# NON-ENANTIOSELECTIVE AND ENANTIOSELECTIVE DETERMINATION OF MICROBIAL VOLATILE ORGANIC COMPOUNDS AS TRACER FOR HUMAN EXPOSURE TO MOULD GROWTH IN BUILDINGS

**Inauguraldissertation**

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

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von

**Sven Heekmann**

aus Orsoy am Niederrhein (Deutschland)

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Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
auf Antrag von

Prof. Dr. Michael Oehme
Prof. Dr. Gabriele Sabbioni

Basel, den 06. Juni 2006

Prof. Dr. Hans-Jakob Wirz
Dekan

This work is dedicated
to my parents

*«Das sogenannte naturwissenschaftliche Wissen ist kein Wissen, denn es besteht nur aus Vermutungen oder Hypothesen – wenn auch zum Teil aus Hypothesen, die durch ein Kreuzfeuer von genialen Überprüfungen hindurchgegangen sind.*

*Wir wissen nicht, sondern wir raten. Obwohl das naturwissenschaftliche Wissen kein Wissen ist, ist es das Beste, das wir auf diesem Gebiet haben. Ich nenne es Vermutungswissen – mehr oder weniger, um die Leute zu trösten, die sicheres Wissen wollen und glauben, es nicht entbehren zu können.»*

*zur Theorie der Wissenschaft aus „Logik der Forschung" (1934)*
*Sir Prof. Dr. Karl Raimund Popper (✳Wien 1902, †London 1994)*
*österreichisch-britischer Philosoph und Wissenschaftstheoretiker*

The presented thesis was carried out under supervision of Prof. Dr. M. Oehme in the Organic Analytical Laboratories of the Department of Chemistry at University of Basel, Basel, Switzerland.

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# Table of contents

# Abbreviations and Definitions

| | |
|---|---|
| AR | acquisition rate |
| $a_w$ | water activity |
| BHT | butylated hydroxytoluene = 2,6-di-*tert*.-butyl-4-methylphenol |
| bp | boiling point |
| BP | basis peak |
| BRN | Beilstein registry number |
| CAS | registry number of the Chemical Abstracts Service |
| CD | cyclodextrin |
| CFU | colony forming unit |
| $cR_S$ | chiral resolution |
| CSP | chiral stationary phase |
| CV | coefficient of variation |
| DE | desorption efficiency |
| ee | enantiomeric excess |
| EI | electron ionization |
| FAME | fatty acid methylesters |
| FID | flame ionization detector |
| GC | gas chromatography or gas chromatograph |
| HRGC | high resolution gas chromatography |
| HVAC | heating, ventilation, and air conditioning system |
| IAQ | indoor air quality |
| ID | inner diameter |
| ISTD | internal standard |
| LOD | limit of detection |
| LOQ | limit of quantification |
| *m/z* | mass-to-charge ratio |
| MCS | multiple chemical sensitivity |
| MEA | malt extract agar |
| mesh | particle size by the U.S. Bureau of Standards |
| MP | molecular peak |
| mp | melting point |
| MS | mass spectrometry |
| MSD | mass selective detection or mass selective detector |
| $M_W$ | molecular weight |
| $M_{W,isotopic}$ | isotopic molecular weight |
| OCIA | organic compounds in indoor air |
| OD | outer diameter |
| PEG | polyethylene glycol |
| POM | particulate organic matter |
| RC | recovery coefficient |

| | |
|---|---|
| RRT | relative retention time (definition in the appendix 8.5, pg. 168) |
| RSD | relative standard deviation |
| RSTD | recovery standard |
| RT | retention time |
| S/N | signal-to-noise ratio |
| SBS | sick-building syndrome |
| SIM | single ion monitoring |
| SR | sampling rate |
| SVOC | semivolatile organic compound |
| t.-o. conc. | threshold-odour concentration |
| TVOC | total volatile organic compound |
| TWA | time weighted average |
| TZ | Trennzahl |
| USP | united states pharmacopoeia |
| v/v | volume per volume (ratio of mixtures of solution in per cent) |
| VOC | volatile organic compound |
| VVOC | very volatile organic compound |
| w/w | weight per weight (ratio of mixtures off solution in per cent) |
| %RSD | relative standard deviation in per cent |
| * | asterisk to mark chiral compounds |
| N.N. | name hitherto unknown (lat. *nomen nominandum*) |

# Organizations

| | |
|---|---|
| ACS | American Chemical Society Committee on Analytical Reagents (Washington, DC, USA) |
| BIA | Berufsgenossenschaftliches Institut für Arbeitsschutz (St. Augustin, Germany) |
| BRE | Building Research Establishment of the British Department of the Environment (Watford, Great Britain) |
| CAS | Chemical Abstracts Service (Columbus, OH, USA) |
| CBS | Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) |
| CEN | European Committee for Standardization (Brussels, Belgium) |
| DOE | British Department of the Environment (London, Great Britain) |
| ECA | European Collaborative Action working group of the European Community (Luxembourg, Luxembourg) |
| ECA-IAQ | European Collaborative Action of Indoor Air Quality and Its Impact on Man (Luxembourg, Luxembourg) |
| EPA | United States Environmental Protection Agency (Washington, DC, USA) |
| HSE | United Kingdom Heath and Safty Executive (Merseyside, Great Britain) |
| KRdL | Kommission Reinhaltung der Luft im VDI und DIN - Normenausschuss (Düsseldorf, Germany) |
| LGA BW | Landesgesundheitsamt Baden-Württemberg (Stuttgart, Germany) |
| NIOSH | United States National Institute for Occupational Safety and Health (Cincinnati, OH, USA) |
| OSHA | United States Occupational Safety and Health Administration (Washington, DC, USA) |
| UBA | Umweltbundesamt (Berlin, Germany) |
| VDI | Verein Deutscher Ingenieure e.V. (Düsseldorf, Germany) |
| WHO | World Health Organization (Geneva, Switzerland) |

# Summary

In the industrialized countries people spend 80 to 90 % of their time in indoor areas. About 5 % of the population are known to be sensitive or allergic to the more than 200 mould species that are found in the indoor environment today.

Identification of mould attacks in the indoor environment, however, is difficult and generally performed by sampling and counting spores and conidia. These procedures are highly dependent on seasonal and environmental parameters.

The chemical analysis of indoor mould mainly focuses on microbial volatile organic compounds (MVOC) in the ambient air. These compounds are present during all stages of the fungal life cycle, are able to penetrate weak barriers (e.g. wallpaper), and can distribute into all regions of the indoor environment. The most commonly reported MVOCs are hydrocarbons (e.g. octane), alcohols (e.g. 2-methyl-1-butanol), aldehydes and ketones (e.g. octan-3-one), esters (e.g. ethyl acetate), ethers and furans (e.g. 2-methylfuran), terpenes and terpene derivatives (e.g. geosmin), nitrogen and sulphur compounds (e.g. pyridine and dimethyl disulfide). A few MVOCs can act as universal fungal signature compounds and sum up to a characteristic marker pattern.

In this work a method was developed to detect mould within a building by tracing and quantifying selected MVOCs in the indoor air. The method was supposed to be applicable at any time and independent of the fungal life cycle. 22 characteristic compounds were chosen as reference compounds to indicate mould contamination even when other signs of microbial growth could not be detected.

Sampling was performed by passive sampling to profit from the advantages above active sampling as simplicity (of field operation), low cost, no need for expensive or complicated equipment, no power requirement, unattended operation, and time-weighted averaged (TWA) concentration of the analyte to gain a representative overview of the sampling site. The passive sampler used throughout this work was the 3M organic vapour monitor (OVM) 3500. This badge-type sampler is a combination of a diffusion and a permeation sampler with an activated charcoal adsorbent and an average sampling rate of 30 mL $min^{-1}$. A sampling period of 28 days was chosen to ensure sufficient detection limits for the trace components. Solvent desorption was performed with diethyl ether.

Separation of the selected 22 MVOCs was carried out by conventional non-enantioselective and enantioselective high resolution gas chromatography (HRGC). The detection was performed by mass spectrometry (MS), but for method development also a flame ionization detector (FID) was applied. The detection of the trace concentration required the selected ion monitoring (SIM) mode of the MS.

Cold on-column injection showed to be most suitable for analyzing the MVOC ether solution. The non-vaporizing technique reduced the thermal stress of the analytes and ensured a quantitative transfer of the solutes into the column. A routinely installed retention gap was used to achieve optimal focussing of the compounds within the capillary column.

Best non-enantioselective chromatographic separation was obtained by a 30 m long DB-Wax capillary (0.25 mm inner diameter and 0.25 µm film thickness) combined with a 30 min temperature program.

The enantioselective analysis of the MVOCs should allow the conclusive differentiation between naturally occurring MVOCs and synthetic/anthropogenic racemates. 11 enantioselective columns from 4 manufacturers were tested with different stationary phases and/or different combinations of chiral selector and polysiloxane solvent. The best enantioselective separation was achieved with the BGB-174, a heptakis-(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin dissolved in BGB-1701 (14 % cyanopropylphenyl 86 % dimethyl polysiloxane). It was able to resolve 13 of the 14 monitored chiral MVOCs.

Quantification of the target compounds accumulated on the passive sampler was performed using the internal standard method. Of the evaluated standards 1-chlorohexane was best suitable as internal standard (ISTD) and chlorocyclohexane as recovery standard (RSTD). The utilized non-enantioselective GC-MS method was validated as a reliable semiquantitative method for trace analysis of MVOCs. It was linear over a concentration range of 0.01 to 5 ng $\mu L^{-1}$ with a coefficient of determination ($r^2$) between 0.96 and 0.99. The recovery rate ranged from 40 to 127 % for the majority of compounds. The between-runs precision was 2 to 7 %. The limit of detection (LOD) was 1 to 86 pg $\mu L^{-1}$ (S/N-ratio 3:1) and the limit of quantification (LOQ) was 2 to 286 pg $\mu L^{-1}$ (S/N-ratio 10:1).

The developed method as described above was successfully able to detect possible fungal contamination on a real case site. 16 of the 22 MVOC compounds contributed to the analytical fingerprint pattern and indicated possible fungal contamination. However, some problems remained. The VOC burden of the indoor air was tremendously high and showed a great variety of compounds that interfered with the MVOC detection. Furthermore, the enantioselective GC-MS analysis of the MVOCs in indoor air was unusable for the given concentration range. The sensitivity of the enantioselective method was too low to

unequivocal differentiate the source of the detected compounds. These problems should be investigated further before enantioselective GC-MS analysis in combination with passive sampling of indoor air can become a reliable, easy, and cheap way of detecting indoor mould.

