with highly concentrated concomitants.

For ¼ inch O.D. tubes, 50 mL/min is the theoretical optimum flow rate. However, negligible variation in retention volume will in fact be observed for pump flow rates varying from 5 to 200 mL/min. Pump flow rates above 10 mL/min are generally used in order to minimise errors due to increase of VOCs via diffusion. Flow rates in excess of 200 mL/min are not recommended for standard ¼-inch sample tubes unless for short term (e.g. 10 min) monitoring (U.S.-EPA, 1997c). Typical example flow rates assigned in EPA-TO-17 method are listed in Table 3.1..

Table 3.1. Typical example pump flow rates (US-EPA)

Flow (mL/min)	Total collected air sample (mL)	Sampling Period
16	1000	1 hour
67	4000	1 hour
10	1800	Over 3 hours
40	7200	Over 3 hours

In this study we took Table 3.1. as reference and we have done preliminary experiments in Yalıncak, METU before we go to Bursa in order to determine suitable flow rate and sampling duration. The results of these experiments are given in Table 3.2.

Table 3.2. The results of the experiments performed at Yalıncak, METU

Exp. #	Flow Rate (mL/min)	Sampling Duration (min)	Total Collected (mL)	Results
1	15	30	450	Not Quantitative
2	15	50	750	Not Quantitative
3	20	120	2400	Quantitative

As can be seen from the Table 3.2., 20 mL/min flow rate and 2 hours sampling duration provides quantitative results.

Considering that Uludağ site is different than Yalıncak some preliminary experiments were also performed in Uludağ using Yalıncak results as guide. The preliminary experiments done in Uludağ is given below in Table 3.3..

Table 3.3. Results of preliminary experiments performed in Uludağ

Exp. #	Flow Rate (mL/min)	Sampling Duration (hrs)	Total Collected (mL)	Results
1	20	2	2400	Not Quantitaive
2	30	2	3600	Not Quantitative
3	30	4	7200	Quantitative

In this study we first aimed to follow terpene concentrations diurnally. That is why we were concentrated on 2 hrs sampling period. The samples collected in Uludağ were sent to Ankara for analyses and only 30 mL/min flow rate and 4 hrs sampling gave quantitative results. The night time sampling was not possible, as the station was not safe. The final sampling schedule obtained after experiments and used in Uludağ is given in Table 3.4..

Table 3.4. Sampling intervals during Uludağ sampling campaign

Start time	Finish time
07:00	11:00
12:00	16:00
17:00	21:00

The total volume of the collected air sample was 5.978-6.735 L, which is similar to the fourth typical sampling program suggested by US-EPA (Table 3.1.).

3.2. Development of Sampling System

In this study sampling system was assembled in our laboratory using commercial and hand-made components. This was one of the time consuming part of the study. Sampling system is composed of special pump, Mass Flow Controller (MFC), flow limiter, flow splitter, and digital flow meter. The system is shown in Figure 2.2.. The experiments to test the sampling system are discussed below.

3.2.1. Construction of the Tubes Free-of Contamination

Details of the construction of adsorption tubes were given in experimental part (section 2.2.2.). In this section the experiments done for the contamination check of the tubes are discussed.

The tubes were cleaned with various chemicals and the contamination check was done after each step. The steps were as follows:

1) Rinsing and rubbing with technical grade acetone and rinsing three times with technical grade acetone.
2) Rinsing with reagent grade (Merck) methanol for 1 hour followed by conditioning for 2 hours under at least 100 mL/min pure N_2 (>99.999%) at 350±10 ^{o}C and contamination check with GC-MS scan mode.
3) Rinsing with reagent grade (Merck) hexane followed by conditioning and contamination check.
4) Rinsing with reagent grade (Riedel) ethanol followed by conditioning for 4 hours and contamination check.
5) Rinsing with reagent grade (Merck) 1:1 methanol + formic acid mixture and rinsing three times with reagent grade methanol in ultrasonic shaker.

The conditioning after this solvent cleaning step provided promising cleanliness and repeated. The initial contamination check after the second step resulted in huge peaks (Figure 3.1.) especially of heavy hydrocarbons like C_{11}-C_{20} and derivatives and un-specifically determined other species. Since we expected a high contamination because of the tube producing conditions, we set up a fast oven program that starts at 40 ^{o}C and reaches 250 ^{o}C with 20 ^{o}C ramp and analysed the tube with MS-Scan mode (35-550 *m/z*).

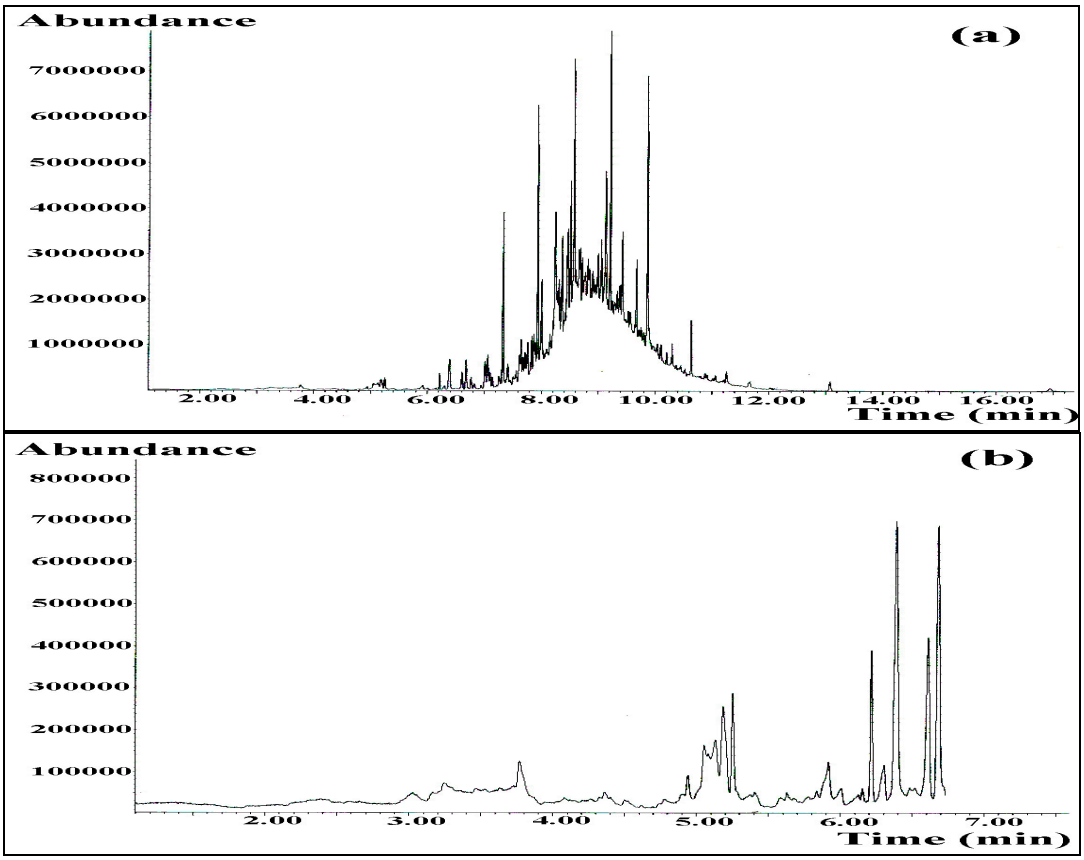


Figure 3.1. Contamination check performed in MS-Scan mode for the empty glass-coated stainless steel tubes.

Figure 3.1.(b) is a focus on the initial part of chromatogram 3.1.(a) to illustrate the extremely high background. Gradual decrease in the contamination

peaks was observed throughout each step and finally 1:1 methanol + formic acid mixture supplied the best results. After observation of heavy hydrocarbon peaks like dodecane, tridecane, tetradecane, etc., that are in general apolar species, hexane was used to clean the tubes since it was an apolar solvent. The resulting chromatogram was better but was not satisfactory. The ethanol usage in turn gave a gradual decrease, but finally it was 1:1 formic acid methanol mixture that was able to provide satisfactory cleaning. The final chromatogram was clean and the back-ground was at desired level.

The tubes were conditioned at 350±15 ^{o}C for 2 hours using the conditioning oven that was constructed with our design. The oven enables conditioning of 10 tubes at the same time under nitrogen stream which was kept equal to or above 100 mL/min . The problem encountered at first usage was the contamination from the flow restrictor on the line and it was solved by cleaning the content with 1:1 formic acid + methanol mixture and rinsing three times with methanol. Final conditioning step was done one by one, for 20min at thermal desorption system under 100mL/min Helium flow.

3.2.2. Control of Flow Rate Fluctuation in the Sampling System

The constant flow provides isokinetic sampling, where the disturbance of the streamline is minimised. Fluctuation in the flow rate can result in poor precision and accuracy. Definitely, it is not possible to keep flow rate constant. But, it is possible to keep the fluctuation in an acceptable range. That is why experiments were conducted to control flow rate during the sampling. The system was checked every half an hour with digital flow meter since the physical difference in the tube throughout the sampling because of the adsorbed species, more likely the water vapour, may cause an attenuation or increase of flow rate that MFC can not compensate. The results are given below in Table 3.5..

Table 3.5. Control of fluctuation in flow during sampling in Uludağ.

Time	**Flow (mL/min)**					
(min)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Initial	29.0	28.0	30.0	29.0	29.5	30.0
30	29.5	32.0	30.0	29.0	28.0	30.0
60	29.5	32.5	30.0	28.5	28.5	30.0
90	29.5	32.5	29.0	28.5	28.0	31.0
180	29.5	32.5	28.5	28.5	29.5	30.0
210	30.0	32.5	28.0	28.5	28.0	30.0
240	29.5	32.5	29.5	28.5	28.5	31.0

Maximum deviation in flow was observed in sample 2 (28.0-32.0 mL/min), which then turned back to acceptable values after first 30 min. The standard deviation (Std. Dev.), averages, and %RSD values are given in Table 3.6..

Table 3.6. Precision of the flow rate during sampling.

Sample	**1**	**2**	**3**	**4**	**5**	**6**
Average	29.5	31.8	29.3	28.6	28.6	30.3
Std Dev.	0.289	1.680	0.809	0.244	0.673	0.488
%RSD	0.98	5.28	2.76	0.85	2.35	1.61
Total collected (L)	6.203	6.735	6.150	6.015	5.978	6.360

In Table 3.6. the %RSD values indicate standard deviation divided by average and multiplied by hundred. Only the second sample lied out of 5% level.

Another important device that has to be considered during BVOC sampling is ozone scrubber. For high concentrations of ozone using scrubber is inevitable since BVOCs can readily react with ozone during sampling. The decomposition can even reach 95-100 % for specific terpenes like terpinolene and α-terpinene in that study. On the other hand, ozone scrubbers may themselves retain BVOC to a considerable degree, and therefore should not be used unless necessary. Ozone scrubber was not used since the concentration of ozone during sampling campaign was 5-30 ppbv which was low compared to the ozone levels studied in literature (80, 100, 120 ppbv and higher).

3.2.3. Breakthrough Volumes (BTV)

In volatile pollution sampling another important point is to verify collection efficiency of 100% or with an approach of 5% error. The term breakthrough volume (BTV) is defined as the determination of the ability of the specific sorbents to hold the analyte(s) of interest. In other words it is the determination of the efficiency of the adsorbent for the selected species. Breakthrough volume can be determined experimentally in two ways. The first way includes frontal and elution techniques in which the specific breakthrough volume is determined and the second one is the back up method, which was more practical for our application since it did not require any modification in the instrument. But, both methods are discussed in the following section and data are given for the one that was used in this study.

3.2.3.1. Frontal and Elution Techniques

For both frontal and elution techniques, a defined amount of adsorbent is filled in an adsorbent tube and the packed tube is placed into a GC-oven and attached to the injector and detector. If the elution technique is applied, a model

substance is injected as a pulse onto the adsorbent bed, and the elution chromatogram at a defined temperature is recorded (Figure 3.2.). In the frontal chromatogram mode, a gas containing the model substance is led continuously through the adsorbent bed at a defined temperature, and a frontal chromatogram is produced (Figure 3.2.).

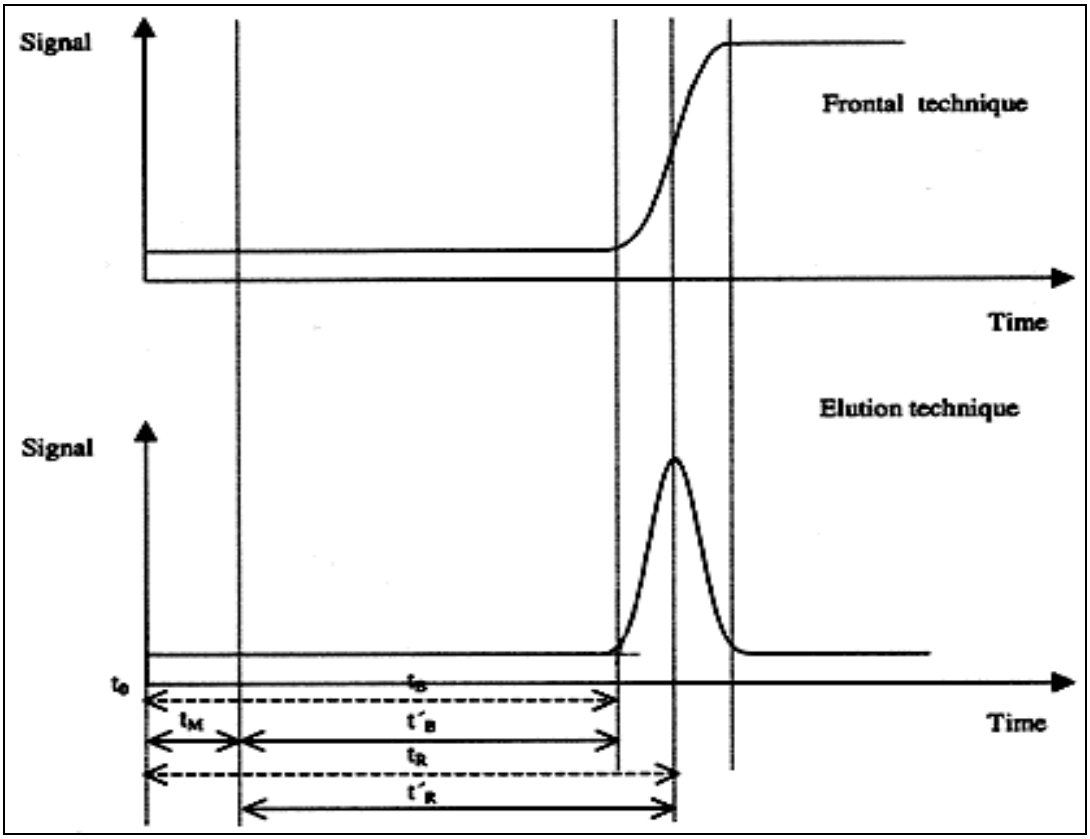


Figure 3.2. Chromatographic techniques used for the determination of the specific breakthrough/retention volume (Dettmer K. and Engewald W., 2002).