# 1 INTRODUCTION

> *"All of us face a variety of risks to our health as we go about our day-to-day lives. Driving in cars, flying in planes, engaging in recreational activities, and being exposed to environmental pollutants all pose varying degrees of risk. Some risks are simply unavoidable. Some we choose to accept because to do otherwise would restrict our ability to lead our lives the way we want. And some are risks we might decide to avoid if we had the opportunity to make informed choices. Indoor air pollution is one risk that you can do something about."*
>
> *(EPA, 1995)*

## 1.1 Indoor Air

### 1.1.1 Indoor air quality

In the course of evolution man has adapted himself to fresh, oxygen-rich ambient air and consumes about 20,000 L a day. Only since the last centuries human beings have developed a need to feel comfortable into an indoor living ambient (Frössel, 2003). In 1972 the comparative time budget research project tabulated data on 25,000 people in 12 countries about human activities throughout the day (Szalai, 1972). This sociological study was reinterpreted by Ott (Ott, 1989). He concluded that average employed persons spend 92 % of their time indoors, 6 % of their time in transit, and only about 2 % of their time outdoors (Klepeis et al., 1999).

As a result of the energy crisis in 1973/74 and 1978/79, changes in building design devised to improve energy efficiency. Homes and offices became more airtight then and advances in construction technology led to a much greater use of synthetic building materials. In addition to more comfortable buildings with lower running costs, these changes led to an increased

accumulation of emitted substances, sometimes exceeding the levels found outside in urban areas.

Today, the understanding of and the emphasis on the relationship between indoor air pollution and health is growing. Over the last 30 to 40 years the prevalence of allergic and other hypersensitivity conditions have increased, particularly in industrialized countries. The reasons for this phenomenon are not clear. Because the increase has been much too rapid to be explained by genetic changes, there is growing concern about the role of environmental factors, including indoor air quality (IAQ) (Nilsson et al., 2003).

IAQ can be understood in terms of physical, chemical, and biological characteristics of indoor air, that all can affect the comfort and health of the occupants. Indoor pollutants can emanate from a wide range of sources, including a) combustion sources such as oil, gas, kerosene, coal, wood, and tobacco products; b) building materials and furnishings as diverse as deteriorated asbestos-containing insulation, wet or damp carpet, and cabinetry or furniture made of certain pressed wood products; c) products for household cleaning and maintenance, personal care, or hobbies; d) central heating, cooling systems, and humidification devices; e) outdoor sources such as radon, pesticides, and outdoor air pollution (EPA, 1995; Nathanson, 1995) (Fig. 1.1).

Criteria for acceptable air quality have existed for many years for the industrial workplace and outdoor environments. However, data for health risk assessment of long term personal exposure at home is lacking. The relationship between ambient background concentrations and personal exposure is often unknown and needs future investigation.

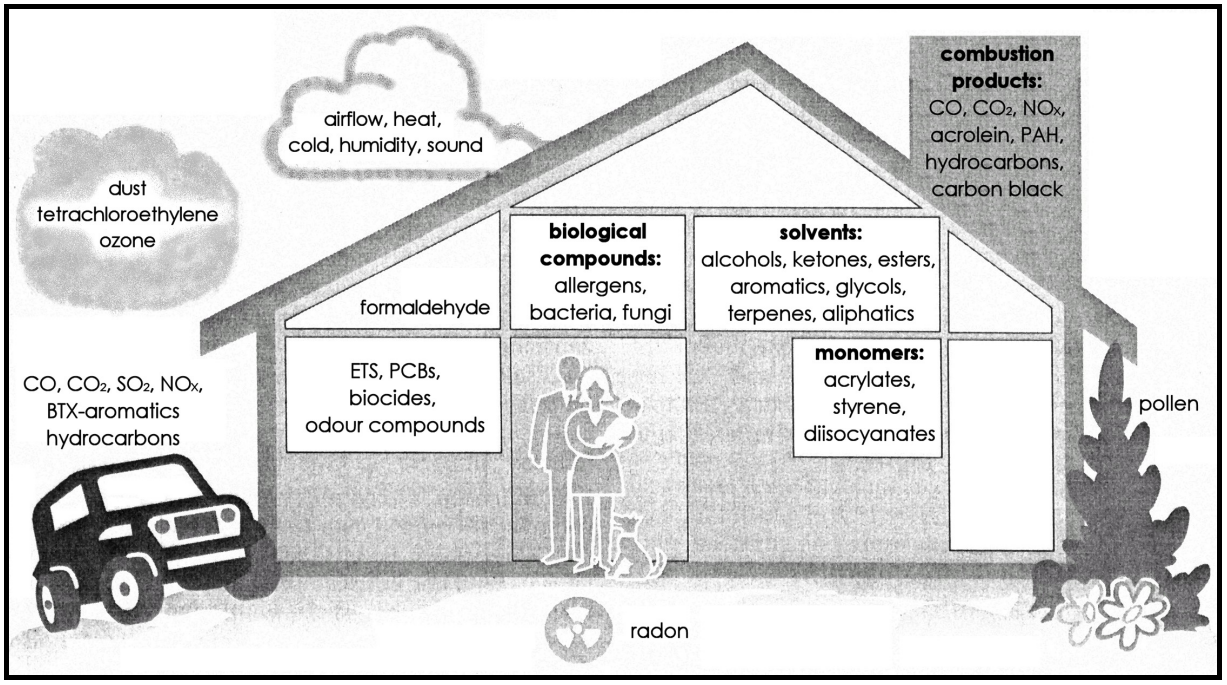



**Fig. 1.1: Indoor air pollutants and their sources (Salthammer, 1999) (ETS: environmental tobacco smoke; PCB: polychlorinated biphenyls; PAH: polycyclic aromatic hydrocarbons; BTX: benzene, toluene, xylene)**

### 1.1.2 Indoor related illnesses

In April 1979, the World Health Organization (WHO) convened a meeting of experts to discuss the health aspects of IAQ. The WHO defines "health" as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (WHO (World Health Organisation), 1948). The experts recommended that health authorities should draw up guidelines for indoor concentrations of air contaminants to protect the health of occupants of homes (Davies et al., 1995).

In 1982 the WHO invited another international working group to review indoor climate research (WHO, 1982). Here the key components of the indoor environment were summarized as air quality, thermal climate, noise, and illumination. The WHO group stated that more than 30 % of all new buildings seems to be affected by indoor climate problems. The major complains were: a) sensory irritation of eyes, nose, and throat; b) neurogenic or

general health symptoms; c) skin irritation and erythema of the skin; d) unspecific hypersensitivity reactions; e) mental fatigue; and f) odour and taste symptoms. These unspecific effects or symptoms often appear in combination.

In the same year the WHO (1982) established the term Sick Building Syndrome (SBS) to describe symptoms that often appear together indoors. Occupants of a building complain that the building is causing them one or more physical problems, mainly discomfort or the feeling of being less than well. The symptoms occur shortly after entering the building, progress while the occupants are inside and cease shortly after leaving. The syndrome includes symptoms and dissatisfaction that occurs for the majority of the occupants in a particular building or part of it. The complaints are related to a reduction of indoor air quality that is apparently linked to the time the occupants spend in the building. Because they cannot be related to any obvious exposure factors, no single cause have been recognized for SBS (Lindvall, 1992; Mølhave, 2003; Seduikyte and Bliudžius, 2003).

In the last several years many studies have been carried out to describe the symptoms and relate them to specific chemical, particulate and/or biological exposures (Fig. 1.2). The National Institute of Occupational Safety and Health (NIOSH) conducted a large number of investigations in offices, schools and other public buildings. The most prevalent problem reported was inadequate ventilation (nearly 50 %). Other problems included humidity, building fabric contamination, hypersensitivity pneumonitis, and cigarette smoke (Levin, 1985).

The decrease of IAQ within homes and other buildings can mainly be correlated to the modern energy efficient and airtight buildings. Inadequate ventilation results in high loads of

organic indoor air pollutants and elevate moisture over long periods of time. An association between dampness in buildings and health effects was recently confirmed in an extensive review of epidemiological data (Bornehag et al., 2001).

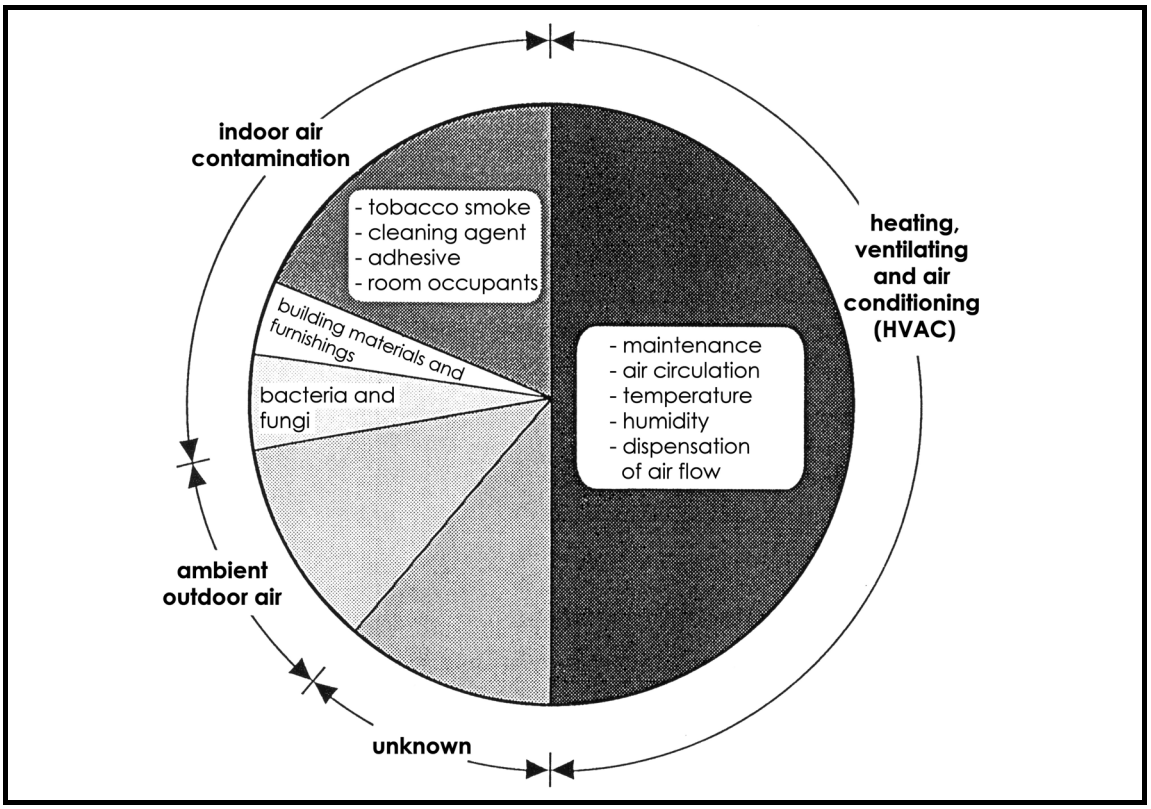



**Fig. 1.2: Reasons for the sick building syndrome assessed out of a study with 350 U.S. buildings (Seidel, 1998; Seifert, 1991)**

### 1.1.3 Volatile organic compounds

The range of possible indoor air pollutants is wide (Fig. 1.1). Types of pollutants that have been determined indoors have changed over the years depending on evolution of new product formulation, product design, and use patterns (Papameletiou, 2003). The major sources of organic indoor air pollution can be divided into the following categories (Salthammer, 1999; Seifert, 1992): a) man and his activities; b) metabolic product of humans, animals, plants, and microorganisms; c) emission of materials and equipment; and d) indoor/outdoor air exchange.

Organic compounds in indoor air (OCIA) (Fig. 1.3) are present in all of the above categories and are diverse. OCIAs as concept for indoor air pollution include gaseous organic compounds, particle bound organic compounds, as well as intermediary species (e.g., organic radicals), and ionic species. This broad definition includes also all biologically relevant organic compounds, non-proteins, non-glucans, etc, (i.e., organic compounds tentatively with molecular weights less than 500–1000 Da) in the indoor environment (Wolkoff and Nielsen, 2001). Although most of these OCIA occur in concentrations near the detection threshold, they include potent irritants and toxins (WHO (World Health Organisation), 1989).

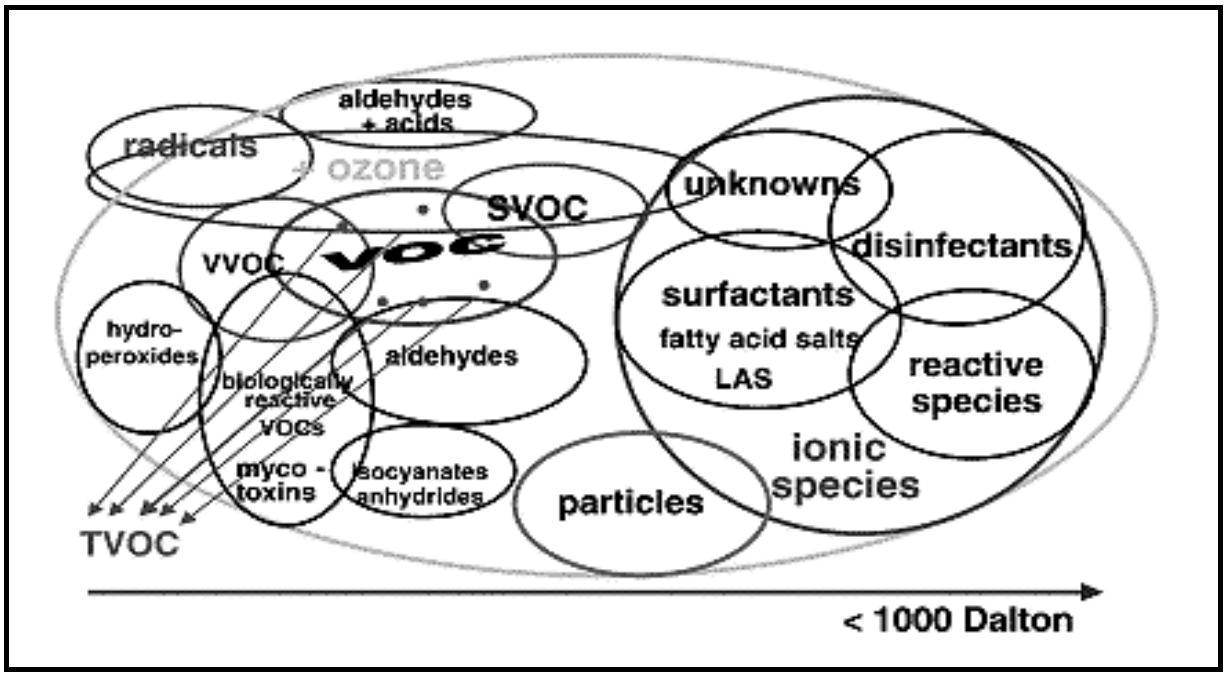



**Fig. 1.3: Schematic presentation of the organic compounds in indoor air (Wolkoff and Nielsen, 2001). (VVOC: volatile organic compound, VOC: volatile organic compound, SVOC: semivolatile organic compound, POM: particulate organic matter, TVOC: total volatile organic compound, LAS: linear alkyl benzene sulfonates)**

A four categories classification of organic air pollutants was given by a WHO working group on IAQ in 1989 (Tab. 1.1). The discrimination between very volatile organic compounds (VVOCs), volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs) and particulate organic matters (POMs) was done according to boiling point ranges. Boiling

points correlate fairly well with the volatility, although no sharp limits exist between the four categories. Also the polarities had a substantial influence on the classification and the VOC class was therefore subdivided into less polar compounds with boiling points between 50 and 100 °C and polar compounds with boiling points between 240 and 260 °C (Mølhave, 1999; WHO (World Health Organisation), 1989). Class intervals were chosen for reasons of sampling and analytical capabilities rather than for health effects (Wolkoff and Nielsen, 2001).

**Tab. 1.1: Classification of indoor organic pollutants according to the WHO (WHO (World Health Organisation), 1989; Zielinska and Fujita, 1994)**

| description | abbreviation | boiling point range (° C) | saturated vapour pressure (Pa) at 25 °C |
|---|---|---|---|
| very volatile (gaseous) organic compounds | VVOC | < 0 to 50…100 | |
| volatile organic compounds | VOC | 50…100 to 240…260 | > 13 |
| semivolatile organic compounds | SVOC | 240…260 to 380…400 | 13…1.3 $10^{-5}$ |
| organic compounds associated with particulate matter or particulate organic matter | POM | > 380 | < 1.3 $10^{-5}$ |

Several thousands of chemicals of anthropogenic and biogenic origin have been identified as VOC. Due to the tremendous diversity and large variety of sources, our knowledge of VOCs is still incomplete. Most of the ambient VOCs are present at air concentrations ranging from ng $m^{-3}$ to a few mg $m^{-3}$. Over 900 of these have been identified in indoor air, with over 250 recorded at concentrations higher than 1 ppb (= µg $m^{-3}$) (Knobloch et al., 1997; Seduikyte and Bliudžius, 2003).

Several field studies throughout the industrialized world (Europe, North America, Australia) have been made to characterize air in closed spaces and to establish the most ubiquitous VOCs (Tab. 1.2).

**Tab. 1.2: Most abundant VOCs in indoor air measured in several European, North American, and Australian field studies (in alphabetic order) (Wolkoff and Nielsen, 2001)**

| | | |
|---|---|---|
| acetone[+] | ethanol[+] | octane |
| alkanes[+] (C7-; C10-; C11-; C12-) | ethyl benzene[+] | p-dichlorobenzene |
| benzene | heptane | phenoxyethanol |
| 2-butanone[+] | hexane | α-, β-pinene[+] |
| 3-carene | isoprene | 2-propanol[+] |
| butoxyethoxyethanol | limonene[+] | styrene |
| butoxypropanol | longifolene | tetrachloroethylene |
| butyldiglycol acetate | methyl cyclohexane | toluene |
| camphene[+] | methylene chloride | trichloroethylene |
| decane | 2-methylbenzene | trichlorofluoromethane |
| dichlorobenzenes | 2-methylhexane | 1,2,4-trimethylbenzene |
| dichlorofluoromethane | 2-methylpentane | undecane |
| dimethyl phthalate | n-nonyl aldehyde | o-, m-, p-xylenes |
| dodecane | nonane | |

[+] These compounds have be correlated to mould emissions (see Tab. 1.3)

Because the identification and measurement of individual VOCs were expensive and time consuming, Mølhave and his working group (Mølhave, 1999) as well as the U.S. Environmental Protection Agency (EPA) developed an indicator concept called total volatile organic compound (TVOC) since 1986. Here a single value represents a mixture of many VOCs as a summation of some or rarely all of the detected compounds present. In 1997 a working group by the European Collaborative Action of Indoor Air Quality (ECA-IAQ) proposed the technical definition of TVOC as a sum of VOC concentrations (in mg $m^{-3}$) within the VOC gas chromatographic window between n-hexane and n-hexadecane (Berglund et al., 1997; Seifert, 1999). The ECA-IAQ working group pointed out, that the TVOC indicator is a good screening method to give a first estimation for VOC exposure and IAQ. However, they also clearly stated, that no documented relationship exists between indoor TVOC concentrations and occupant health (Wolkoff and Nielsen, 2001), and that this average

VOC concentration may give an inaccurate indication of personal exposure. Two possible approaches for deriving IAQ guidelines for VOCs have been proposed by TVOC values. The approach by Mølhave (Mølhave, 1990) suggested 4 exposure ranges of increasing concentrations: a comfort range (< 0.2 mg $m^{-3}$) a multifactorial exposure range (0.2…3 mg $m^{-3}$), a discomfort range (3…25 mg $m^{-3}$), and a toxic range (> 25 mg $m^{-3}$). In the approach by Seifert (Seifert, 1992; Seifert, 1999) an upper TVOC concentration of 300 µg $m^{-3}$ was suggested which should not be exceeded (Berglund et al., 1997). Large scale studies of TVOC in indoor air in the U.K. (1996), Germany (1993), Sweden (1993), Denmark (1991), and the USA (1986) came up with average concentration of TVOC of about 200…500 µg $m^{-3}$ in air (Jones, 1999).

## 1.2 Mould

Mould, bacteria, and other microorganisms have been identified by several studies as important contributors to the indoor air quality (Ebbehøj et al., 2002; Jovanovic et al., 2001; Stadler and Kennedy Jr, 1996). These microorganisms spread a wide variety of compounds during all stage of their life cycle, that might cause adverse health effects.

### 1.2.1 Mould in the indoor area

Mould spores can be found almost everywhere in the indoor and outdoor environment. However, they should not automatically be regarded as health thread as approximately one quarter of the biomass of the earth consist of fungi (Miller, 1990). The ideal habitat of mould is damp ground or concentrated nutrition media with a high humidity. Nevertheless, these decay organisms have the ability to grow under unfavourable ambient conditions and can often, as so called pioneer species, colonize at a time where no other microorganisms have been able to settle yet. Mould are highly adaptive to their environment, that allows their growth on virtually any substrate including glass, jet fuel, paint, rubber, textiles, electrical

equipment etc. (Miller, 1990). Even though they can survive extreme situations like the cold or high temperatures, they have individual optimum growth and breeding conditions (Tab. 1.3 and pg. 15).