3.2.3.2. Back-up method for determining Breakthrough Volumes

In this study, back-up method was used to assure the safe sampling conditions for the sampling parameters that were determined. Frontal and elution

techniques are far more difficult than back-up method since they imply a change in the configuration of the chromatographic system. Back-up method was described by US-EPA and used by many studies in literature (U.S.-EPA, 1997a). According to this definition, Breakthrough Volume (BTV) is the volume of air containing a constant concentration of analyte, which may be passed through a sorbent tube before a detectable level, typically 5%, of the analyte concentration elutes from the non-sampling end. Alternatively, the volume sampled when the amount of analyte collected in a back-up sorbent tube reaches a certain percentage, that is typically 5% of the total amount collected by both sorbent tubes.

In this study we have done both laboratory and field back-up experiments to assure the complete sampling with accepted tolerance of 5%.

3.2.2.3. BTV experiments at laboratory

For laboratory BTV experiments a terpene standard which contained approximately 24 ng of each terpene in 2 μL ethanol was injected through the Tenax TA® containing side of the sorbent tube and it is combined to another clean tube in series with a brass joint. The four joints (screw threads) were well fitted with Teflon tape to prevent leakage. The system was demonstrated in Figure 3.3.

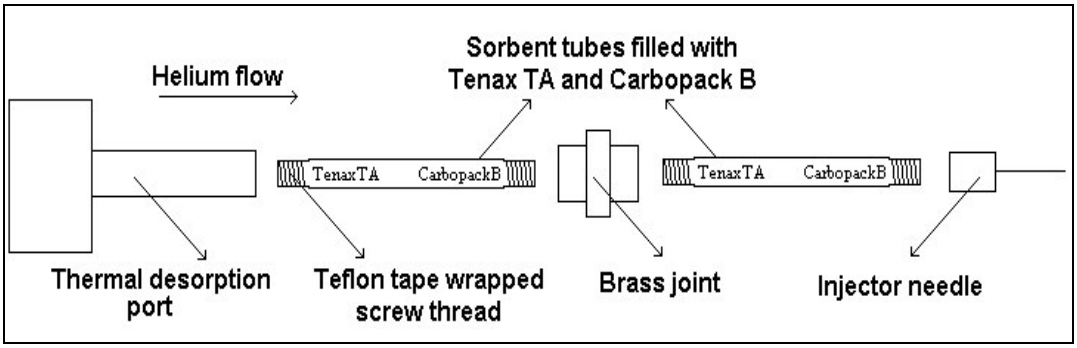


Figure 3.3. Breakthrough test in laboratory.

He flow of desired value was applied for the required duration and then the back-up tube, the tube on right-hand side in Figure 3.3., was analysed.

As a start point the experiments conducted at Yalıncak were taken as a base and the initial test was done for 2 hours at 20 mL/min flow. No breakthrough at room temperature was observed as a result of the analysis of the back-up tube with GC-MS. Then, the time interval was increased gradually in order to obtain further information before the sampling campaign at Uludağ. The results are given below in Table 3.7..

Table 3.7. Results of the laboratory BTV experiments

Exp.#	Flow Rate (mL/min)	Duration (hours)	Total Volume (mL)	Results
1	20	2	2400	No BT
2	30	2	3600	No BT
2	30	3	5400	No BT
3	30	4	7200	Menthol (<5%)

As a result of the replicate analyses for the experiments stated in Table 3.7., the conditions up to 4 hours at 30 mL/min flow resulted in no breakthrough. It was only one of the replicates for the last experiment that have suggested breakthrough of menthol in acceptable amount (<5%). So 4 hours at 30 mL/min was chosen to be the highest Safe Sampling Volume (SSV) for this study. Successively increasing the time interval and flow-rate, the SSV might be increased.

These results are in agreement with the literature. The sorbent tube style that we have chosen was similar to that of Tube Style 1 described by U.S.-EPA-TO-17 method in terms of analytes aimed to be measured (U.S.-EPA, 1997c). The tube

they describe consists of 30 mm Tenax®GR plus 25 mm of Carbopack™ B separated by 3 mm of unsilanized, preconditioned glass or quartz wool. They have noted that air volume >5 L can be handled for compounds ranging in volatility starting from n-C_7. Isoprene (C_5) was reported to have breakthrough volume of >5 L of trace levels in humidified zero air for humidities of 20% and 65% RH at 25^oC. It is important to note that BTV is affected adversly by humidity. M. Komenda *et al.* have used Tenax TA (60/80 mesh, 100 mg) and Carbotrap (20/40 mesh, 50 mg) as sorbent and for upto 7 ppb no breakthrough of monoterpenes were observed for volumes ranging from 1 L to 18 L (Komenda M. *et al.,* 2001). As we consider that the terpenes in this study are heavier hydrocarbons than those studied by US-EPA and it was expected that they can be handled for volumes higher than 5 L safely.

3.2.2.4. BTV experiments at sampling site

BTV test should also be applied during sampling. The idea is similar to laboratory experiments and the only substantial difference is that the system (Figure 3.4.) works under continuous flow of sample air instead of He.

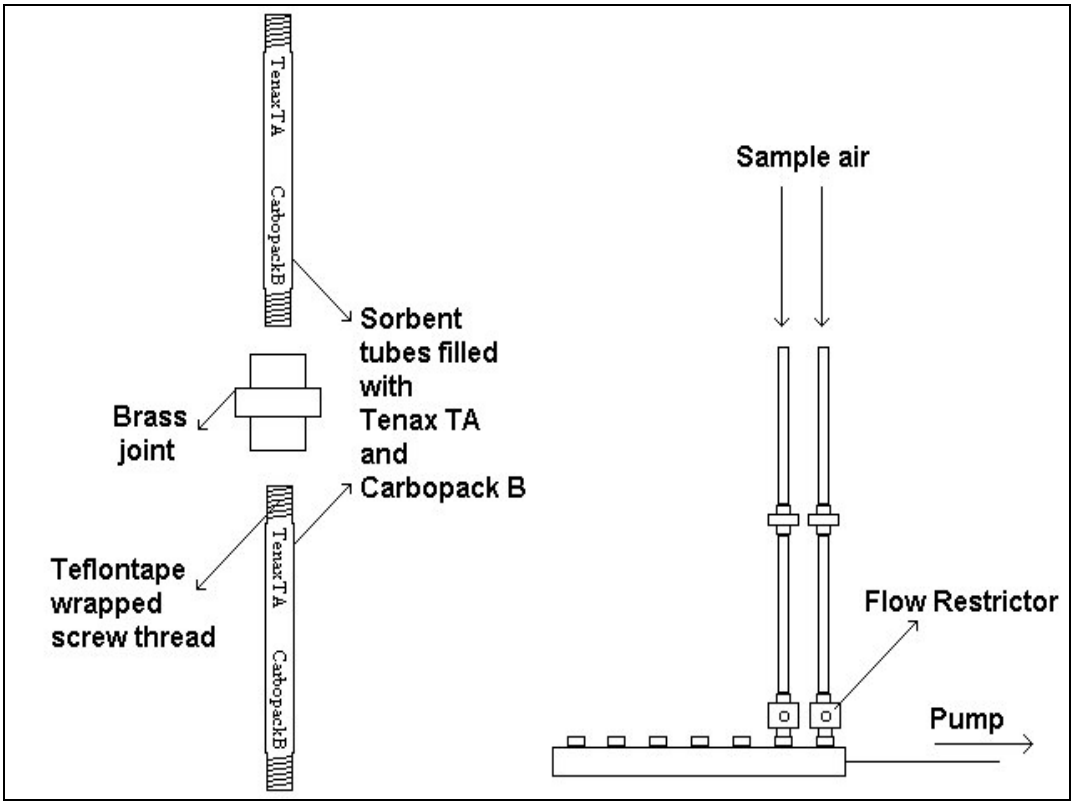


Figure 3.4. Breakthrough test sampling site.

The chromatograms for the back-up tubes of sampling site breakthrough experiments is not as clean as the one for laboratory, since the sample air includes many species of different structure and therefore may not be successfully adsorbed by the first tube in series. As a result, we could not observed any breakthrough of analytes for 4 hours at 30 mL/min.

3.3. Optimisation of Gas Chromatography

Gas chromatograph is simply composed of inlet (containing liner), cryo-trap, oven, column and GC-MS interface.

Inlet serves as a part of GC that provides the introduction of analytes in homogenous vapor phase. For liquid samples it, direct injection of liquid with micro syringes provides both vaporisation and introduction of the sample directly to oven. However, in thermal desorption the desorption process is itself a step, which brings the sample desorbed from the adsorbent to vapor phase. Therefore, the inlet in thermal desorption serves as a part where the sample, which is already in gas phase, is homogenously mixed with carrier gas and then introduced to cryotrap. So, using inlet at the same temperature with thermal desorber (260 ^{o}C) was suitable.

As the name implies, cryotrap is the device which cools a small section of the column and the analytes are retained on this section during desorption step by the help of a cold surface. It can be considered as a preconcentration step for introduction of gaseous samples using canisters. It should be kept as cold as possible, while the most important limitation is the possible clogging due to the adsorption of the highly abundant concomitants such as water, solvent, or even CO_2 when liquid nitrogen is used as coolant. The minimum temperature that could be maintained with the available assembly was -40 ^{o}C. Theoretically it would be possible to cool further ($b.p._{CO_2}$ = -78.5 ^{o}C) but -40 ^{o}C was the lowest constant temperature that could be obtained with our system.

Since the optimum desorption temperature was 260 ^{o}C for the terpenes that were studied, using the inlet and the GC-MS interface at the same temperature was suitable. Among the analytes, the maximum boiling was belong to geraniol, which was 229-230 ^{o}C. Consequently, using 260 ^{o}C was consistent for the analytes transfer through this line without deposition.

The system was used in constant flow mode and as the first step, the flow rate was selected to be the minimum that is compatible with the pressure to keep the foreline at lowest value since highest vacuum provides highest detector

efficiency. The step by step increase of flow-rate did not provide better separation or narrower peak widths. So it was chosen to be 1.3 mL/min.

3.3.1. Optimisation of Oven Parameters

After selection of the suitable column, one of the most important step was the optimisation of the oven temperature program. The initial temperature, ramps applied, and the duration of the intermediate steps has a big influence on separation and behaviour of the peaks. Several temperature programs were applied to perform best resolution of the analytes. Some of these temperature programs are listed in Table 3.8.

Table 3.8. Oven temperature programs and corresponding remarks.

Temperature Program				**Remarks**
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Broadening of almost all species
40	0	40	10	
	8	280	15	
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Broadening of the initial 4 species, other peaks are better
40	0	40	5	
	10	250	5	
	20	280	10	
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Similar to previous one
40	0	40	10	
	7	200	5	
	20	280	10	
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Similar to previous one
40	0	40	10	
	4	200	0	
	50	280	10	
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Similar to first one
50	0	50	35	
	50	280	10	
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Especially the initial species are overlapped
50	0	50	7	
	8	220	5	
	50	280	10	
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Similar to previous one
50	0	50	7	
	10	220	0	
	50	280	10	
Initial (^{o}C)	**Ramp (^{o}C/min)**	**Final (^{o}C)**	**Hold (min)**	Except for citronellol and pulegone satisfactory resolution. Broadening is minimum
40	0	40	0	
	12	60	0	
	8	180	0	
	20	280	10	

The temperature program given at the end of Table 3.8. provided good resolution of the analytes, and the peak drawbacks (i.e. tailing or fronting or splitting) were minimised. Application of the successive ramps by this temperature program, the chromatogram in Figure 3.5 was obtained. However, citronellol and pulogone peaks were coeluted. For that reason, they were quantified together.