**Tab. 1.3: Some charactering values of growth and breeding conditions of five selected mould species typically found in indoor air (Böck et al., 1998; Sedlbauer et al., 2002) (For details about water activity ($a_W$) see pg. 15)**

| mould species | tolerated temperature range (°C) | minimum water demand in terms of $a_w$ | tolerated pH values |
|---|---|---|---|
| *Aspergillus niger* | 6…47 | 0.88…0.96 | 1.5…9.8 |
| *Aspergillus versicolor* | 4…40 | 0.75…0.95 | |
| *Alternaria alternata* | -2…32 | 0.85…0.98 | 2.7…8 |
| *Cladosporium herbarum* | -3…35 | 0.85…0.95 | |
| *Penicillium expansum* | -7…32 | 0.82…0.95 | |

Since the energy crises in the 1970s the style of building construction and insulation changed and created a significant degradation of the air change rate. An ideal habitat for mould was created due to the increased use of household amenities such as washing machines and dishwashers, and the behaviour of the occupants.

The majority of mould enter a building through outdoor air intakes of the heating, ventilation, and air conditioning system (HVAC), through doors and windows, as contaminants on building materials and contents, and by people entering (Shelton et al., 2002). Airborne concentrations of mould spores in indoor environments vary with the amount of mechanical and/or human activity (Levetin, 1995).

Variation of fungal spore levels in general throughout the year is very high resulting in seasonally variable fungal intake rates. Spore concentrations in outdoor air peak during the moist weather in late summer or early fall. Lowest spore concentrations are observed in winter and spring (Shelton et al., 2002).

Several factors influence the growth and breeding of mould. A first decisive factor is the availability of free water. The availability of water on the surface of an object is dependent on the relative humidity and the temperature in the room, as well as the chemical composition and the pH value of the materials. It is described by the concept of water activity ($\mathbf{a_w}$) as a criterion of water availability to microorganisms (Scott, 1957). Microbial colonization of building materials is a dynamic process in which population composition changes in response to the $a_w$ of the materials. An $a_w$ value of above 0.75 involves a certain danger of mould growth.

Beside humidity, temperature is a second important factor for mould growth. Generally, mould are able to tolerate a wide range of temperatures, but have a well defined temperature optimum that typically lies in the temperature range of most indoor areas. Temperature preferences and requirements have led to a division of mould into three categories: a) Mesophilic mould is predominant indoor and has optimum mycelia growth between 25 °C and 35 °C. Minimum temperature for growth is about 0 °C and maximum temperature is 30 °C to 40 °C. b) Thermotolerant mould has a much wider acceptable temperature range between 0 °C and 55 °C. Their optimum mycelia growth is found between 30 °C and 40 °C. c) Thermophilic organisms are very rare in the indoor environment. Their minimum temperature for mycelia growth is around 20 °C to 25 °C, the optimum temperature is between 35 °C and 55 °C and the maximum temperature is between 55 °C and 60 °C (Reiß, 1998; Schwantes, 1996).

A third factor is the general low nutrient demand. Mould only needs some organic carbon compounds for their ingestion. Indoors organic carbon compounds can be found e.g. in carpets, upholstered furniture, soap films on shower walls, shower curtains, and other

bathroom fixtures, or wallpaper. Only small and water-soluble molecules (e.g. glucose, maltose, saccharose) are able to pass the cell wall of mould. Larger compounds such as proteins, lipids, starch, or pectin are generally first digested by excreted exoenzymes into amino- and fatty acids, before they pass the cell wall. The recalcitrant molecules cellulose and lignin can be barely digested. Like all organisms mould require trace amounts of elements like zinc, iron, cupper, molybdenum, boron, or manganese. Absence can lead to a retarded growth and to abnormalities of the conidia (Reiß, 1998).

A fourth factor affecting mould growth is the pH value of the colonized material. Most building materials are slightly alkaline. Although, mould prefer a lightly acidophilic environment ($4.5 < pH < 6.5$), they have the ability to excrete alkalic and/or acidic compounds in order to adjust the pH value of their surroundings to their own needs.

The composition of the surrounding atmospheric air is a fifth factor influencing the growth and breeding condition of mould. Although, mould have a low demand for molecular oxygen, most do need traces of oxygen for their metabolism. Carbon dioxide as another atmospheric gas, that is also produced by mould, can influence the metabolism and the mould growth velocity, too. High carbon dioxide levels and reduced oxygen content cause stress. This sometimes results in higher MVOC production and can even change the whole biosynthetic catabolism to an anaerobic fermentation (Reiß, 1998).

Solar radiation is a sixth factor, even so, mould are no heliotropic organisms and are able to grow in any direction. High solar radiation can retard growth by harming the fungal cell.

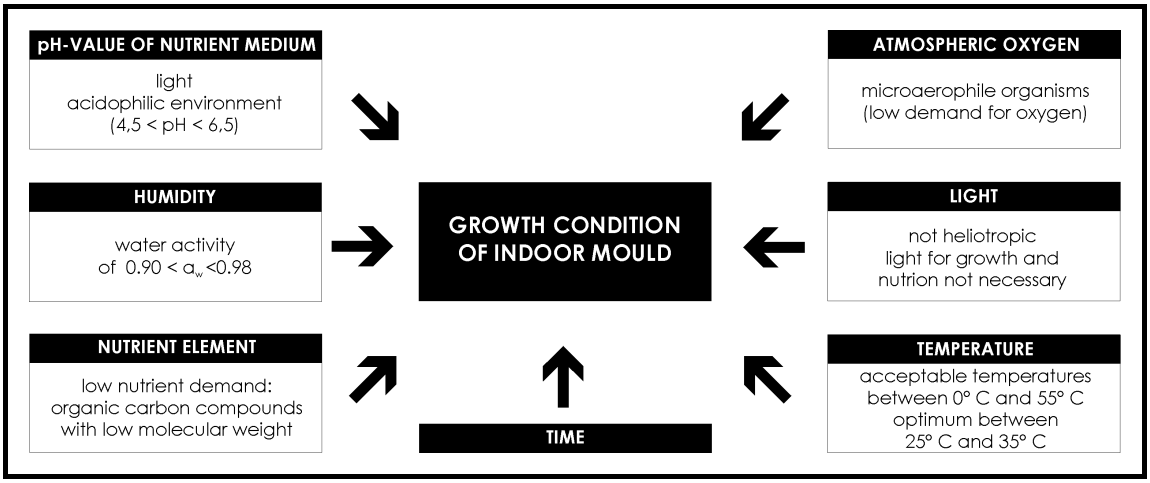



**Fig. 1.4: Optimal growth and breeding conditions for most indoor mould (Frössel, 2003).**

### 1.2.2 Impact of mould on human health

In a random survey that investigated 5,530 randomized habitations of houses in German, 21.9 % showed visible humidity damages and 9.3 % of indoor areas showed high profiles of mould contaminations (Brasche et al., 2003). The Danish research programme "Moulds in buildings, 1998–2002" pointed out that 15 to 20 % of Danish homes show visible signs of moisture or mould growth and 13 % of apartments had visible mould growth (By og Byg, 2003). In 1982 Sanders and Cornish of the Building Research Establishment (BRE) of the British Department of the Environment (DOE) estimated that 2 million dwellings (11.8 %) in England might have serious problems of dampness (Hunter et al., 1988). Miller et al. estimated in 2004 that between 10 and 30 % of homes in Canada have moisture and therefore mould problems (Miller, 2004).

In most public and private buildings, indoor concentrations of mould are lower than outdoor concentrations, and the species mix is similar. In consequence, the health risk of fungal exposure should not be increased indoors. However, conditions in some buildings promote the growth of mould resulting in higher indoor concentrations and/or a different species mix. In

several case reports such situations have been associated with adverse health effects such as allergies, infections, toxic effects, irritation and general symptoms, and have been correlated to the SBS (Davies et al., 1995; Nielsen, 2003).

The most common symptoms reported from exposures to fungal indoor environments are similar to SBS symptoms as like runny nose, eye irritation, cough, congestion, headache, aggravation of asthma and breathing (Environmental & Occupational Disease Epidemiology, 2002; Levetin, 1995) (compare chapter 1.1.2, pg. 7). Some symptoms are non-specific such as discomfort, inability to concentrate, and fatigue. These health problems observed in mouldy and damp buildings have be grouped into three major categories as listed in Tab. 1.4 (Nielsen, 2002).

**Tab. 1.4: Three major groups of health problems associated with mouldy and damp buildings (Nielsen, 2002).**

| general symptoms (incl. symptoms of the central nervous system) | mucosal symptoms | lung symptoms |
|---|---|---|
| • extreme fatigue | • blocked nose | • wheeze |
| • lack of concentration and memory, in extreme causes as cognitive impairment | • itching eyes<br>• burning sensation of the skin | • cough<br>• bronchitis |
| | • hoarseness | • asthma |
| • lowered immune function due to a misbalance in the lymphocytes subpopulations or chronic stimulation of some of these | • recurrent airway infections, especially sinusitis | • pulmonary hemosiderosis in infants |

### 1.2.3 Mould as source of microbial volatile organic compounds

Mould are important decomposers in most ecosystems and part of the nutrient cycle as they are depending on non-living organic material. They recycle carbon, nitrogen, and essential mineral nutrients.

Most mould are aerobic (heterotrophic) organisms, that depend to some extent on the presence of molecular oxygen. Carbohydrates, fats and amino acids serve as energy sources. The combustion of these nutrients is mainly done by the glycolysis, the pentose phosphate pathway, the Krebs citric acid cycle, and the respiratory chain (Frössel, 2003) (Fig. 1.5).