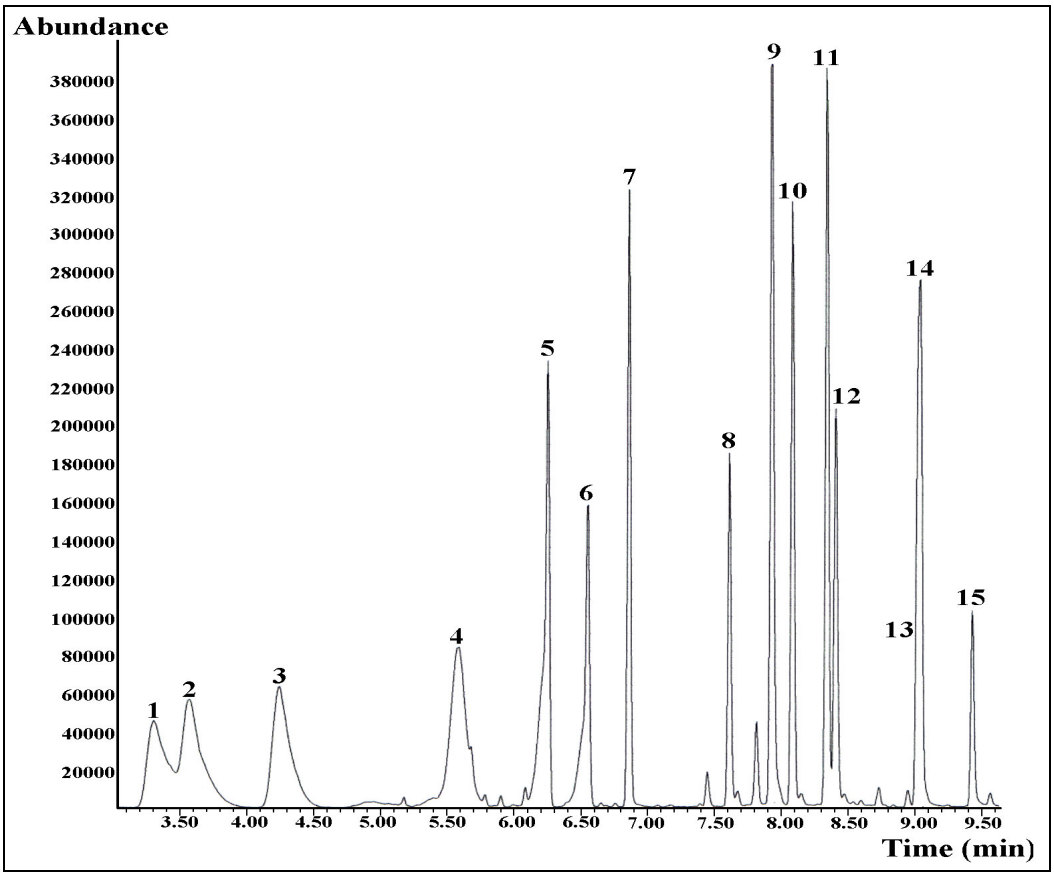


Figure 3.5. Total Ion Chromatogram for Terpenes (1: α-pinene, 2: camphene, 3: β-pinene, 4: d-limonene, 5: γ-terpinene, 6: linalool, 7: fenchone (ISTD), 8: 1-isopulegol, 9: 1-borneol, 10: dl-menthol, 11: alpha-terpineol, 12: dihydrocarveol, 13 and 14: citronellol + pulegone, 15: geraniol)

As can be seen from the TIC (Figure 3.5) the first 4 peaks were broadened. α-pinene, camphene, β-pinene, and: d-limonene are highly volatile compounds and neither cryotrap temperature nor column temperature was sufficient to provide better resolution. In order to decrease the cryotrap temperature further, liquid nitrogen instead of CO_2 could be used but our cryotrap was designed just for CO_2. Further reduction of the oven temperature was not practical since cooling of the oven was provided by a fan and ambient temperature acts as the limiting factor.

3.3.2. Effect of solvent and column cooling on determination of isoprene

Isoprene determination was one of the objectives of this study. We aimed to determine isoprene and monoterpenes simultaneously. However, we have faced some limitations during the study and isoprene could not be measured satisfactorily both in the laboratory and sampling site. The first limitation was that, the terpene standards were prepared in ethanol and isoprene was diluted with ethanol so that the desired concentration levels were reached. However as can be seen in Figure 3.6. the peak was obscured extremely by ethanol and the selected ions in that extreme case could not be expected to obtain satisfactory quantitation.

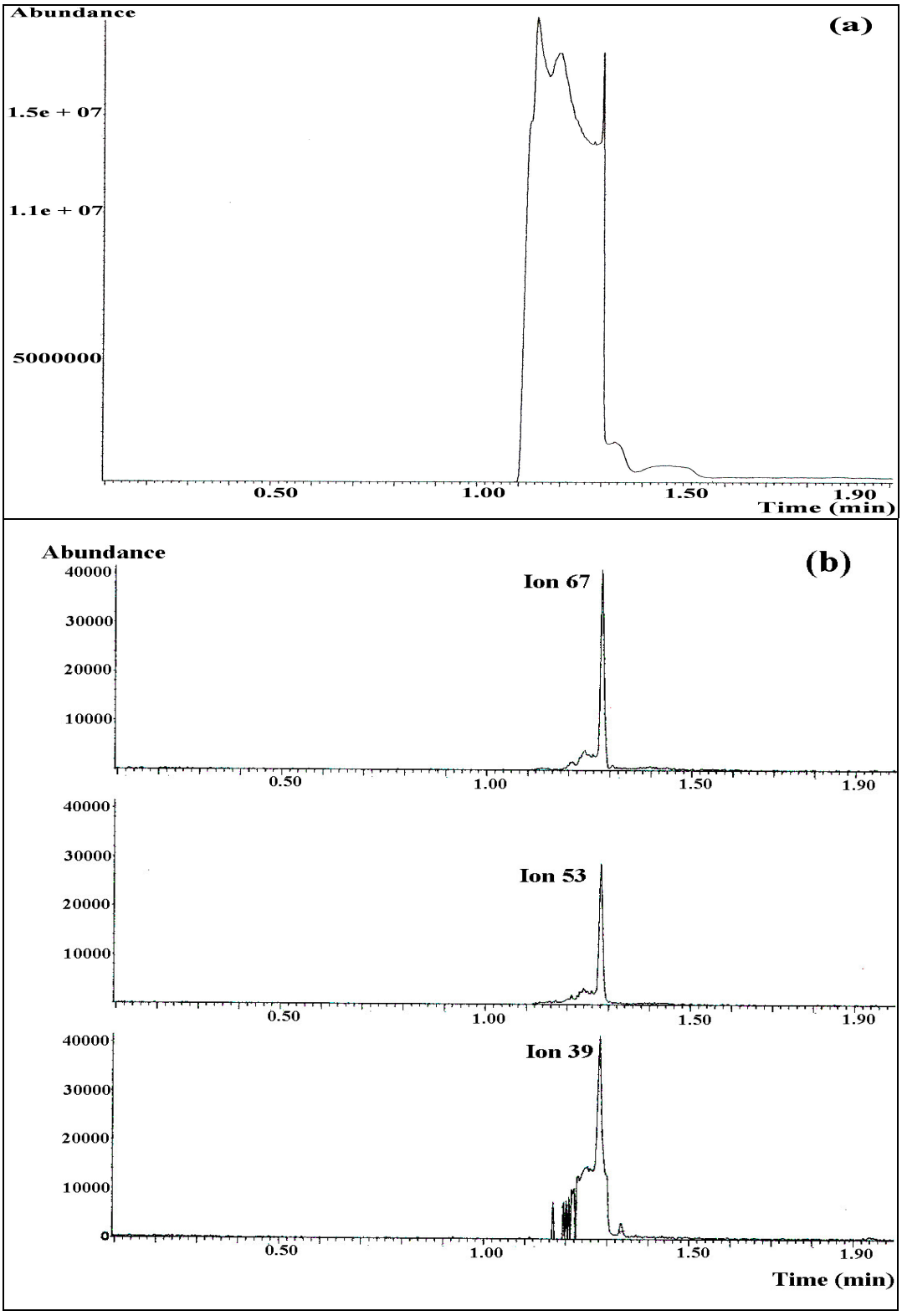


Figure 3.6. Effect of solvent (ethanol) on detection of isoprene.

This drawback might be eliminated by using either standards free-of solvent and prepared in gaseous phase, or by simply cooling the column further to provide the resolution of the solvent and isoprene peaks. Unfortunately it was not possible to reduce the temperature of the oven further and therefore isoprene was eliminated from the analyte list.

Figure 3.6.a. is the total ion chromatogram of the singly injected isoprene standard. Figure 3.6.b., 3.6.c., and 3.6.d. are the extracted ion monitoring chromatograms of the same experiment. Extracted ion monitoring is a soft-ware utility of GC-MS which provides to visualise one or several ions of interest. As ions 67, 53 and 39 *m/z* are investigated the evidence of isoprene existence under solvent peak is acquired.

3.4. Effect of Cryotrap Cooling and Desorption Temperature to Recovery of Terpenes

Cryotrap cooling and desorption temperature are important parameters to be determined for thermal desorption operation. Following sections give the experiments performed to investigate their effect.

3.4.1. Effect of Cryotrap Cooling to Recovery of Terpenes

To investigate the efficiency of cryo-trap cooling on recovery of terpenes, the same standard was injected in 6 different temperatures, keeping the other thermal desorption and GC-MS parameters constant. The terpenes which were more prone to leak through the cryogenic-trap were the most volatile ones (alpha-pinene, camphene, beta-pinene, d-limonene, fenchone and gamma-terpinene) as expected. The safe working conditions require to keep the cryo-trap as cold as possible. Data were collected for different terpenes at different temperatures

starting from – 20 to – 40 ^{o}C as our cryogenic trap system was not able to keep the temperature satisfactorily constant under – 40 ^{o}C. These data are shown in Figure 3.7..

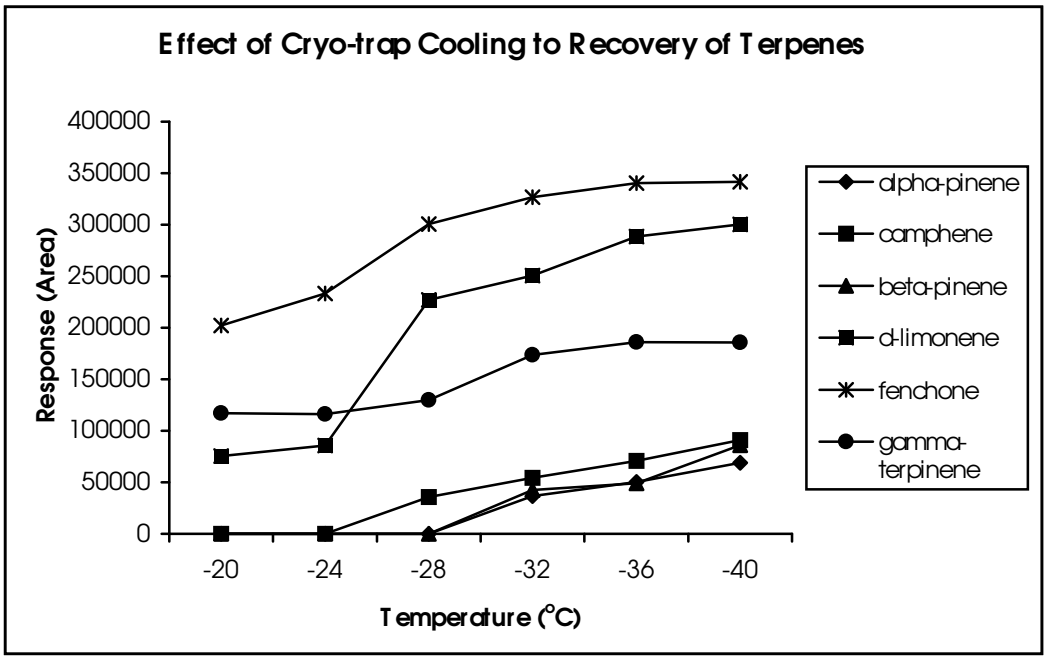


Figure 3.7. Effect of Cryo-trap Cooling to Recovery of Terpenes

As can be seen from the Figure 3.7., except for β-pinene and camphene for all the terpenes recovery stay constant after – 36 ^{o}C. That is why – 40 ^{o}C was selected as optimal temperature. Recovery may be improved a little more by using N_2 instead of CO_2. Nevertheless, the recovery results obtained with current settings were satisfactory.

3.4.2. Effect of Desorption Temperature to Recovery of Terpenes

The desorption temperature has to be selected so that the analytes are essentially desorbed from the sorbent bed. Temperatures lower than required

values will lead to inefficient recovery. The check can be performed by reanalysing the sorbent tube after desorption of the sample. If any removal of >5% is suggested, then the temperature can be increased step by step or alternatively the desorption time can be increased. On the other hand the usage of higher temperatures results in decomposition of analytes and will shorten the lifetime of the sorbents. The typical temperatures that can be used with terpenes that was studied lies in 250 to 280 ^{o}C. 250 ^{o}C is required to maintain the desorption of the terpene with highest boiling point (geraniol), while 280 ^{o}C should not be exceeded in order not to decrease the sorbent multi-usage capability. The results of replicate experiments conducted at same operational conditions using different desorption temperatures are given in Figure 3.8..

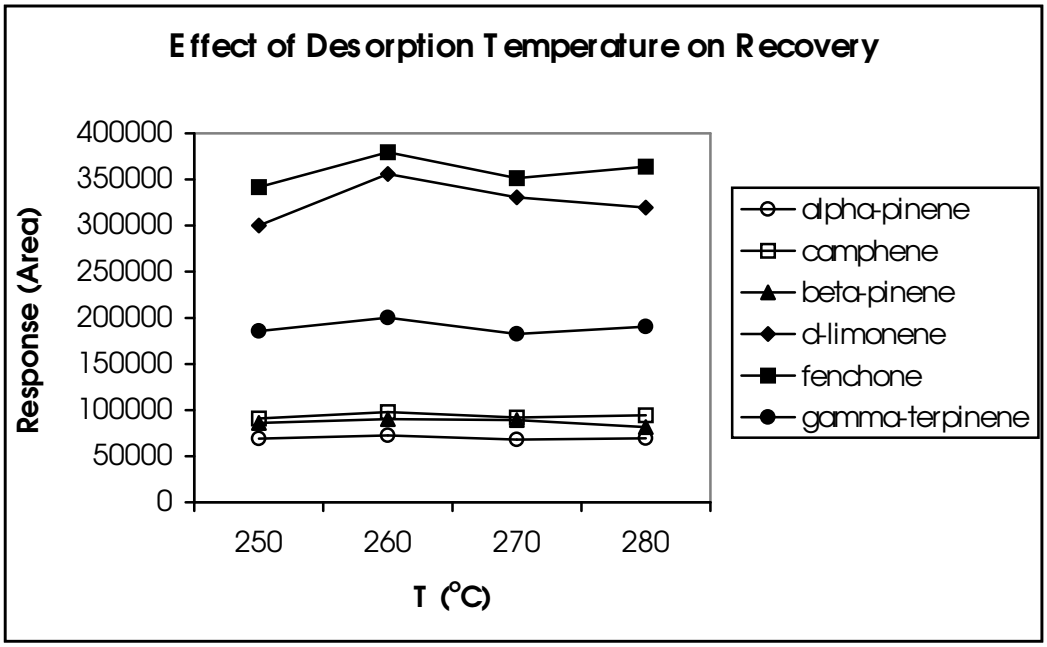


Figure 3.8. Effect of Desorption Temperature to Recovery of Terpenes

As can be seen from Figure 3.8. there was not a considerable change for most of the terpenes within this temperature-range. But, desorption temperature

was chosen as 260 ^{o}C, because the recoveries of the α-pinene, camphene, and β-pinene were maximum at that point.