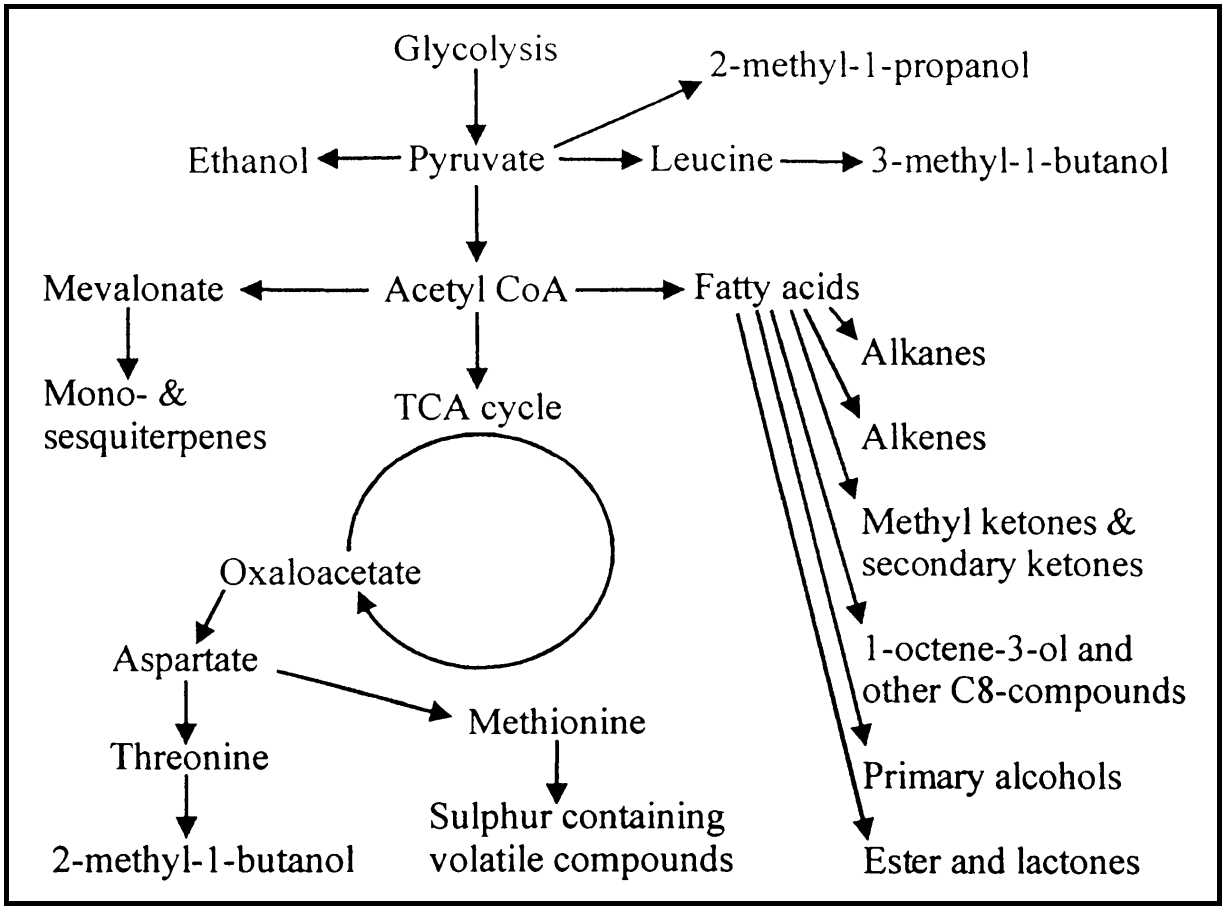



**Fig. 1.5: Overview of metabolic pathways for biosynthesis of the main fungal volatile metabolites (Schnürer et al., 1999) (Acetyl CoA: acetyl coenzyme A; TCA cycle: citric-acid [tricarboxylic-acid] cycle)**

The life cycle of mould is subdivided into two different stages: the trophophase and the idiophase. In the trophophase, also referred to as nutritional phase, energy is gained and cell mass is formed. Low molecular components and intermediate products of the metabolism are produced and immediately used by the organism. With the depletion of nutrients media and changes in living conditions, the conversion to the idiophase (product formation phase) takes

place. Now, mainly secondary metabolites are formed, which are unnecessary for growth. Rather the entire organism than the single cell benefits from these compounds for instance by acting as a chemical defence or by promoting sporulation.

Mainly during the idiophase (Fig. 1.6), a wide range of compounds are produced, that are either emitted and accumulated in the surrounding environment or stored within the fungus. These are generally referred to as microbial volatile organic compounds (MVOCs). Although, the dominant MVOC of mould is ethanol, there is a large species-specific range of alcohols, ketones, aldehydes, esters, carboxylic acids, lactones, terpenes, sulfur and nitrogen compounds, aliphatic and aromatic hydrocarbons. Far over 500 VOCs have been described from various fungal species, some with characteristic odour (Jelen and Wasowicz, 1998; Kaminski et al., 1974; Miller, 1990) (Tab. 1.5). The compounds commonly reported as MVOCs follow the WHO definition for polar volatile organic compounds (see Tab. 1.1).

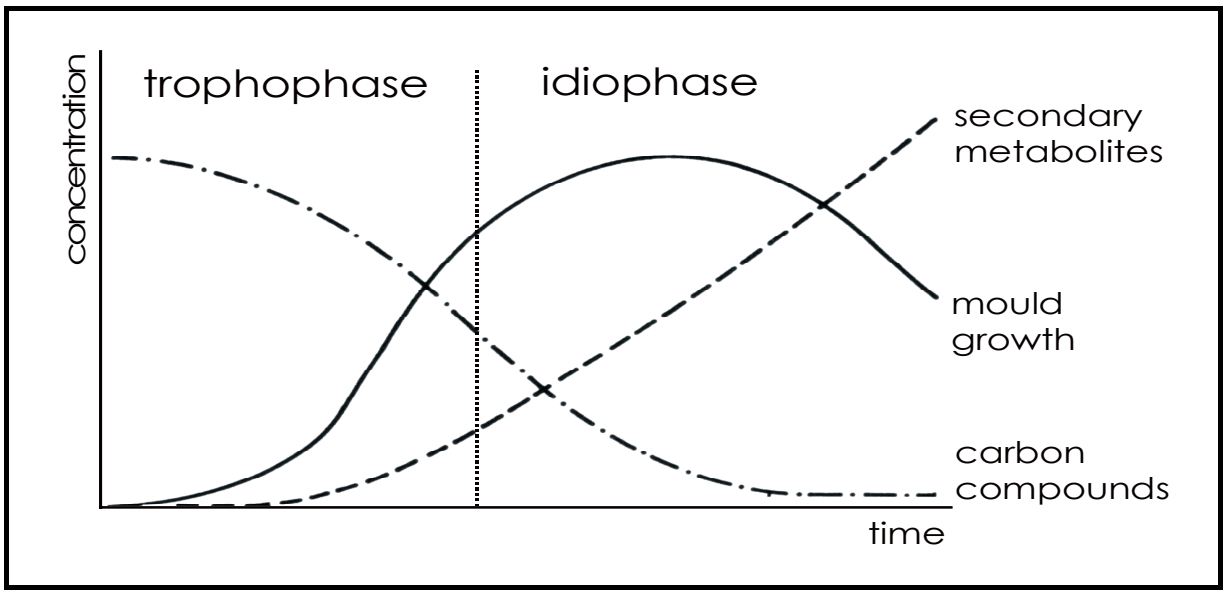



**Fig. 1.6: Dependency of the secondary metabolites on the life cycle of fungi (Reiß, 1998).**

The central intermediate catabolic product for the biosynthesis of MVOCs is acetate, in its acetyl-coenzyme A (CoA) form (Fig. 1.5). It has been established as the main precursor of

volatile fungal metabolites. Many factors, such as substrate, temperature, oxygen concentration, age of culture and microbial species affect the composition of MVOC emissions, but their role in controlling the formation of MVOCs and the MVOC formation itself is still poorly understood (Pasanen et al., 1996).

**Tab. 1.5: Overview of volatile compounds reported as VOC emission from mould (Bjurman, 1999; Bjurman and Kristensson, 1992; Böck, 2001; Claeson et al., 2002; Fischer and Dott, 2003; Fischer et al., 1999; Kaminski et al., 1974; Keller et al., 1998; Larsen and Frisvad, 1994; Lorenz, 2001; Pasanen et al., 1996; Pasanen et al., 1997; Ström et al., 1994; Sunesson et al., 1995; Wessén et al., 1995; Wilkins and Larsen, 1995; Wilkins et al., 2000).**

**ALCOHOLS**

butanol, 1-
butanol, 2-
butanol, 2-methyl-1-
butanol, 3-methyl-1-
butanol, 3-methyl-2-
buten-1-ol, 3-methyl-2-
buten-1-ol, 3-methyl-3-
buten-2-ol, 2-methyl-3-
cyclohexen-1-ol, 4-Methyl-1,3-
decanol, 1-
ethanol
heptanol, 1-
heptanol, 2-
heptanol, 2-
hexanol, 1-
hexanol, 4-methyl-1-
octanol, 3-
octen-1-ol, 2-
octen-3-ol, 1-
octen-3-ol, l-
pentanol, 1-
pentanol, 2-
propanol, 1-
propanol, 2-
propanol, 2-methyl-1- (isobutanol)

**ESTERS**

acetate, 1-propyl-
acetate, 2-methyl-1-propyl-
acetate, 3-methyl-l-butyl-
acetate, ethyl
acetate, methyl-
acetate, n-propyl-
acetic acid
acetic acid, pentyl ester
butanoate, ethyl
butanoate , ethyl 2-methyl-
butanoate, ethyl 3-methyl-
butanoate, propyl 3-methyl-
butyrate, ethyl-2-methyl-
isobutyrate, ethyl-
propanoic acid ester
propionate, ethyl 2-methyl-
propionate, ethyl

**ETHERS and FURANS**

benzene, 1-ethyl-4-methoxy-
benzene, 1-methoxy-3-methyl-
furan, 2,5-dimethyl-
furan, 2-hexyl-
furan, 2-methyl-
furan, 2-pentyl-
furan, 3-methyl-
furan, butyl-
furan, pentyl-
furan, propyl-
isobutylether, methyl-
isopentylether, 2-methyl-

**HYDROCARBONS**

benzene, 1,3-dimethoxy-
benzene, 1-methyl-4-(1-methylethyl)-
benzene, ethyl-
butadiene, 2-methyl-1,3- (isoprene)
cyclopentene, 1,2,4,4-tetramethyl-
cyclopentene, 3-ethylidene-1-methyl-
heptane
heptane, 2,4-dimethyl-
heptene, 1-
hexadiene, 2,5 dimethyl-2,4-
hexadiene, dimethyl-
hexane
hexane, 4-methyl-l-
hexene, 1-
nonadiene, 1,3-
nonane
nonene, 1-
octadiene, 1,3- (isomer)
octane
octane, 4-methyl-
octatriene
octene, l-
styrene
toluene, 4-(1,5-dimethyl-4-hexenyl)-
toluene, 4-(1-methylethenyl)-
xylene

**KETONES**

acetone
bicycloheptan-3-one, 2,6,6-trimethyl-
butan-2-one
butanone , 3-hydroxy-2-
butanone, 2-
cyclohepten-1-one, 3-
cyclohexanone, 2-methyl-1,5-
cyclopentanone
heptanone, 2-
heptanone, 3-
hexanone, 2-
hexanone, 3-
hexanone, 4-methyl-2-
hexanone, 4-methyl-3-
nonanone, 2-
octanone, 2-
octanone, 3-
pentanedione, 2,4-
pentanone, 2-
pentanone, 3-methyl-2-
pulegone

**NITROGEN COMPOUNDS**

ammonia
hexanenitrile
nitromethane
pyridine
pyridine, 2-methyl-

**SULPHUR COMPOUNDS**

dimethyl disulfide
dimethyl sulfide
dimethyl trisulfide
diphenyl sulfone

**TERPENES**

borneol
borneol, 2-methyliso-
borneol, endo-
camphene
camphor
carvone
cuminol
fenchone
franesene, β-
geosmin
limonene
pinene, α-
pinene, β-
terpinen-4-ol
terpineol, α-
thujopsene
tricycloheptane, 1,3,3-trimethyl-
verbenol
verbenone

## 1.3 State of the art of indoor mould detection

The sampling strategy for the detection of indoor mould is fundamental for the exposure assessment. Up to now, no single method is appropriate for the identification of fungal growth and exposure. Each of the so far applied methods have advantages and disadvantages, and might not even show the same results. Differentiations have to be made between the biological detection of the fungal organism itself (e.g. spores or conidia) and the chemical detection of their metabolic products (e.g. MVOCs).