3.5. Strength of the Mass Selective Detector in the Analysis of Organic Samples

Mass selective detection not only provides enhanced sensitivity for many species, but also gives a definite fingerprint about the analytes. Using traditional detectors, like FID, TCD, etc., the problem encountered, especially with real samples is that, the analyte may be interfered with (a) concomitant(s) that has similar or same retention time. The most difficult case can even mislead the analyst to define a concomitant that has replaced the analyte, and the analyte even never existed in the sample. However in GC-MS peak identification is done using more than one parameter. For example, the existence of selected target and qualifier ions does not indicate that, the compound is purely your analyte, unless the ion ratios are within the allowed levels.

Before the discussion of applied tests some definitions are given in order not to mislead the reader. Target ion is defined as the peak chosen for quantitation and it is generally selected to be the base-peak, which is the most abundant peak in mass spectrum, to obtain the highest sensitivity. Qualifier ion is defined as the follow-up peaks that has substantial abundance and used in characterisation of the species by using the ratio against target ion. In order to identify an analyte the ratio of the qualifier ions to the target ion should be in acceptable levels (±20%) suggested by the library data base.

As the name implies scan mode covers the whole molecular ion and fragments and therefore provides characterisation of the species at initial step by comparing with library results. The quality tests of terpene standards were done in scan mode since we want to view any possible impurity, or even the decomposed

standard, that can have other ions than the target and qualifier ions selected for that compound. The tests of standards are given in the following section.

On the other hand, selected ion monitoring (SIM) mode is mass spectra containing only the target and qualifier ions of the selected species. With enhanced sensitivity provided by elimination of the ions which are not under interest, it provides better means of quantitation.

The strength of the mass detector over the others were demonstrated in this work by performing the following tests for standards, which could not be obtained with other traditional detectors. These tests are also indicating the reliability of the standards that we have used during the experiments.

3.5.1. Quality Tests of Terpene Standards

In order to check the quality of the terpene standards they were analysed in scan mode and observed peaks were compared with the library data of NBS75K.L and WILEY.L. Initially there were 19 standards including myrcene ($H_2C=CHC(=CH_2)CH_2CH_2CH=C(CH_3)_2$, 136.24 g/mol), citral, 2-piperidone, and α-terpinene. Probably because of unsuitable transportation some of the standards were unsuitable. Myrcene standard was prepared in acetone, while all the other standards were prepared in ethanol by the manufacturer. In order not to face problems encountered by usage of different solvent, we omitted myrcene. The first standard mixture was prepared in ethanol using alpha-pinene, beta-pinene, geraniol, camphene, linalool, d-limonene, alpha-terpineol, citronellol, dl-menthol, 1-borneol, dihydrocarveol, 1-isopulegol, pulegone, gamma-terpinene, α-terpinene, citral, and 2 piperidone

The scan of the first multi-standard gave evidence that citral and 2 piperidone standards were not satisfactorily pure or even degragated. The target

and qualifier ions for α-terpinene did not even match with NBS75K.L library results. In the following sections, first, the γ-terpinene was evaluated to demonstrate the case of a suitable standard and the cases for unsuitable standards were demonstrated by detailed investigation of citral, α-terpinene and 2-piperidone.

γ-terpinene

As it is seen in Figure 3.9., the γ-terpinene standard has single peak which was well correlated with library search results. Here it is good to indicate that, if that result was obtained for selected ions of γ-terpinene instead of complete scan, the conclusion would be misleading, since there would still be question marks about the purity of the compound. The reason is that, the impurities with different fragments and molecular ion would not be detected since they have already been eliminated.

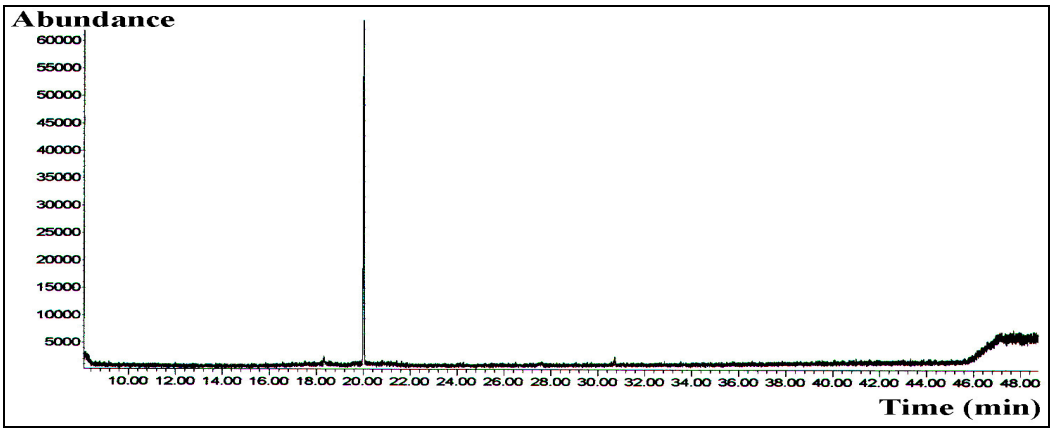


Figure 3.9. Full-scan chromatogram (35-550 *m/z*) of γ-terpinene

To check whether that single peak was γ-terpinene or not, mass spectra obtained with scan-mode and that for the library NBS75K.L were compared. The spectra are given in Figure 3.10.

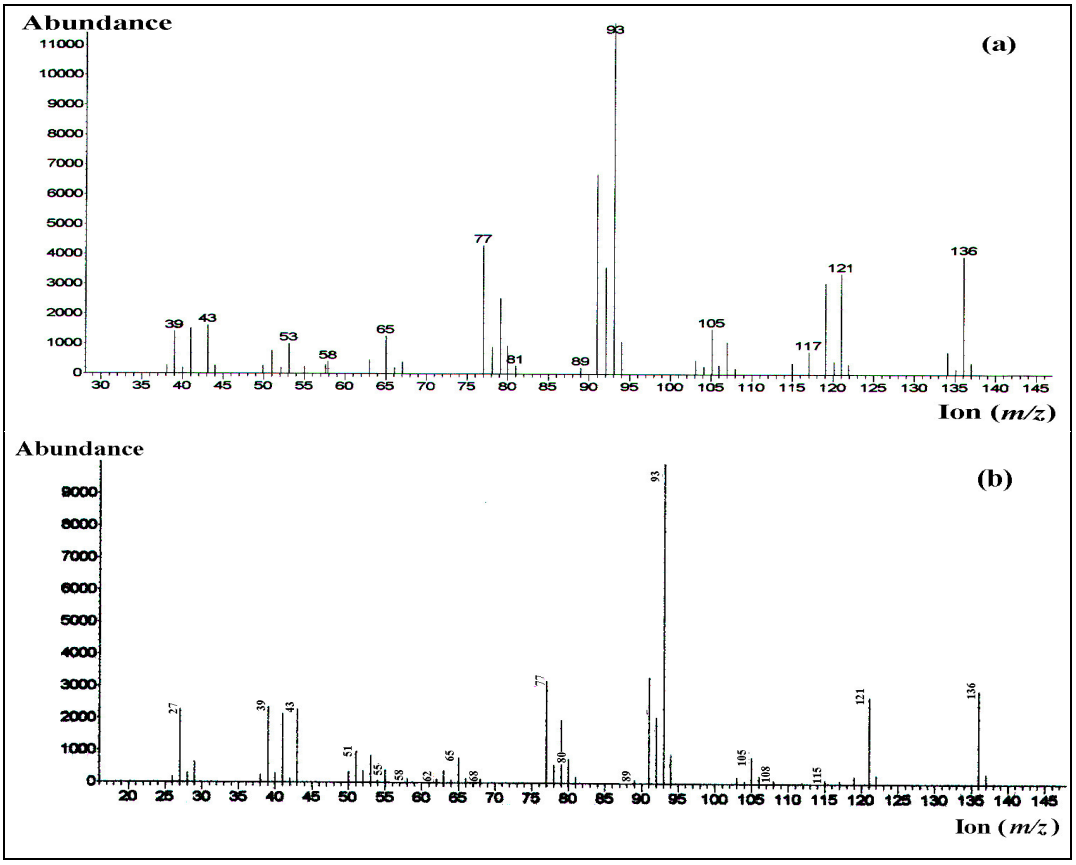


Figure 3.10. Mass spectrum of gamma terpinene (a), and corresponding NBS75K.L library mass spectrum (#6612: 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-) (b).

The mass spectrum of single standard full-scan (35-550 *m/z*) chromatogram of γ-terpinene (Figure 3.10.a.) demonstrated 93% correlation with the chromatogram supplied by library (Figure 3.10.b.).

The ion abundances obtained match that of library 94%. Since both criteria of purity and quality were satisfied γ-terpinene was accepted to be a suitable standard. The cases for other terpenes except for α-terpinene, citral, and 2-piperidone were similar.

Citral

The results for citral (3,7-dimethyl-2,6-octadienal) suggested the removal from standard list like α-terpinene and 2-piperidone. The full-scan chromatogram (Figure 3.11.) of citral displayed two major defined peaks, labelled as 1 and 2, and a series of peaks which were not well-correlated with library.

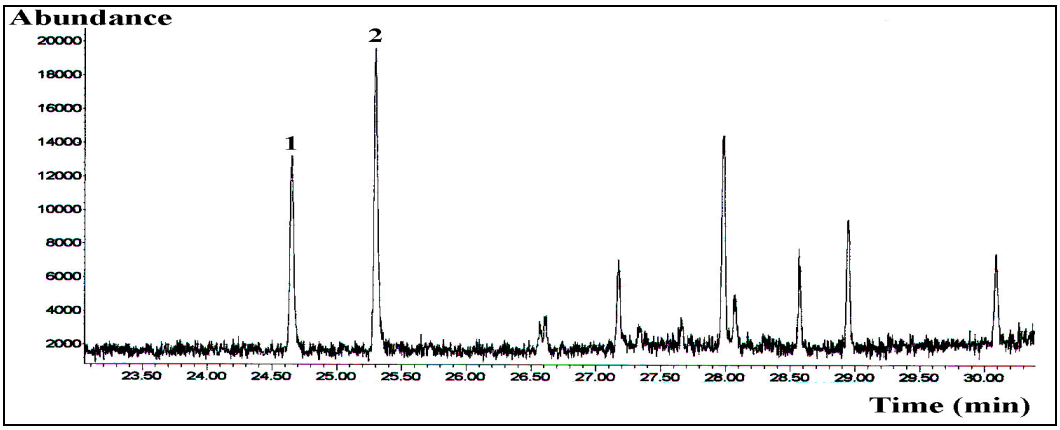


Figure 3.11. Full-scan chromatogram of citral (35-550 *m/z*).

We have compared the mass spectrum of both defined peaks (Figure 3.12.a. and Figure 3.12.c.) with library (Figure 3.12.b., Figure 3.12.d. and 3.12.e). According to those comparison the compound consisted of (Z) and (E) isomers of citral, (the peak assigned with 1 and 2 in Figure 3.11.), and undefined impurities, respectively.

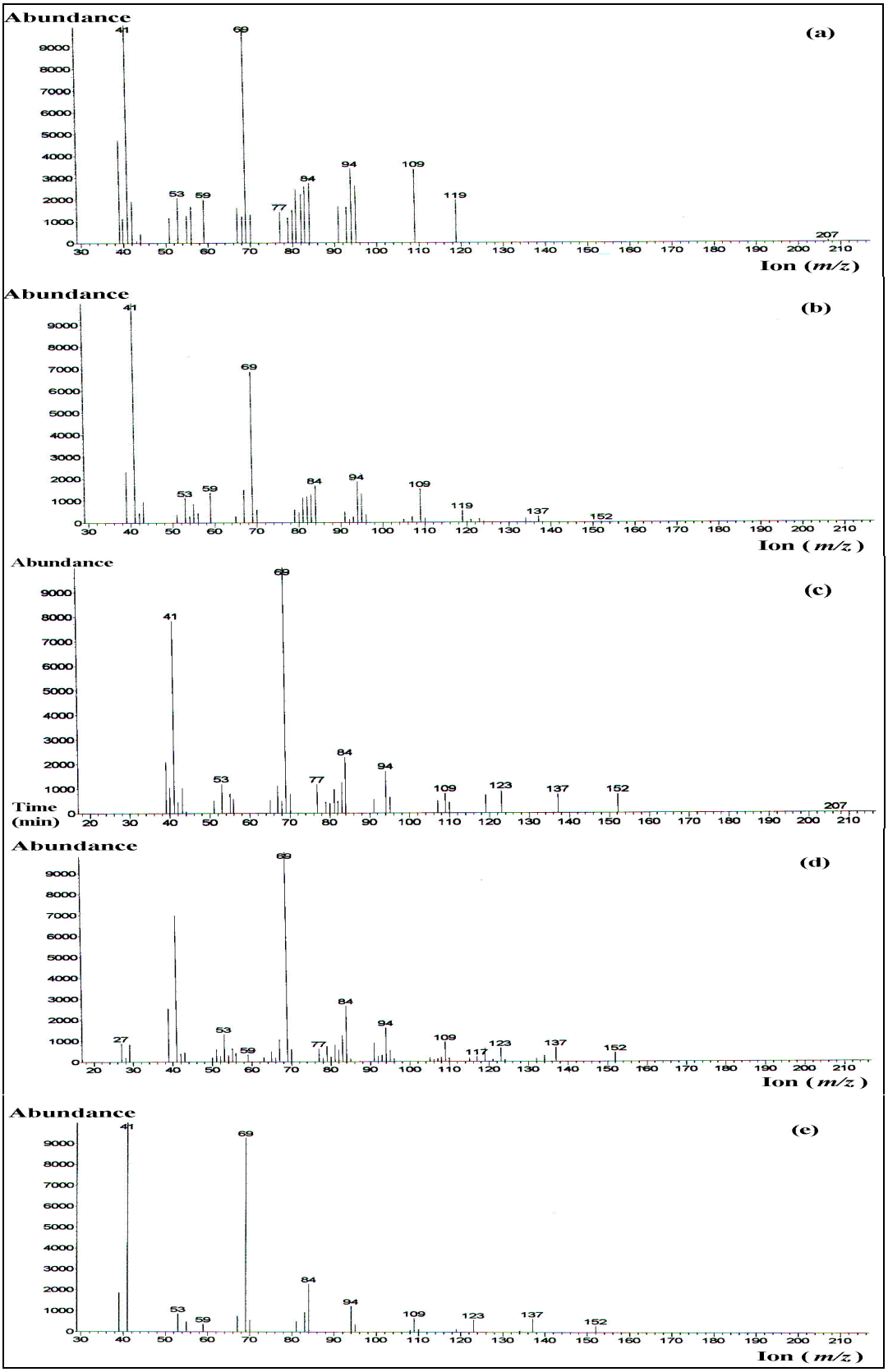


Figure 3.12. Scan mode and library mass spectra for citral.

As we have compared our mass spectrum of the first peak (Figure 3.12.a.) with the library (Figure 3.12.b.), the first peak in full-scan chromatogram of citral (Figure 3.11.) was confirmed to be 78 % as (Z)-citral.

The second peak in Figure 3.11. consisted the mixture of (E) and (Z) isomers. The comparison of our mass spectrum (Figure 3.12.c.) with library (Figure 3.12.d.) in this case supplied correlation of 95 % as mixture of isomers and 90 % as (E)-citral (Figure 3.12.e.).

The group of species (Figure 3.11.) were not correlated when compared to library. They were probably the mixture of degradation products of the standard. The base-peaks for most of these side reagents were at 69 and 44 *m/z*. The abundance of the first two peaks were 50.473 %, while the impurities accounted for the rest. It must have been decomposed during transportation or storage before we received the standards. Therefore, it was rejected from standard list.

3.5.2. Scan and SIM Modes and Sensitivity

Depending on the nature of the sample like number of analytes, purity, concentration etc., the Quadrupole-MS can be operated in Scan or SIM modes. For identifying a sample of unknown mass and fragments, Scan-mode is employed. Scan-mode counts all the ions in the selected interval. But, if the analysis aims to detect target compounds, with known spectral characteristics that were determined by scan-mode analysis and/or library database, with maximum sensitivity, selected ion monitoring (SIM) is useful. Thus, when we choose to detect a terpene by monitoring three characteristic fragments, we switch the analyser rapidly from one mass to another, by excluding the other ions in between the fragments in concern.

In the scan mode, complete spectra are repeatedly measured between two extreme masses, which contained 35 amu to 550 amu for this study. Suppose that

the time width of the chromatogram peak is 10 s at mid-height. At least one spectrum must be measured every 5 s in order to maintain that one of them lies entirely within the chromatographic peak. If the scan covers masses 50-550 amu at low resolution, 0.01 s per mass can be used. All of the ions reaching the detector during that time span are counted. Increasing this span time increases the sensitivity, as the total number of counted ions increase. To increase the sensitivity, either the range of masses scanned is decreased, or the scan time is increased. In the first case, analytical information may be lost, and in the second case, a good mass spectrum may not be obtained, or two eluted compounds in the same peak may not be distinguished. The sensitivity gain can be enormous. Sometimes S/N ratio of SIM mode can be 50 fold higher than that of Scan mode, which can be attributed to the decrease in the background and the increase in the integration time for the selected ions (Hoffmann E.D. *et al.,* 1996).

In this study, we have selected to use SIM-mode, which is the most sensitive option. Because, the terpenes exist in trace amount in the atmosphere which can not be determined with scan mode. Moreover, in most cases the complex matrix leads to overlaps unless the specific ions are selected. The ion ratios provide to identify the analyte in SIM-mode, which can be considered as a selective and more sensitive means of analysis.

3.5.3. Selection of Target and Qualifier Ions

Definition of the terms target and qualifier ions and scan and SIM modes were given in sections 3.5. and 3.5.2.. Working with real air samples in trace amounts restricts the usage of scan mode for determination of the concentrations. After obtaining the information about the qualifier ions of the terpene standards, SIM-mode was used for the determination of the analytes. Full scan mass spectra, which were obtained during quality checks, were investigated to select the target and qualifier ions. The ions obtained at scan mode and that of literature were both

considered during selection. The most virtual way of obtaining the library spectra is to find the CAS#'s (Chemical Abstracts Service, which is a division of American Chemical Society) and then search the library using this definite identity number (Table 3.9.). The ions (*m/z*) with the highest abundances were chosen as target ions and 3 of the remaining ions (*m/z*) which has high intensity and can provide additional information to identify the related terpene were chosen as qualifier ions. The selected target and qualifier ions and the retention times (t_r) for the terpenes analysed in this study are given in Table 3.9. below.

Table 3.9. Target and qualifiers ions for terpenes.

Compound	CAS #	t_r (min)	Target Ion	Qualifier Ion-1	Qualifier Ion-2	Qualifier Ion-3
α-pinene	78-79-5	3.30	93	77	39	121
Camphene	80-56-8	3.57	93	121	41	79
β-pinene	79-92-5	4.25	93	41	69	77
d-limonene	5989-27-5	5.59	68	93	79	41
γ-terpinene	99-85-4	6.25	93	91	77	136
Fenchone	4695-62-9	6.55	81	69	41	152
Linalool	78-70-6	6.86	71	93	41	55
Isopulegol	59905-53-2	7.61	81	69	41	121
1-Borneol	507-70-0	7.92	95	110	41	67
Menthol	10482-56-1	8.08	71	81	95	41
α-terpineol	1490-04-6	8.34	59	93	121	136
Dihydrocarveol	619-01-2	8.40	93	107	121	41
Pulegone	89-82-7	9.03	81	67	152	109
Citronellol	106-22-9	9.04	69	41	55	81
Geraniol	106-24-1	9.43	69	41	93	43

Ideally the neighbouring ions should have different target ions for ease of determination. However, since the intensity of the target ion was primary concern in this study, some of the neighbours had to have same target ions. As soon as

there is not a chromatographic interference this application does not bring about any difficulty during determination of the species.

3.5.4. Construction of SIM Windows

When target and qualifier ions were selected the SIM windows were constructed. Window is a portion of chromatogram that the analyst selects group of ions in this section, which can be examined apart from the other analytes. The window must be selected so that, each window should demonstrate well resolved spectra of ions taking the retention time shifts into account, and the group of ions consist of the ones those enable minimum number of ions to be counted. As it was mentioned before lower the number of ions to be counted, higher the time of span , Consequently more counting and higher sensitivity is achieved. The SIM windows constructed for this purpose and the ions included in windows are given in Table 3.10..

Table 3.10. SIM windows and the included ions.

Window	Time (min)	Ions (m/z)	Terpenes
1	1.00-5.20	39, 41, 69, 77, 79, **93**, 121	α-pinene, camphene, β-pinene
2	5.20-7.30	41, 55, 67, 68, **69, 71**, 77, 79, **81**, 91, **93**, 121, 136	d-Limonene, γ-terpinene, Fenchone (ISTD), Linalool
3	7.30-8.70	41, 43, **59**, 67, 69, **71**, 79, **81**, **93**, **95**, 107, 110, 121, 123, 136	Isopulegol, 1-borneol, Menthol, α-terpineol, dihydrocarveol
4	8.70-31.67	41, 55, 67, 68, **69**, **81**, 82, 93, 152, 109	Citronellol, Pulegone, Geraniol

The ions in bold are target ions.

3.5.5. Use of Qualifier Ions in Identification of Terpenes

In this section the use of qualifier ions in improving quantitative separation of the closely spaced or overlapped peaks are discussed. The ideal way of obtaining qualitative information about the analytes is possible by having well-resolved peaks. Nevertheless, by the use of target and qualifier ions the neighbouring peaks, which are not well-resolved, or even co-eluted, up to three molecules, can also be evaluated by the use of target and qualifier ions. The condition for separation of overlapping peaks is that, they should have completely different target ions to decide on the quantity and a few different qualifier ions to

satisfy the ion ratios. For the case of neighbouring terpenes, the qualifier ions and the ion ratios can give suitable information as long as the target ions are different.

Discussion below covers the advantages of using qualifier ions in the parameters that we are concerned. Each one is discussed separately by comparing the Total Ion Chromatograms (TIC; chromatograms established by using all ions included in the window of interest) and Extracted Ion Monitoring Chromatograms (EIM; chromatograms including only target and qualifier ions of the selected species).

α-Pinene and Camphene

As we have a look at the Total Ion Chromatogram (TIC) of α-pinene and camphene peaks it is hard to decide at which time the peaks are separated. Investigating Figure 3.13.a. (TIC chromatogram), at 3.45 min, where the aboundence was minimized, they seem to be separated . However, the target ion 93 is far from background even when they are minimized and therefore the error brought about may be high when we only consider this ion. But taking qualifier ions into account can decrease the uncertainty to much lower level. As we investigate the qualifier ions in Figure 3.13.b., ion of 121 *m/z* offers more thrustworthy decision about the separation point of the analytes.

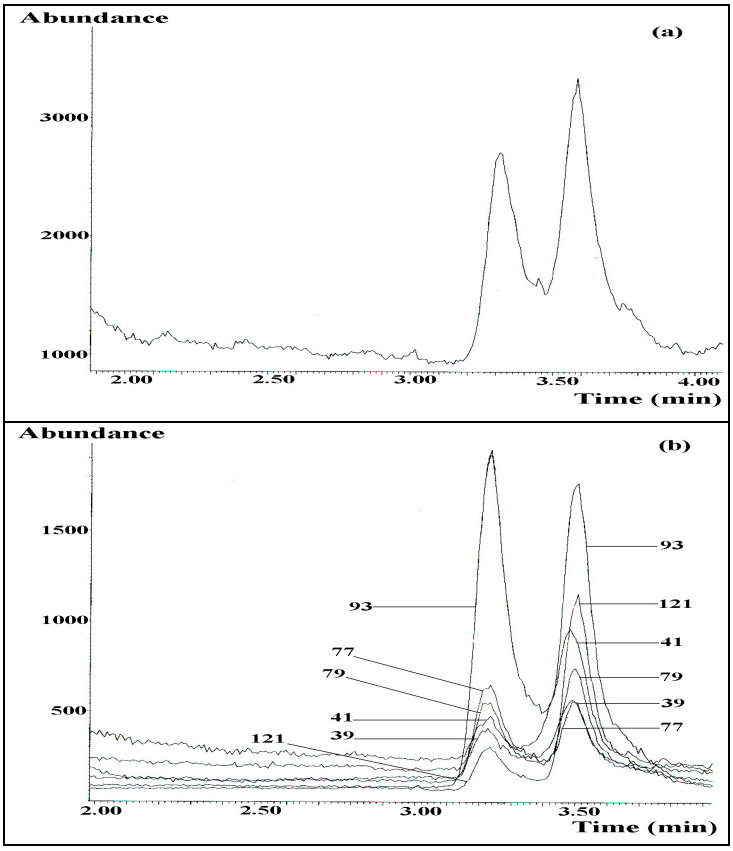


Figure 3.13. TIC and EIM of α-pinene and camphene in SIM-mode

In this case the guidance of 121 *m/z* ion helped us to decide where the peaks were most likely to be separated. Since the structure and chemical properties of these terpenes were similar, it was not possible to choose different target ions. The main concern during selection of target ion was achieving the highest sensitivity.

α-Terpineol and Dihydrocarveol

The case of α-terpineol and dihydrocarveol is similar to that of α–pinene and camphene. The peaks were not well resolved in TIC (Figure 3.14.a.), however the qualifier ions in extracted ion monitoring chromatogram (Figure 3.14.b.) that

were closer to base line provided assistance to distinguish the separation point. WILEY275.L library was used to confirm the dihydrocarveol, and the corresponding correlation value was 91%.

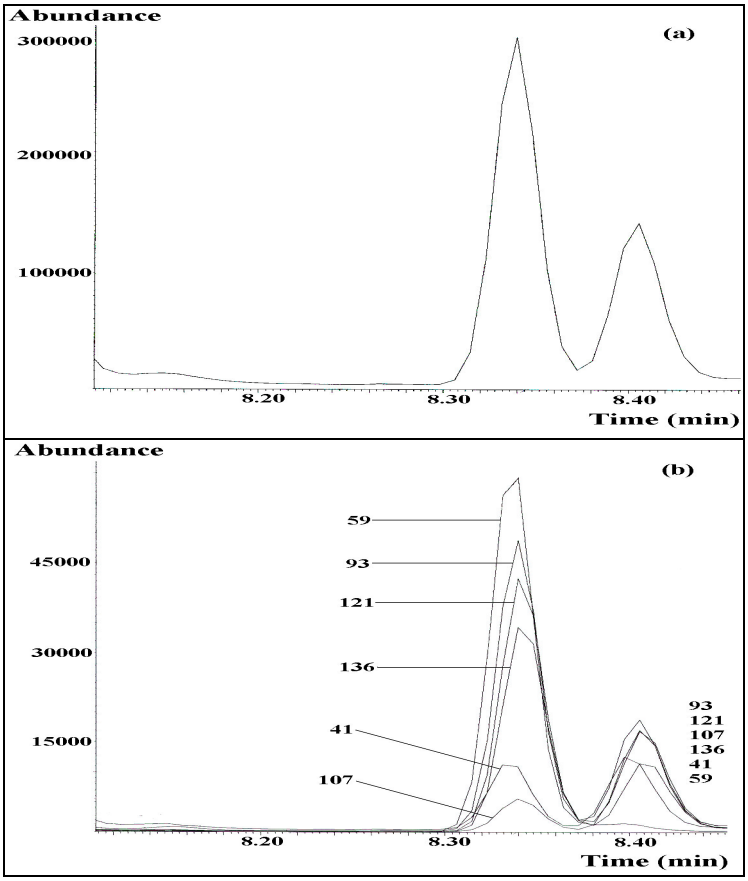


Figure 3.14. Extracted Ion Monitoring Chromatogram of alpha-terpineol and dihydrocarveol

Different to the case of α-pinene and camphene, the terpenes α-terpineol and dihydrocarveol had different target ions, 59 and 93 *m/z* respectively. The 107 *m/z* ion was found most reliable in deciding the separation point.

Pulegone and Citronellol

We have mentioned that even co-eluting species can be distinguished provided the compounds had totally different target ions. In this study the only co-eluting couple was pulegone and citronellol. These species unfortunately did not have different highly abundant ions which do not exist in each others mass spectra.