Additionally, two different strategies for the identification of indoor contaminations are pursued: First, the detection of specific tracer-pollutants in indoor air, and second, the detection of deviations from standard respectively surrounding values.

Fundamental to all mould investigations is an extensive anamnesis of the sampling site as suggested by the Commission on Air Pollution Prevention of VDI and DIN - Standards Committee (KRdL) (e.g. VDI 4300 (Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 1999)). The on-site investigation gives important background information of the interior and the neighbourhood, and prevents data misinterpretation due to of cross-contaminations.

### 1.3.1 Biological detection

The biologically based methods for assessing potential exposures to (airborne) mould in indoor environments involve the collection and identification of fungal propagules (e.g. spores). Determining types and prevalence of various species of fungi present on surfaces and in the air allows the assessment of (active) mould growth within buildings. The procedures used for sampling these bioaerosols can be classified into source sampling and air sampling.

In source sampling, areas of visible or suspected fungal growth may be sampled directly by taking bulk material, swabs or tape samples, as well as contact plate samples. Alternatively, settled dust may be collected from the contaminated surface for identification. Analysis of the collected material can be done by direct optical and microscopic examination, and/or by culture methods.

The majority of these mainly non-destructive source sampling techniques give only qualitative results. They provide rapid information and are easily performed, but require expert knowledge on bioaerosols identification (AKA, 2002; Fradkin, 1985; Gabrio et al., 2005; Neville et al., 2002; Pasanen, 2001; Trautmann et al., 2005; Trautmann et al., 2005).

Air sampling is used primarily to assess the presence of fungal disseminators in a building, but may not indicate the location or source (Neville et al., 2002). The principles of air sampling techniques are based on sedimentation, filtration, impingement, precipitation, centrifugal, separation, and impaction. Several air sampling techniques using volumetric devices are available, e.g. the sieve type impactor, the spore trap, the liquid impinger, or the filter cassette (Burge, 1990; Neville et al., 2002; Pasanen, 2001).

The most common used technique is the sieve impactor technique. An air sample (30 to 150 L during 2 to 5 min) is drawn through several sieve plates providing a differentiation according to particle size (and therefore different fungal spores). On each stage of the sterilized sampler, a selected agar culture medium (e.g. dichloran glycerol agar (DG 18) or malt extract agar (MEA), see Fig. 1.7) is placed depending on the sampling conditions and the target disseminators.

Although, biological detection is most commonly used for air sampling, studies performed by Hodgson and other working groups indicated that up to 50 % of known contaminated buildings produce false-negative results when culturable air samples are collected (Hodgson, 1995). Other studies suggested that culture analysis may detect less than 10 % of spores present. In addition, huge variations (up to 1,000 fold) between essentially identical specimens can occur (Kuhn and Ghannoum, 2003; Neville et al., 2002).

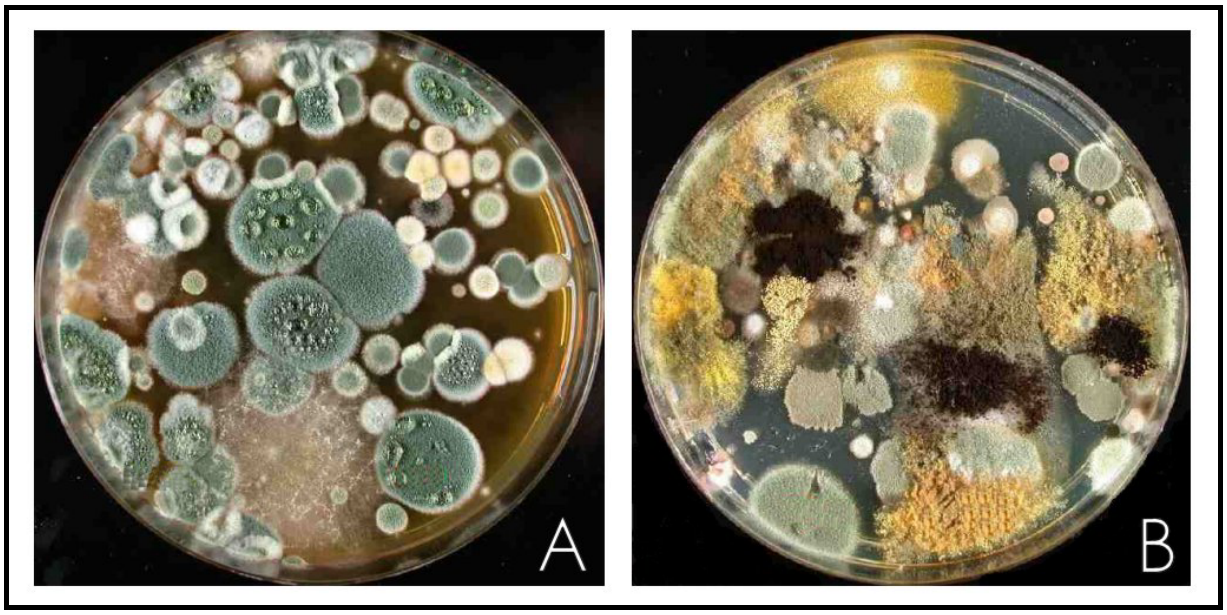



**Fig. 1.7: Example of air sampled and incubated Petri dishes with two different and commonly used cultivation agars: (A) malt extract agar (MEA) preferred for hydrophilic fungi, and (B) dichloran glycerol agar (DG 18) preferred for xerophilic fungi (Gabrio and Samson, 2003)**

Possible cause for these false results can be that the growth of mould, and consequently fungal spore release, depends on the stage of the metamorphosis, the capability of spores to become airborne, the fungal species, the building material, the relative humidity, the temperature, the age of the mycelia, the mechanical vibrations in construction materials, and the airflow over the surface. This can cause significant temporal variations in the sporulation and the air spore concentration (Burge, 1990; Kildesø et al., 2003). Another possible cause can be particle size discrimination by the sampling system. Furthermore, not all spores are

culturable and incubation is highly dependent on the selected culture media (Fig. 1.7) (Nielsen, 2002).

### 1.3.2 Chemical detection

The chemical analysis of indoor mould is mainly focused on microbial volatile organic compounds (MVOCs) (Tab. 1.5) as these are a significant part of the fungal metabolism (chapter 1.2.3). Although mould may not be detected in indoor air, a wide range of MVOCs can permeate building materials and diffuse into the surrounding air. Thus, identification of MVOCs may indicate microbial contamination when other signs of microbial growth cannot be detected (Korpi et al., 1998).

The complex air matrix contains gases, aerosols, and particles with VOCs present at trace levels (ppb(v)…ppt(v)). In addition, air is a dynamic medium. Analytes move and diffuse depending on air movement and air exchange, and can undergo chemical reactions in the atmosphere (Dettmer and Engewald, 2002; Dettmer and Engewald, 2003).

Three different principles have been applied for sampling MVOCs, and VOCs in general, from (indoor) air (Knöppel, 1992): a) The grab method takes a whole air sample by either opening the valve of an evacuated cylinder or pumping air into it (using e.g. a membrane pump). b) During active sampling an appropriate amount of air is sucked through a solid or liquid sorbent, where the pollutants are trapped. c) Passive sampling is based on the penetration of pollutants from the entrance opening of a sampling cylinder to the surface of a well defined sorbent by gradient-driven diffusion.

In general, the grab method is not sensitive enough for the detection of VOCs at trace level. This compound analysis in ambient air normally requires a pre-concentration, that is often

combined with the sampling step. In passive and active sampling this can be done by adsorptive enrichment on adsorbents. The selection of the sorbent material has a major influence on the sensitivity and selectivity of the sampling. Mainly, three general types of solid sorbents are used for trapping VOCs in air (Dettmer and Engewald, 2002) (Tab. 1.6). While the surface area of the sorbent has a tremendous impact on the amount of VOCs that can be withheld by the medium, the surface polarity determines the type of compounds that a sorbent can be used for.

**Tab. 1.6: Properties of solid sorbents for air sampling summarized by Uhde (Knöppel, 1992; Uhde, 1999)**

| type | structure | surface area ($m^2 g^{-1}$) | products | desorption | compounds tested | polarity | thermal stability (°C) | water affinity |
|---|---|---|---|---|---|---|---|---|
| inorganic sorbents | silica gel | 1…30 | Volasphere | solvent | PCBs, pesticides | high | ~400 | |
| | molecular sieve | | | solvent | permanent gases | high | < 400 | |
| | aluminium oxide | ~300 | Alumina F1 | solvent | hydrocarbons | high | 300 | |
| porous organic polymer resins | styrene polymers | 300…800 | Porapak, XAD, Chromosorb | thermal / solvent | non polar and moderately polar VOCs (> 40 °C) | variable | < 250 | low |
| | phenyl-phenylene oxide polymers | 20…35 | TENAX® | thermal | non polar VOCs (> 60 °C) | low | < 350 | low |
| | PU foams | | | solvent | pesticides | low | - | low |
| porous materials based on carbon | activated charcoal | 800…1500 | | solvent | non polar and slightly polar VOCs (> 50 °C) | medium | < 400 | high |
| | carbon molecular sieves | 400…1000 | Carbsieve, Ambesorb | solvent / thermal | non polar and slightly polar VOCs (> 80 °C) | low | < 400 | low |
| | graphitised carbon black | 12…100 | Carbotrap, Anasorb | thermal | non polar VOCs (> 60 °C) | low | < 400 | low |

Inorganic sorbents such as silica gel, zeolites, or alumina are of minor importance mainly because of the higher hydrophilicity of these materials.

Porous organic polymer resins are a large group of adsorbents with different surface areas and polarities. Many of these adsorbents have their origin as cross-linked stationary phase in packed GC columns. Tenax® [poly-(2,6-diphenyl-p-phenylene oxide)] developed by Supelco (Bellefonte, PA, USA) is the most important material for air analysis among the porous organic polymer resins. The high-purity Tenax® TA (Trapping Agent) with a particles size of 60 to 80 mesh and a density of 0.25 g $mL^{-1}$ is a very hydrophobic material that is excellent for trapping non-polar compounds. However, due to its low specific surface area of 30 to 50 $m^2$ $g^{-1}$ and a pore volume of 0.05 mL $g^{-1}$, it has relatively poor capacities for sampling highly volatile organics. Although Tenax® is an excellent adsorbent with a very high thermal stability (up to 350 °C), it tends to form artefacts during sampling in the presence of some reactive gases and during thermal desorption. In former times the polymer decomposed under thermal stress into toluene, benzene, and other aromatics and degraded by the presents of organic acids with modern materials this could largely be excluded (Dettmer and Engewald, 2002; Restek Corporation, 2003; Zielinska and Fujita, 1994).