Figure 3.15.a. and Figure 3.15.c. consist of the mass spectrum obtained from the single injected standards analysed with full-scan mode (35-550 *m/z*). Figure 3.15.b. and Figure 3.15.d. give the corresponding library chromatograms of NBS75K.L data base.

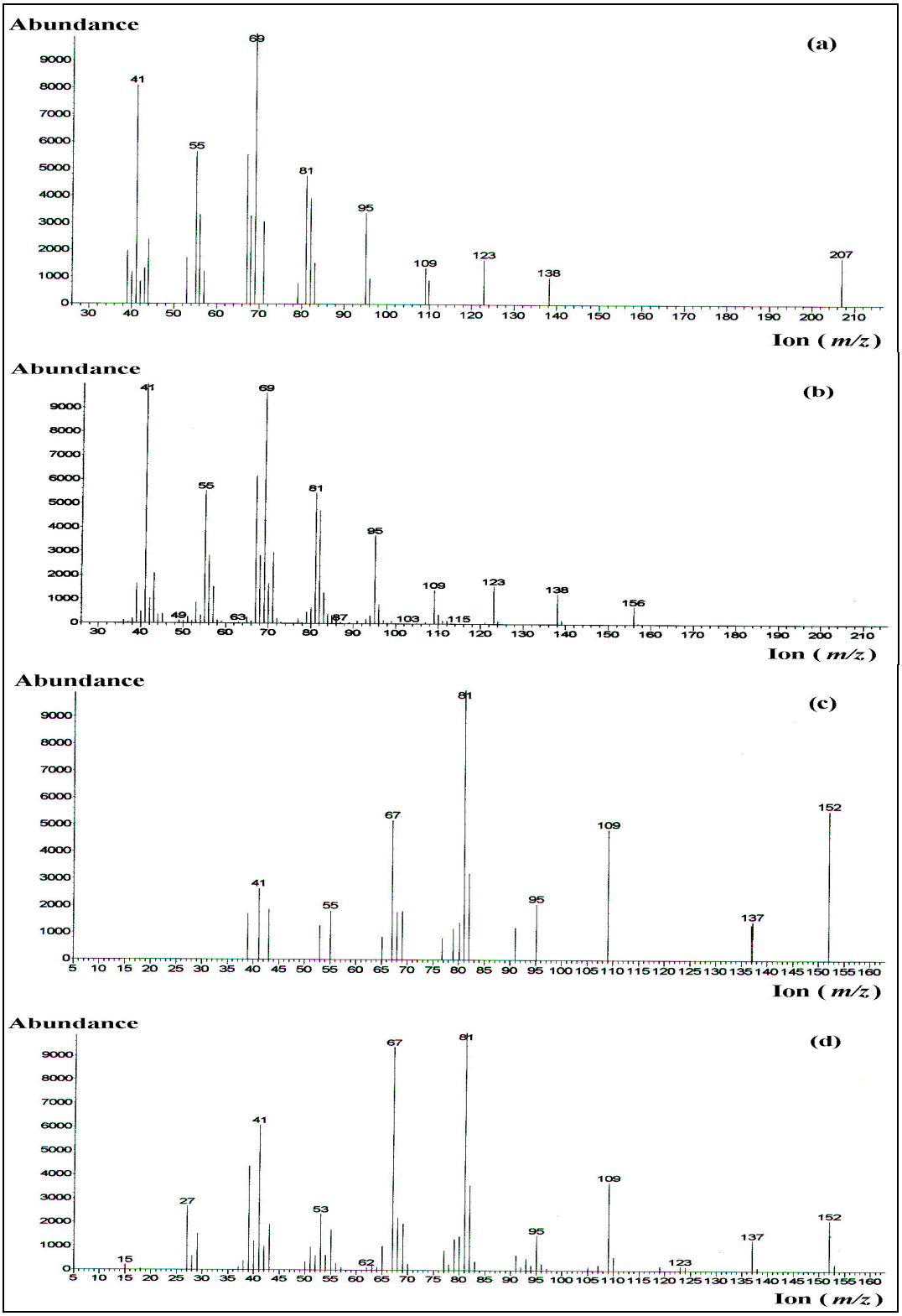


Figure 3.15. Mass spectra for citronellol and pulegone, and the corresponding NBS75K.L spectra.

Citronellol had highly aboundant ions at 69, 41, 55, 81, and 95 *m/z*. The target ion selected was 69 for citronellol before the case of co-elution. 69, 41, 55 were also included in SIM-window (window #4) but it was not possible to keep the ion ratios in control since pulegone also had 69 *m/z* ion (approximately 25 % of the base-peak)

Figure 3.16. is the extracted ion monitoring chromatogram of the standard mixture analysed in scan-mode.

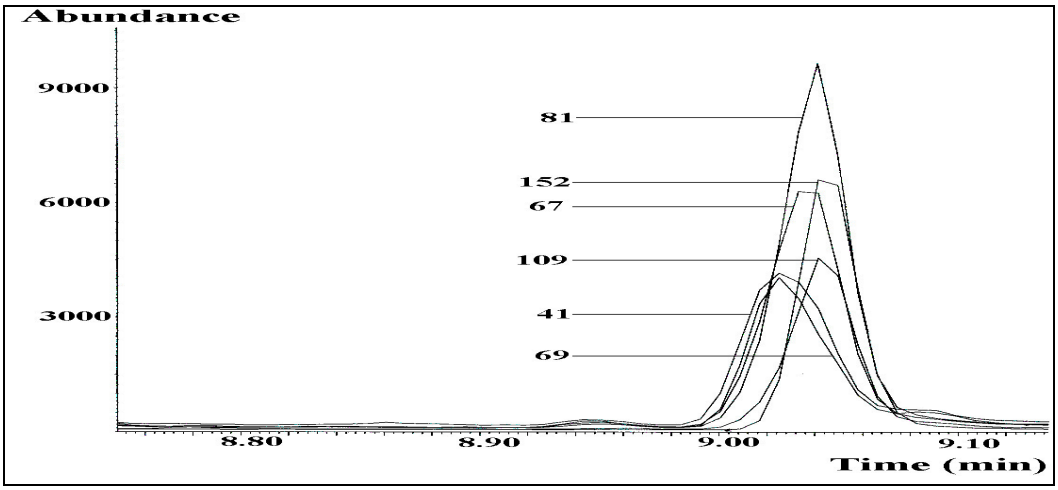


Figure 3.16. Extracted Ion Chromatogram of overlapped pulegone and citronellol.

The co-elution of the species can be seen obviously in this chromatogram. 81 *m/z* ion was decided to be used for the total quantitation of citronellol and pulegone peaks, since 81 *m/z* is still more than 50 % of the base peak (69 *m/z*) for citronellol.

3.6. Quality Assurance of Mass Spectrometer

Mass Spectrometer is a widely used, sensitive detector. However, the repeatibility can be disturbed from even small changes in response factor of the detector, which may take place indeterminently. Moreover the errors that can be brought about during sample introduction (may be instrumental and/or personal error) should be considered. The analyst must take these into account and control the response factor by daily autotunes, and by using internal standard calibration and intermediate standard checks. The following sections cover the discussion of these subjects and experimental results obtained.

3.6.1. Auto-tune

Auto-tune was performed daily to control unexpected sensitivity and resolution variations, which may possibly resulted from a serious hardware problem or from the contamination gradually depositing on the detector compartment (especially, the ion source is the most important part of the detector that should be kept clean). Perfluorotri-n-butylamine (PTFBA) or perfluorokerosene are often used for this purpose (Barker J., 1999). Our instrument consists of PTFBA as auto-tune standard by which we checked daily response and resolution for the ions covering 69, 219, and 502 *m/z* ions. The most prominent peaks of PTFBA (*m/z* 31, 69, 100, 114, 119, 131, 219, 264, 414, 464, 502, 576, and 614) sufficiently cover the range of scan-mode for our application.

3.6.2. Internal Standard Calibration

The autotune alone can not investigate the detector reproducibility. Even after a single analysis the response may vary because of the organics somewhat deposited on the ion source. To check the response and correlate the differences,

internal standard calibration was used in this study. The other factors which may also display unnegligible variations through the analysis, such as sample introduction efficiency, are also can also be controlled by ISTD calibration.

Internal standard (ISTD) is a compound that is added to every standard and sample. The internal standard is added at an exact, known concentration. It is required for the calculation of relative response factors for target compounds. The internal standard should be similar to the target compound chemically. But some properties, like retention time and/or ions, must be distinct to differentiate it from analytes.

The concentrations of the analytes are evaluated using the relative response factor (RRF) which can be calculated using the following equation:

$$RRF = \frac{A_c C_{is}}{A_{is} C_c}$$

where;

RRF: Relative Response Factor
A_c: Area of the target analyte
A_{is}: Area of the corresponding internal standard
C_{is}: Concentration of the corresponding internal standard
C_c: Concentration of the target analyte

This equation is valid only if the same amount of internal standard is introduced into each level of the initial calibration, and the volume of each initial calibration standard is constant as the was we have proceeded in this study (Alltech Associates Inc., 1997)

Fenchone was used (24 ng) as internal standard (Gershenzon *et al.,* 2000; http://www.eosdis.ornl.gov/boreas/TGB/tgb8mono/comp/TGB08_Mono terpenes_Conc.txt) since it was already an oxygenated terpene with similar chemical behaviours (most importantly retention time), and was not expected to exist in troposphere. The addition to the standards was directly during preparation. It was injected to the vial just before the analysis so that the 2 μL injection from the standard in the vial would provide 16.840 ng Fenchone. On the other hand, the addition to the samples were done prior to analysis followed by purging with Helium to eliminate excess solvent. The addition of Fenchone to standards in 2 μL ethanol also provides matrix matching since the difference in solvent content would otherwise lead to extreme retention time differences, and possibly variable background levels.

3.6.3. Results of ISTD Calibration

Using internal standard calibration the typical correlation values (R^2) obtained (Table 3.11.) were above 0.995 with an exception of α-pinene (0.990). These high correlation values suggest linear response through calibration range for each standard. Number of levels indicates how many calibration points were used for the plot. For evaluation of the precision and detection limits, 7 replicates of the lowest standard was analysed. To establish the %RSD, the standard deviation of these replicates was divided by the mean of these replicates (m_r). The detection limit (D.L.) was calculated by multiplying the standard deviation by the factor of 3 and then dividing by the mean sampling volume (V_m = 6.24 L).

$$\%RSD = (s/m_r) \times 100$$

$$D.L. = 3s/V_m$$

Table 3.11. Calibration data and Detection Limits for terpenes.

Terpene	Calibration Range (ng)	Number of Levels	R^2	% RSD	D.L. (ppbv)
α-pinene	0.97 – 30.94	5	0.990	5.20	0.025
Camphene	0.97 – 30.94	6	0.996	8.11	0.039
β-pinene	0.96 – 30.75	6	1.000	4.37	0.021
d-limonene	0.96 – 30.94	6	0.998	4.99	0.024
γ-terpinene	0.99 – 31.71	5	0.997	7.07	0.034
Linalool	0.97 – 31.13	6	0.997	4.78	0.023
1-isopulegol	1.03 – 32.08	6	0.997	6.03	0.029
1-borneol	1.02 – 32.64	6	0.999	9.78	0.047
dl-menthol	1.04 – 32.45	6	0.996	6.03	0.029
α-terpineol	0.97 –31.14	6	0.996	7.28	0.035
Dihydrocarv.	0.92 – 29.62	6	0.995	9.36	0.045
Citr. + Puleg.	1.98 – 63.39	5	0.991	13.94	0.067
Geraniol	0.98 – 31.71	5	0.989	9.98	0.048

In Table 3.11. number of levels indicate how many points were used when plotting the calibration line. The %RSD values were within 10% except for the composite peak of citronellol (Citr.) and pulegone (Puleg.). The detection limits were at sub-ppbv levels for the method. The lowest detection limit was belong to β-pinene.

3.6.4. Investigation of the fenchone peaks

The fenchone peaks were investigated separately to have an idea about what would happen if internal standard calibration was not used. The particular results which include the area of the fenchone peak during analysis of 6-level calibration standards (Table 3.12.) and ambient air samples (Table 3.13.) demonstrate the necessity for internal standard calibration.

Table 3.12. Response for fenchone during 6-level calibration standards.

Std. Level	Area ISTD ($x10^6$)
1	2.53
2	2.29
3	2.34
4	2.19
5	2.56
6	2.32
std	0.146
mean	2.37
std/mean	0.060
%RSD	6.041

Table 3.13. Response for fenchone during ambient air sample analysis.

Sample	Area ISTD (x10^6)	Sample	Area (ISTD)
1	1.76	**9**	2.11
2	1.92	**10**	2.58
3	2.58	**11**	2.62
4	2.45	**12**	2.26
5	2.50	**13**	2.25
6	2.38	**14**	1.90
7	2.74	**15**	1.74
8	2.50	**16**	1.91
		std	0.331
		mean	2.26
		std/mean	0.146
		%RSD	14.64

In Table 3.13., Std. Level is the different concentrations of the standard mixture which contained of 14 basic terpenes. Area (ISTD) is the peak area of the spiked fenchone. The %RSD gives an idea about the fluctuation of the measurements.

The %RSD resulting from the 6-level standards was 6.04% while that of samples was 14.637%. This picture emphasises the requirement for ISTD during working with such a sensitive detector and a procedure and sample introduction with too many intermediate steps.

3.6.5. Intermediate Standard Check

Especially for long term measurements, the response of the detector is highly influenced by the depositions on the detector. As we have already observed

through the particular evaluation of fenchone for calibration standard and sample injections the detector can not always stand robust and as there exist too many parameters during sample introduction. The error that could be introduced because of these factors were checked daily to assure if the calibration plot was still valid or not. Table 3.14. gives the $\%E_r$ and the %RSD accompanied by the intermediate standards to demonstrate the day to day variations. In table 3.14., D.Std. is the abbreviation for the daily intermediate standard of the consequent day.

Table 3.14. Results of intermediate standards.

Terpene	D.Std.1 (ng)	D.Std.2 (ng)	D.Std.3 (ng)	% E_r (1-2)	%E_r (1-3)
alpha-pinene	9.23	9.21	9.12	-0.22	-1.20
camphene	7.41	7.21	7.48	-2.72	0.95
beta-pinene	9.05	9.15	9.10	1.10	0.55
d-limonene	9.18	9.50	9.29	0.76	1.19
γ-terpinene	9.56	9.62	9.72	0.62	1.62
linalool	11.40	11.48	11.50	0.70	0.87
1-isopulegol	10.18	10.35	10.42	1.65	2.33
1-borneol	7.43	7.47	7.25	0.54	-2.44
dl-menthol	7.98	8.11	8.02	1.62	0.50
α-terpineol	10.19	10.18	9.15	-0.10	-6.40
Dihydrocar.	8.12	7.29	8.08	-10.60	-0.51
c.lol + puleg.	21.47	21.29	19.76	-0.86	-8.21
geraniol	10.36	9.90	9.78	-4.59	-5.82

The $\%E_r$ was calculated by subtracting the related intermediate standards and dividing by the mean of three, and multiplying by 100. Here, we demonstrated the error introduced by the daily variations in conditions by comparing the analysis days with the initial day. $\%E_r$ (1-2) indicated the error between first and second day and $\%E_r$ (1-3) gives the error brought about between first and third days. If

these injections indicated consistent error greater than 5%, fresh calibration would have been required.

3.7. Results of Analysis of the Ambient Air Samples for Selected Terpenes

In the previous sections experiments which were done to set-up sampling and analysis were discussed in detail. In this section the results obtained using this set-up for ambient air samples are discussed.

3.7.1. Typical Total Ion and Extracted Ion Chromatograms of the Analytes

We have mentioned the necessity of using selected ion monitoring mode for complex matrices to obtain a spectra of low base-line. The selected cases for the measured terpenes to illustrate the use of SIM during interpretation of the data were given in Figure 3.17.. Total ion chromatogram (TIC) stands for all of the ions included in the consequent window and exracted ion monitoring chromatogram (SIM) stands for only the target and qualifier ions of the species of interest.

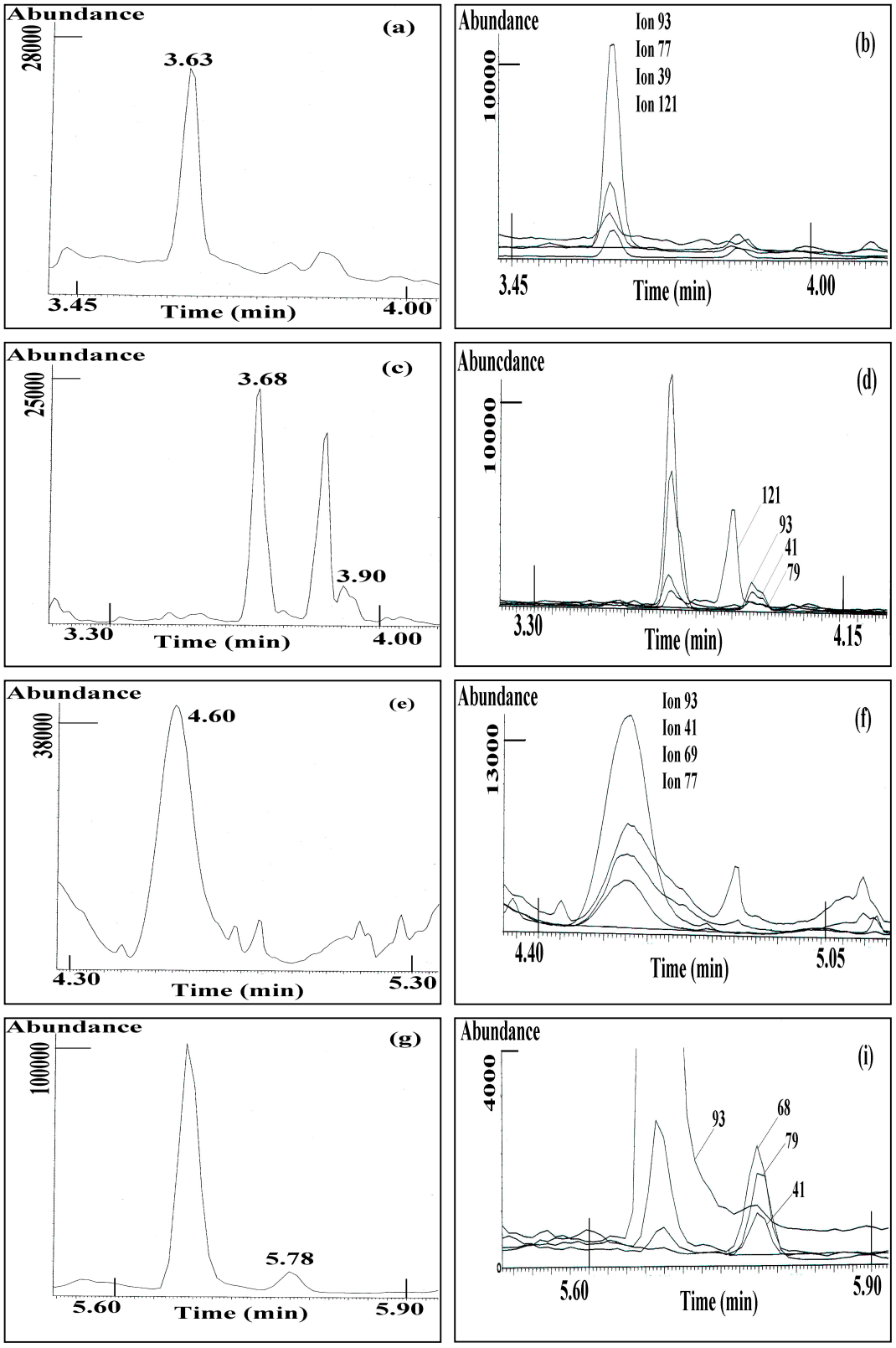


Figure 3.17. TIC and SIM chromatograms of analytes.

In Figure 3.17. a, c, e, and g are TIC; b, d, f, and i are SIM chromatograms for the measured terpenes alpha-pinene, camphene, beta-pinene, and d-limonene, respectively. As the figures side by side are investigated it can be seen that the simplicity of peaks and reduced backgrounds for SIM chromatograms provides more effective and sensitive means of evaluation.

In some conditions retention time shift was observed. The reason for this observation was attributed to the concomitants mainly composed of humidity.

3.7.2. Ambient Concentrations of Terpenes

The ambient air samples collected at Uludağ Sarıalan region were analysed for determination of alpha-pinene, beta-pinene, camphene, d-limonene, gamma-terpinene, alpha-terpineol, 1-isopulegol, geraniol, dihydrocarveol, citronellol, linalool, 1-borneol, pulegone and dl-menthol using the methodology described in previous sections. Among these analytes alpha-pinene, camphene, beta-pinene, and d-limonene were measured quantitatively. Other terpenes were below the detection limits of our method. The results of the analysis were summarised in Table 3.15. below.

Table 3.15. Results of terpene measurements at Uludağ Mountain.

			Measured Terpenes			
Sample #	***Sampling V. (L)***	***Date and Time***	**α-pinene (ng/L)**	**β-pinene (ng/L)**	**Camphene (ng/L)**	**d-limonene (ng/L)**
1	6.20	Oct. 12 07-11	0.372	0.186	0.113	0.222
2	6.74	Oct. 12 12-16	N.P.	0.229	N.P.	N.D.
3	6.15	Oct. 13 17-21	0.256	N.D.	0.109	0.206
4	6.02	Oct. 14 07-11	0.346	0.149	N.P	0.073
5	5.98	Oct. 14 12-16	0.569	N.P.	N.P.	0.287
6	6.36	Oct. 14 17-21	N.P.	0.107	N.P.	0.232

N.P.: No peak is observed.

N.D.: Lover than detection limit.

As can be seen from this table, it was not possible to see every terpene in each measured day and time. Highest concentrations was observed for alpha-pinene which is expected, as α-pinene is the most abundant terpene emitted in forest areas (Komenda M. *et al.,* 2001; Peters R.J.B. *et al.,* 1994).

With the limited data at hand, we looked at the diurnal variation of the mesured terpenes in Uludağ. These results are given in Table 3.16. and Figure 3.18. below.

Table. 3.16. Variation of terpenes during daily sampling periods.

	Concentration of terpene (ppbv)		
Parameter	**Morning (07:00-11:00)**	**Afternoon (12:00-16:00)**	**Evening (17:00-21:00)**
α-*Pinene*	0.359	0.799	0.256
β-*Pinene*	0.168	0.229	0.107
Camphene	0.113	-	0.109
d-Limonene	0.148	0.287	0.219

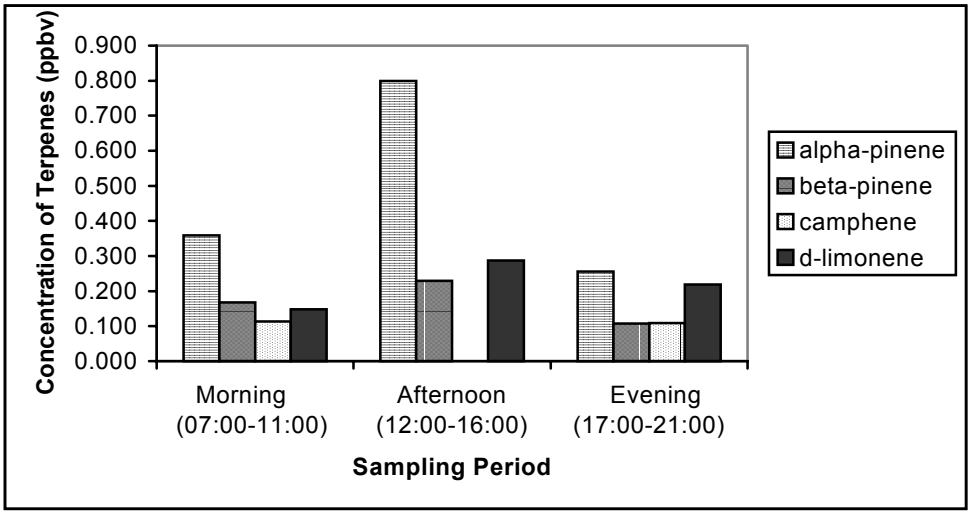


Figure 3.18. Diurnal variation of terpenes measured at Uludağ.

As can be seen from this figure, each terpene has its high concentration in the period of the day with high solar flux and temperature. The concentrations of α-pinene and β-pinene were maximum at noon time, and the following highest concentration was observed in the morning period. This observation was very striking for α-pinene. Its noon time concentration was almost a factor of four higher than its average concentration. This could be because of more dependency

of α-pinene on photochemical production compared to other terpenes (Owen S. M. *et al.,* 2002). D-limonene concentration was also maximum at noon time, and the following highest concentration belongs to evening period, which still includes sunny hours. These data are in accordance with expectations since the direct dependency of the terpenes on light and temperature was observed by several other researchers (see section 1.3.3.1.).

3.7.3. Behaviour of Ozone during Sampling Campaign

In this section the measurements of ozone and NO_x performed during the sampling campaign is discussed. As it was mentioned in the previous chapters these parameters are measured using commercial monitoring devices. Ozone measurements in Uludağ Mountain has been done on and off since from 1993. The of O_3 and its relation with NO_x has been published before (Tuncel S. G. *et al.,* 1994).

In this study the O_3 is measured for examination of its relation with terpenes. Ozone showed its expected behaviour throughout this sampling campaign too. Very strong correlation of ozone with temperature, which in turn could mean solar flux, was very obvious (Figures 3.19. and 3.20.). This is a direct indication of photochemical production.

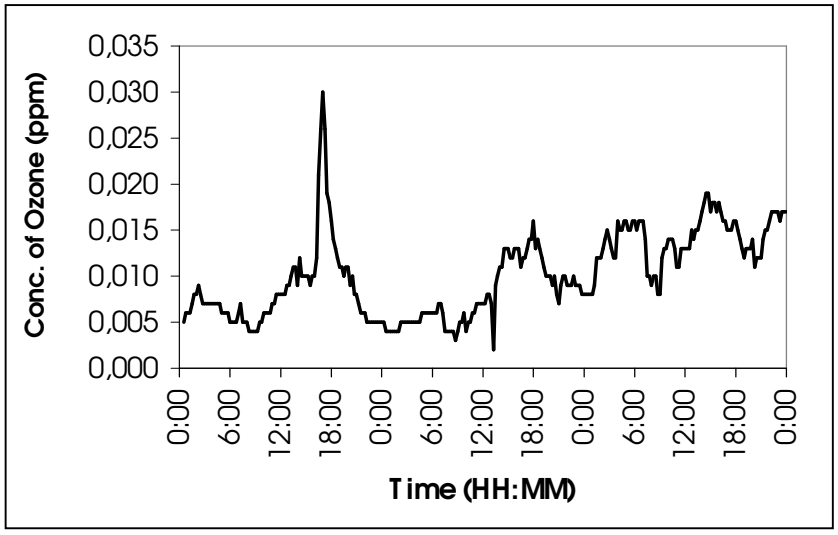


Figure 3.19. The ozone profile during BVOC sampling campaign.

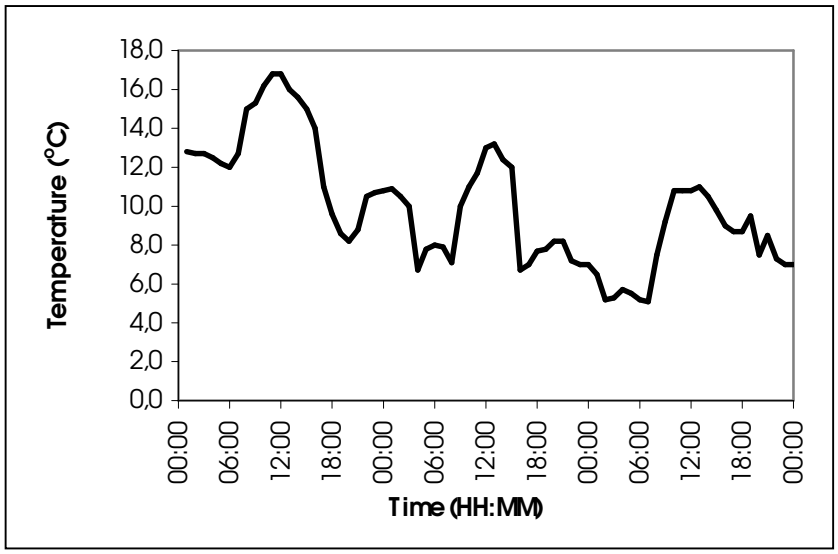


Figure 3.20. Temperature profile during BVOC sampling campaign.