Porous materials based on carbon are the most widely used sorbent materials and can be sub-classified into activated carbon (charcoal), carbon molecular sieves and graphitized carbon blacks. The most important carbon adsorbent material is activated carbon. It is made out of carbon-containing biological materials, such as wood or coconut shells, synthetic polymers, lignite or petroleum. The physical and chemical properties of this material are influenced by the starting material, and the manufacturing process.

Activated carbon has an average density of 0.44 g $mL^{-1}$ and a particle size of 20 to 40 mesh. It has a very high specific surface area of 800 to 1.500 $m^2$ $g^{-1}$ and a huge pore volume of 0.5 mL $g^{-1}$. In addition, this adsorbent possesses a chemical heterogeneous surface with

several functional groups, such as hydroxyl-, carbonyl- and carboxylic-functions that cause non-specific and specific interactions during the enrichment. Therefore, activated carbon shows a strong adsorptive behaviour especially for higher volatile and lower molecular weight compounds. This sometimes results in the disadvantage of very low recoveries depending on the desorption method, the trap compounds, and chemical reactions, that may occur especially during storage and desorption of the samplers.

**Tab. 1.7: Characterization of adsorbent materials commonly used in ambient air analysis (Dettmer and Engewald, 2002) ($V^{\theta}_{g}$: specific retention volume)**

| Adsorbent (provider) | **Activated carbon** (Supelco) | **Tenax TA** (Supelco) |
|---|---|---|
| Type | Coconut charcoal | Poly-(2,6-diphenyl-)-p-phenylenoxide |
| Particle size | 20…40 mesh | 60…80 mesh |
| Sampling range (ideal) | C2…C8 | C7…C26 |
| Maximum temperature | 220 °C | 350 °C |
| Density | 0.44 g $mL^{-1}$ | 0.25 g $mL^{-1}$ |
| Micro pore volume | 0.5 mL $g^{-1}$ | 0.002 mL $g^{-1}$ |
| Total pore volume | 0.54 mL $g^{-1}$ | 0.05 mL $g^{-1}$ |
| Elemental composition (measured) (mass %) | C: 93.7 %; H: 0.9 %; S: 0.1; O: 3 %; Cl: 0.2 % | C: 84.5 %; H: 4.4 %; S: 0.1 %; O: 6.4 %; Cl: 0.4 % |
| Specific surface area | 1070 $m^2$ $g^{-1}$ | 35 $m^2$ $g^{-1}$ |
| $V^{\theta}_{g}$ of water at 20 °C | 1454 mL $g^{-1}$ | 39 mL $g^{-1}$ |

Activated charcoal, one of the porous materials based on carbon, shows a very low background. It is hydrophobic, but does retain a relatively high amount of water vapour compared to other adsorbent materials e.g. Tenax® TA (see the specific retention volume $V^{\theta}_{g}$ in Tab. 1.7). It is a thermally stable material up to maximum temperatures of 220 °C allowing the application of thermal desorption, but solvent desorption is mostly favoured (Dettmer and Engewald, 2002; Zielinska and Fujita, 1994). All these properties qualify activated charcoal as an excellent material for air sampling.

#### 1.3.2.1 Active sampling and thermal desorption on Tenax

The most favoured method for sampling MVOCs in the indoor area is the direct active sampling in combination with the thermal desorption followed by gas chromatographic (GC) separation.

Most commonly the phenyl-phenylene oxide polymer TENAX® TA is used as solid sorbent. Approximately 150 to 200 mg TENAX® TA in the glass or stainless steel sampling tube are applied per analysis. Several suitability studies have proven that TENAX® is convenient for the sampling of VOCs especially regarding analyte recovery and background (Uhde, 1999). Nevertheless, the conditioning of whole sampling devise at about 270 °C with an inert gas flow (usually helium) for a two hours straight before the sampling is an absolute requirement. During the active sampling process an air volume of 1 to 12 L with an airflow of about 10…200 mL $min^{-1}$ is sucked through the adsorbent material. The sampling period ranges from several minutes to one hour. The accuracy of the sampling is determined by the pump calibration and mass flow controller. After the sampling period the adsorbent tubes are normally sealed with Teflon caps and stored below 4 °C until analysis (Gabrio, 2001).

The first step of the GC analysis is the primary thermal desorption of the coated sampler at 250 to 280 °C for about 2 to 3 minutes with a desorption flow of 100 mL $min^{-1}$. The desorbed compounds are reconcentrated either on a small adsorbent tube or by cryogenic trapping at -30 °C. In both cases the trap is flash heated up to 260 to 290 °C for several minutes in order to guarantee a rapid direct injection onto the capillary column. This is done either in the split or splitless mode. The chromatographic separation is performed by long thickfilm capillary columns with apolar dimethylsiloxane or diphenyl/dimethylsiloxane phases (Claeson et al., 2002; Fischer et al., 1999; Gabrio, 2001; Keller, 2001; Korpi et al., 1998; Pasanen et al., 1997; Wilkins et al., 2003; Wilkins et al., 2000).

A common problem in the GC is a high water content in the sample. Ice can lead to a clogging. In addition, water gives a high background noise level in GC-MS chromatograms and can influence the signal of other detectors. It is also known to cause damage on fused silica columns due to a hydrolysis of the polysiloxane stationary phase at elevated temperatures (Knöppel, 1992; Pekar, 2000; Uhde, 1999).

The main advantage of thermal desorption is the complete and solventless transfer of all analytes into the GC system resulting in high sensitivity. Due to the elevated temperatures of the thermal desorption system, the ability of the sorbent to retain compounds decreases dramatically. A continuous flow of an inert carrier gas in reverse direction of the sampling flow (backflush technique) supports the complete release and transfer of the adsorbed compounds. Limits are given by the thermal stability of the sorbent and the (thermal) reactivity of the analytes.

#### 1.3.2.2 Active sampling and solvent desorption on Anasorb

Alternatively, the active sampling of MVOCs is combined with solvent desorption followed by GC separation. Unlike the thermal desorption method, the airflow during the sampling through a porous carbon based sorbent (e.g. Anasorb or Carbotrap, Tab. 1.6) is about 200 to 1'000 mL $min^{-1}$. The sampling period varies from several minutes up to several hours with a total sampling volume of 1 to 250 L or even more. The amount of adsorbents used and the handling of the sample until analysis is the same as for the thermal desorption method.

Extraction is achieved by different organic solvents (e.g. dichloromethane or carbon disulphide) (Uhde, 1999). In general, the desorption of the sorbent takes place in the sampler

body with a solvent volume of 1…3 mL during 30…60 min. The GC analysis is carried out by a splitless injection on long apolar thickfilm columns (Palmgren, 2003).

The problematic nature of solvent extraction is the dilution of the sampled compounds in the solvent. Therefore only a small aliquot of the trapped pollutant extract can be injected in the HRGC system, but several analysis are possible.

#### 1.3.2.3 Passive sampling for the detection of VOCs

A widely used and since 1927 (Gordon and Lowe, 1927) well established sampling technique for the detection of general VOCs in the air is the passive sampling (Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 1999; Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 1999; Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 2001; Ullrich, 2000). In 1986 during the first international symposium on diffusive sampling the following definition was made (Brown, 2003):

> *"A diffusive§ sampler is a device which is capable of taking samples of gas or vapour pollutants from the atmosphere, at a rate controlled by a physical process such as diffusion through a static air layer, or permeation through a membrane, but which does not involve the active movement of air through the sampler."*
>
> *§ The adjective "diffusive" should be regarded as synonymous with "passive", which is preferred in describing these samplers as opposed to "active" (Brown, 1993).*

The main principle of the passive sampling is based on Fick's first law of diffusion, that describes the diffusion rate of molecules onto a sampling medium (Blome and Hennig, 1985):

$$\frac{dn}{dt} = -D * A * \frac{dc}{dl} \qquad (1)$$

where **n** is the molar amount of transported substance (mol), **t** is the time of exposure (s), $\frac{dn}{dt}$ is the rate of diffusion mass transport, **D** is the molecular diffusion coefficient of a

particular gas or vapour in air ($cm^2\ s^{-1}$) (refer to (Lide, 1993)), **A** is the cross sectional area ($cm^2$) and **l** is the length (cm) of the diffusion path, **c** is the pollutant concentration in air (mol $cm^3$), and $\frac{dc}{dl}$ is the concentration gradient above the diffusion path along the axis.

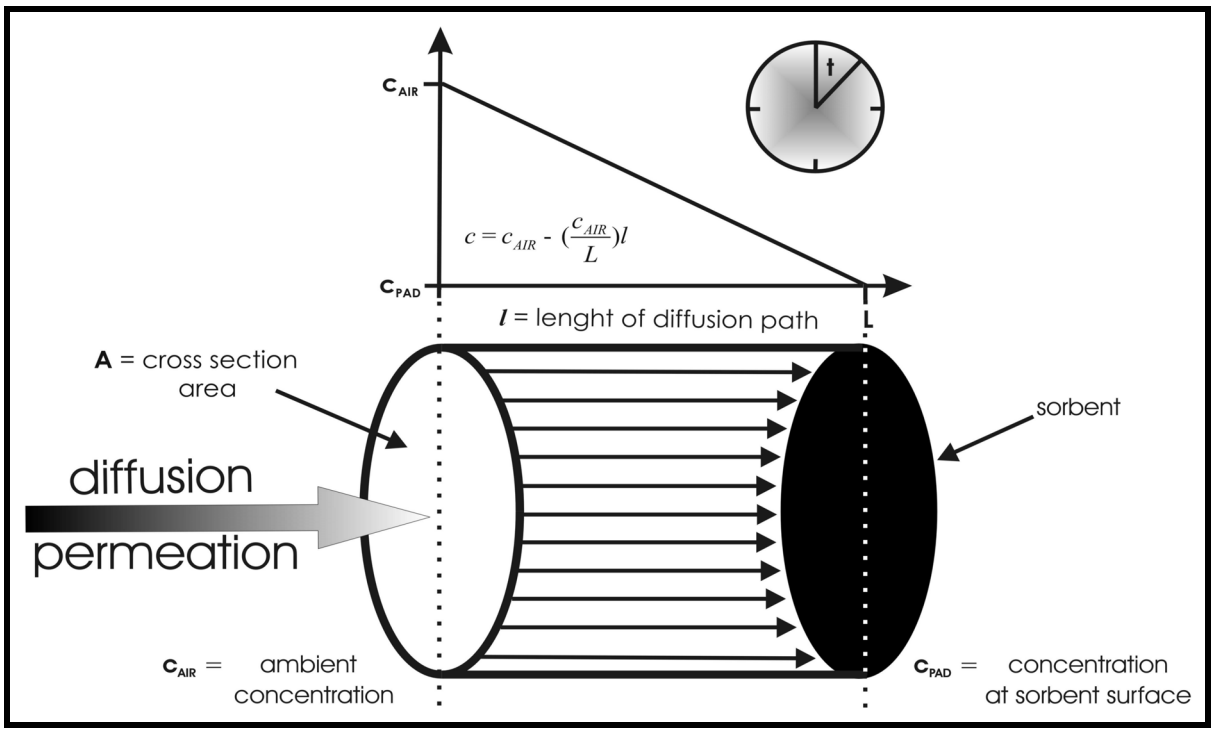



**Fig. 1.8: Scheme of diffusion and permeation**

Following Fick's first law of diffusion assuming that a) the concentration of the compounds in question above the sampler medium (in the sampler body) behaves like a perfect sink and is zero ($\mathbf{c_{PAD}}$) (zero-sink-condition), that b) the concentration at the face of the sampler equals the ambient air concentration ($\mathbf{c_{AIR}}$), and that c) the concentration gradient is linear, the transported amount of pollutant is proportional to the exposition time (t) and pollutant concentration (c) in air, then **n** can be described as follows (Eq. (2)):