This finding is also supported by the Figure 3.21., which gives the diurnal variation of O_3.

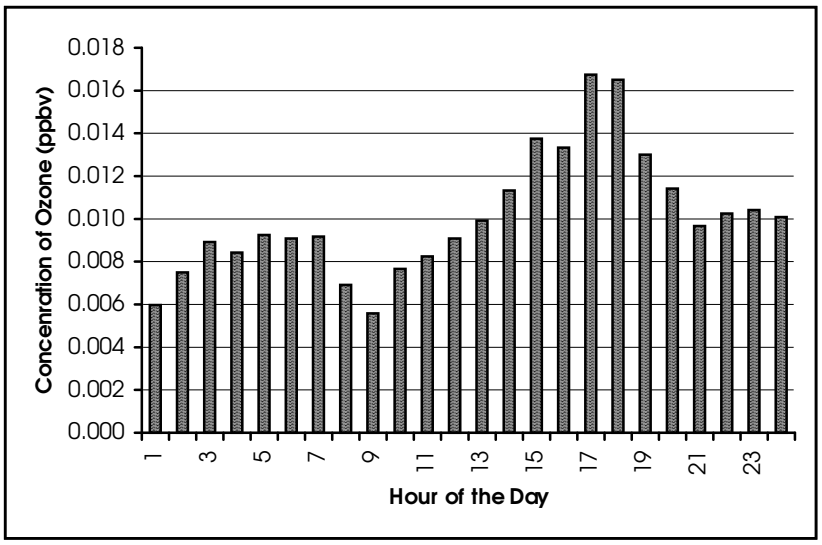


Figure 3.21. Diurnal variation of ozone.

In Figure 3.21. the concentration values represent the avarages of the ozone concentrations during the sampling campaign. The typical behaviour of ozone indicates the photochemical production of the ozone. A morning and afternoon maxima is typical for photochemical O_3 production and this is clearly seen in Figure 3.21.

3.7.4. Relation of Ozone to Terpenes

It is well known in the scientific community that O_3 and BVOC are affecting each other (see section 1.4.4.). We also aimed to look at the relation in between BVOCs and O_3 but unfortunately we did not have enough number of samples to look at the behaviour of O_3 and terpenes in detail.

Figure 3.22. below shows the diurnal variation of terpenes with ozone.

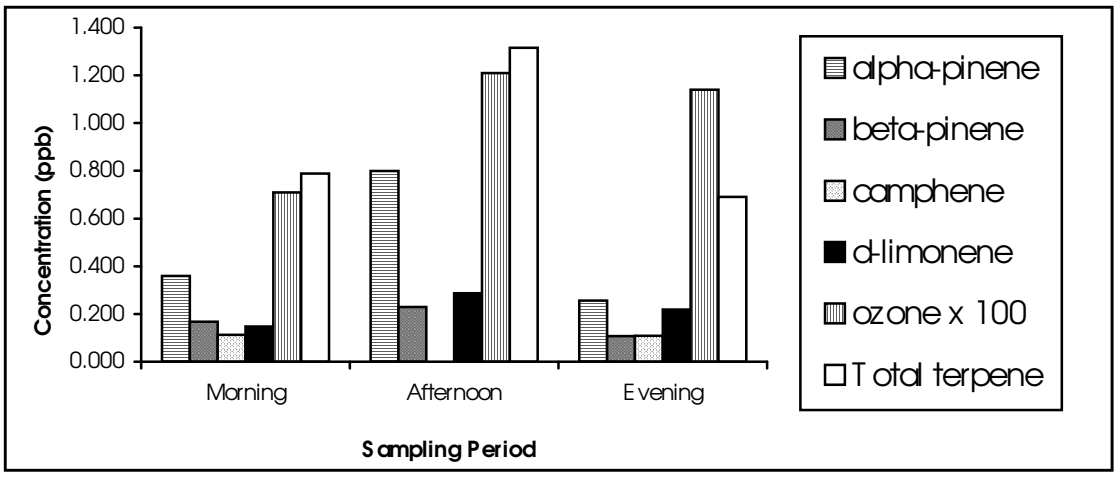


Figure 3.22. Diurnal variation of terpenes and ozone.

As can be seen from this figure, the total terpene concentration followed similar pattern with ozone. Highest concentration at noon time indicates a contribution to the photochemical ozone production by monoterpenes.

Day to day changes in terpene concentrations with ozone is given in Figure 3.23. below.

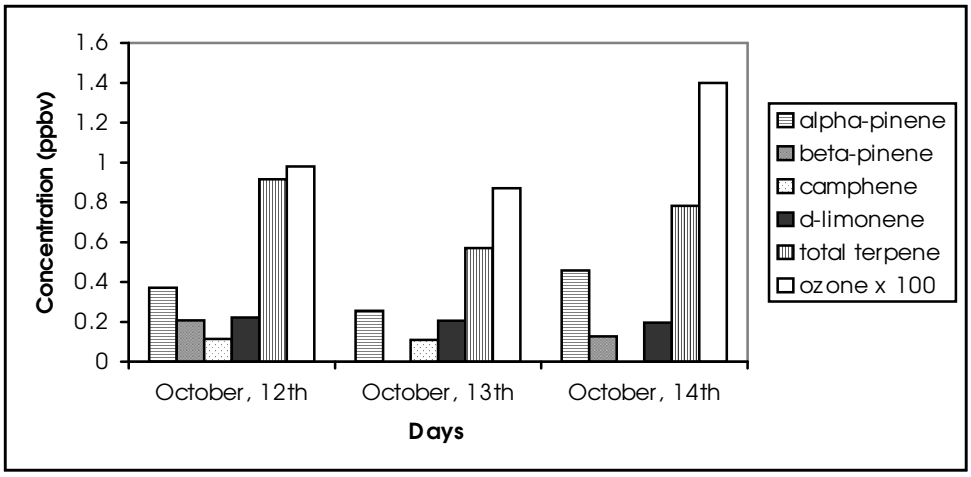


Figure 3.23. Daily variation of terpenes and ozone.

Figure 3.23 clearly indicates that α-pinene and β-pinene show the same daily variation with O_3. They have lower concentrations on Oct. 13th and highest on Oct. 14th. When we looked at meteorological data in these specific days the major difference was observed for Oct 14th , which was the day of highest relative humidity. The effect of humidity on speeding up the monoterpene production was also observed by other researchers (Reissel A. *et al.,* 2003).

3.7.5. Results of NO_x Measurements

NO and NO_2 (NO_x) were measured simultaneously during the sampling campaign to investigate the relationship between the BVOCs and NO_x. Figure 3.24. and 3.25 show the diurnal variation of NO and NO_2. The data points are the hourly averages of days of sampling campaign.

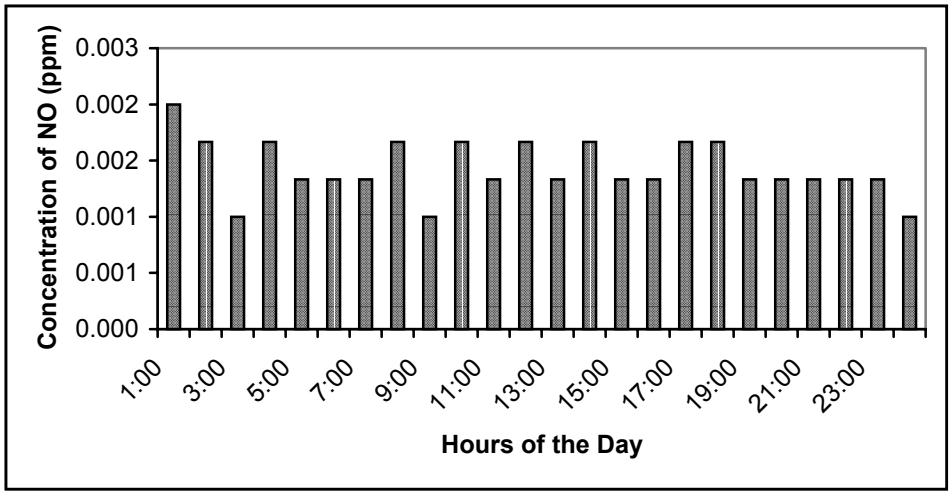


Figure 3.24. Hourly averages of NO (ppm) measured on Uludağ (October 12-14)

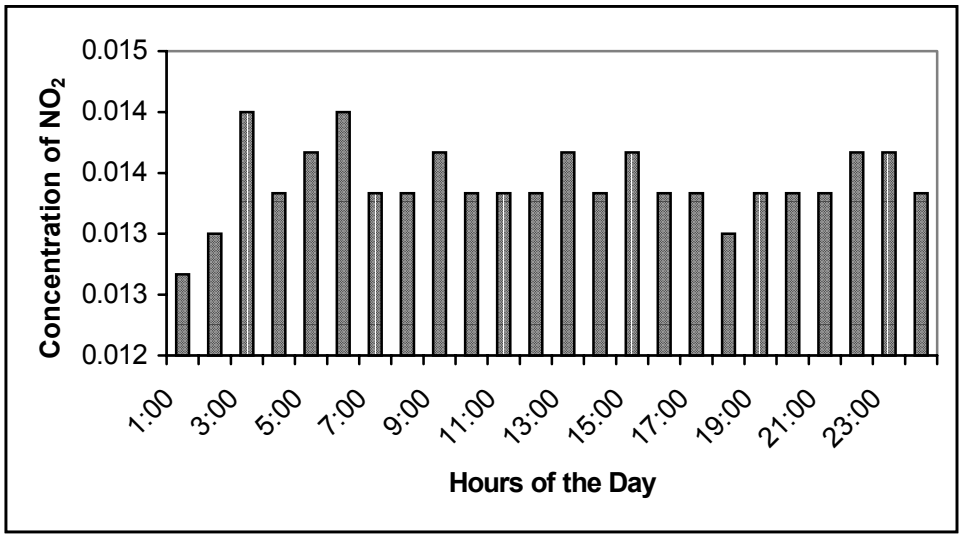


Figure 3.25. Hourly averages of NO (ppm) measured on Uludağ (October 12-14)

From these figures one can easily see that no diurnal variation was observed for NO and NO_2. The concentrations represented a considerably constant behaviour through out Oct. 12-14, the days, which are covering the quantitative measurements of monoterpenes.

Figure 3.26. shows daily variation of NO and NO_2 with terpenes.

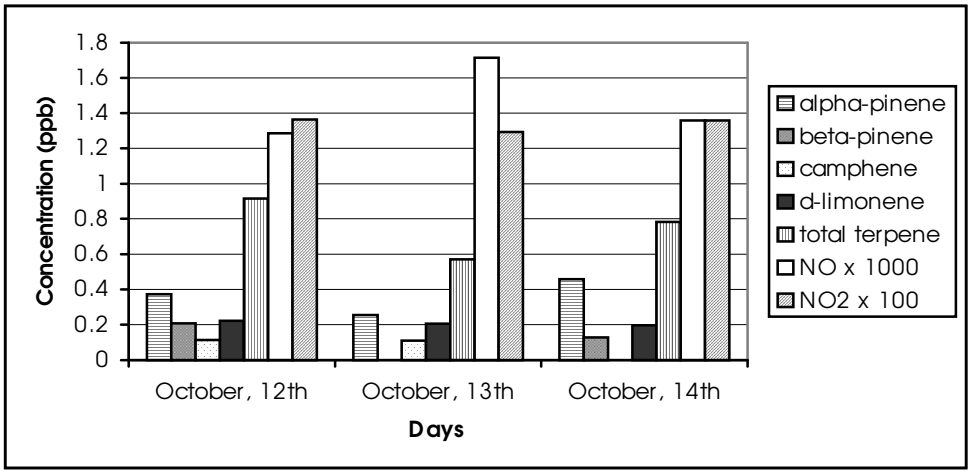


Figure 3.26. Daily variation of NO and NO_2 with terpenes

No clear relation was observed in between NO, NO_2, and terpenes. These are very preliminary observations. More detailed interpretations require more number of samples.

As can be seen from these figures NO and NO_2 do not show concentration change from one day to another. But NO_2 shows diurnal variation like O_3. The concentrations represented a considerably constant behaviour through out October 12-14, the days including the quantitative mesurements of monoterpenes. We have not observed noteworthy variations in the concentrations of the parameters, therefore it was not possible to state any relation between BVOCs and NO_x.

CHAPTER 4

CONCLUSION

Applied sampling strategy and sampling system for collection of monoterpenes by means of active sampling onto sorbent tubes was successful at Uludağ mountain. Tenax® TA and Carbopack™ B used in conjunction were found to be suitable for sampling.

The optimum operation conditions for thermal desorption unit was studied and applied to real samples. The cryo-trap efficiency can be improved slightly by using liquid nitrogen instead of carbon dioxide.

Several monoterpenes were studied including α-pinene, camphene, β-pinene, d-limonene, γ-terpinene, linalool, 1-isopulegol, 1-borneol, dl-menthol, alpha-terpineol, dihydrocarveol, citronellol, pulegone, and geraniol. Internal standard calibration was employed by using fenchone. Calibrations with high correlation coefficients and detection limits of sub-ppb levels were obtained for the species. %RSD values were within 10% except for citronellol and pulegone.

α-pinene, β-pinene, camphene, and d-limonene were determined quantitatively. α-pinene was found to be the most abundant terpene at Uludağ mountain. Terpene emission was higher at mid-day and humid days.

Simultaneous measurements of O_3, and NO_x (NO and NO_2), and meteorological parameters were performed. Ozone showed strong diurnal variations which indicates photochemical production of this pollutant. Similar daily and diurnal behaviours between terpenes and ozone were observed which implies BVOCs contribute to the photochemical ozone production in Uludağ mountain. With the current data, no consistent relation was observed in between terpenes and NO_x. More data is required for detailed investigation of the relation between terpenes and NO_x. among the meteorological parameters temperature and humidity had direct effect on the production of terpenes.

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