$$\frac{n}{t} = \frac{D^* A}{L} * \left(c_{AIR} - c_{PAD}\right) \Rightarrow \underbrace{\text{if } c_{PAD} = 0}_{\text{zero-sink-condition}} \Rightarrow n = \frac{D^* A}{L} * c_{AIR} * t \qquad (2)$$

where **L** is the length of diffusion path (cm) and $\frac{D*A*c}{L}$ is the diffusive transport rate (mol $s^{-1}$).

The sampling rate (**SR**) (unit by convention: ng $ppm^{-1}$ (m(mg) $v(m^3)^{-1}$) $min^{-1}$) of a passive sampler corresponds to the sample flow rate (mL $min^{-1}$) in a dynamic device. SR depends on the geometry (size and shape) of the sampler. Both rates match to the amount of analyte present in the given volume (x mL) of ambient air that is accumulated during a 1 min interval.

$$(2) \Leftrightarrow \underbrace{\frac{D*A}{L}}_{\text{geometry of sampler}} = \underbrace{\frac{n}{c*t}}_{\text{sampling rate (SR)}} \tag{3}$$

(If the concentration **c** is viewed in the dimension of g $cm^{-3}$ or g $mL^{-1}$. **n** can be summarized as mass uptake in the dimension of g.)

Furthermore, Fick's first law is also applicable if the rate of analyte mass transfer is controlled by permeation through a microporous membrane. Here, the amount of gaseous analyte, collected onto an ideal collecting medium during a defined sampling period, can be described by the following equation (Eq.(4)):

$$n = \frac{S*A}{L_M} * p*t \tag{4}$$

where **n** is the molar amount of transported substance (mol), **S** is the (membrane) permeability coefficient of a given analyte ($cm^2$ s), **A** is the cross-sectional area of the permeation path ($cm^2$), **p** is the partial pressure of the analyte near the external membrane surface (that can easily be converted into a concentration by p = a * c), $\mathbf{L_M}$ is the membrane thickness (cm), and **t** is the time of exposure (s).

However, the effect and influence of the much slower permeation compared to the diffusion of the compounds in the same medium is typically several orders of magnitude lower. Therefore permeation can often be neglected, if viewed in combination with the diffusion.

Commercially available passive samplers mainly fall into three geometrical categories (Fig. 1.9): a) Devices of an open-ended tube shape are characterized by a long axial diffusion path length and a small cross sectional area resulting in relatively low SRs in the order of 1 mL $min^{-1}$ or less. b) Badge-type samplers show a shorter diffusion path length and a larger cross-sectional area. They typically exhibit intermediate SRs in the order of 10 mL $min^{-1}$. c) Radial devices, with a tube-type shape sampling through the side walls of a microporous membrane, have the largest SRs in the order of 100 mL $min^{-1}$ (Begerow et al., 1999; Brown, 2003).

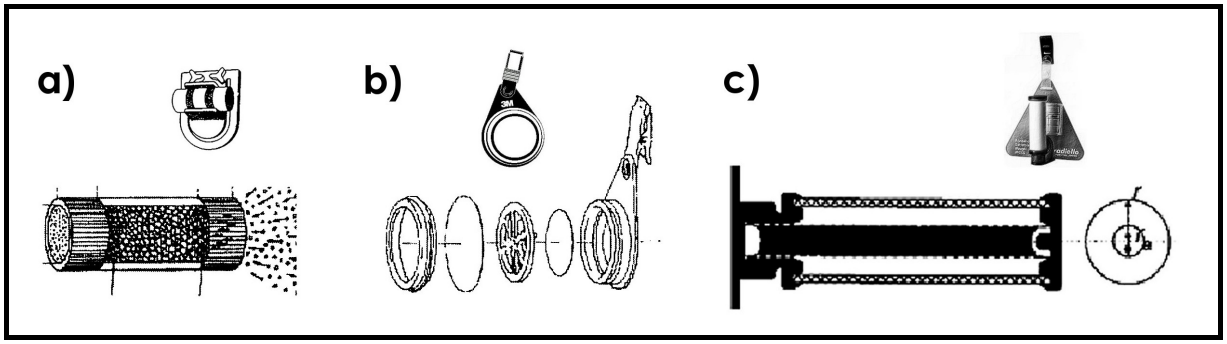



**Fig. 1.9: Types of passive sampler: a) tube-type (e.g. ORSA 5 by Dräger, Lübeck, Germany), b) badge-type (e.g. OVM 3500 by 3M Company, St. Paul, MN, USA), c) radial-type (e.g. Radiello 3310 by Fondazione Salvatore Maugeri-IRCCS, Padova, Italy)**

Besides the geometry of the diffusive sampler, that is described by its geometrical constant (A $L^{-1}$) and the value of D, secondary factors affect the sampler performance: temperature, pressure, humidity, pollutant concentration, exposition time, type of pollutant and solvent composition, velocity of the surrounding air, and variation in compound concentrations (Blome and Hennig, 1985; Brown, 2003).

Temperature and pressure are the most important factors, that influence the diffusion coefficient D directly from the kinetic theory of gases following the Maxwell's equation (Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 2003; Pozzoli and Cottica, 1986):

$$D = f\left(\frac{T^{\frac{3}{2}}}{p}\right) \tag{5}$$

However, in practice the temperature dependency of D has been determined in the range of 0.2…0.4 % per °C (Bailey and Hollingdale-Smith, 1981; Górecki and Namiesnik, 2002; Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 2001) and can therefore be neglected in routine application. Additionally, high humidity can affect the sorbent strength by influencing the saturation capacity of the sampler and steam droplets compete with the analytes for sorption. If the sampler becomes saturated, the zero-sink-condition cannot be assumed anymore ($c_{PAD} \neq 0$; equation (2)) and the SR becomes non-linear (Occupational Medicine and Hygiene Laboratory, 1993). The sorption capacity is highly dependent on the type and amount of sorbent material (e.g. activated charcoal), as well as the type of analytes, that are competing for sorption in dependence of their physical and chemical properties (e.g. polarity).

Furthermore, ambient air movement at the face of the sampler body influences the passive sampling. If the air velocity becomes to slow, a static air layer above the membrane is formed and causes an extension of the diffusive path. This results either in a flattening of the concentration gradient ($\frac{dc}{dl}$) or reduces the diffusion effectively to zero by the formation of a boundary layer. The required air velocity is mainly dependent on the SR of the sampler that again depends on design. The higher the SR the higher the necessary air velocity. Overall, a

threshold value of at least 0.13 m $s^{-1}$ linear air velocity has to be maintained for reproducible and effective sampling if a badge-type sampler is applied.

To overcome most limitations of a) environmental conditions, b) non-ideal behaviour of sorbents, and c) missing direct control of the SR (by means of a sampling pump), a calibration of each sampler model is necessary dependent on the target compound. In 1995 the European Committee for Standardization (CEN; Brussels, Belgium) developed a standardized evaluation protocol for passive samplers, describing tests to assess effects of analyte concentration, environmental conditions and exposure time on sampler performance (CEN EN 838 (Arbeitsausschuß Gefahrstoffe/Arbeitsschutz (AGSA) im VDI und DIN, 1995)). For a large number organic vapours the SRs have been measured experimentally in an exposure chamber (e.g. (Lugg, 1968)), or have been calculated from the diffusion coefficient based on empirical relations (Hirschfelder equation (Friz and Freise, 1951), Pannwitz calculations (Pannwitz, 1983)). They are available from the manufacturers of the commercial samplers.

However, even if the theory behind the passive sampling is extensive, the application is simple and many-sided. The advantages of passive sampling can be summarized with the following keywords: simplicity (of field operation), low cost, no need for expensive and complicated equipment, no power requirement, unattended operation, ability to produce reasonable accurate results, time-weighted averaged (TWA) concentration of the analyte that give a real overview of the field situation.

So far, no publications have been reported applying passive sampling instead of active sampling for the detection of MVOCs in indoor air. A single study by Larsen and Frisvad

published in 1995 reported on the collection of fungal volatiles by diffusive sampling onto tubes with carbon black (Larsen and Frisvad, 1995; Larsen and Frisvad, 1995).

## 1.4 Chirality

### 1.4.1 Chirality in nature

Many of the compounds associated with living organisms show the phenomenon of asymmetry called chirality, e.g. DNA, enzymes, antibodies and hormones. The term chiral is derived from the Greek name χειρ (= cheir) meaning "hand" and in simple terms, chirality is "handedness" - that is, the existence of left/right opposition or a mirror image of one another. The two enantiomers of a chiral molecule feature the same atoms, bonded by the same sequence of bonds, not interchangeable different three-dimensional structures (configurations) and stereogenic (asymmetric) centres.

Biology is very sensitive to chirality and enantiomers of compounds may have distinctly different biological activity as receptors are extremely enantioselective. Additionally, compound synthesis and reactions in nature are often asymmetrical as most of them depend on chiral enzymes. Therefore, both forms of chiral molecules are not equally common. Nature mainly uses one of the two enantiomers or elicit different biological effects by the enantiomers. This applies to all living material including the metabolism of mould. In contrast, the majority of industrial synthesis is symmetrical in the sense that equal amounts of both enantiomers are produced and most the time racemic mixture are released in the environment.

### 1.4.2 Enantioselective chromatographic separation

Enantiomers possess identical properties in a symmetrical environments, but their properties may differ in asymmetrical environments. This basic principle forms the background for enantioselective chromatography. Enantiomers exhibit identical physical and chemical properties except that they rotate the plane of linearly polarized light in opposite directions and that they react at different rates with other chiral compounds.

Today, enantioselective analysis is commonly performed with HRGC capillaries containing cyclodextrin (CD) derivatives as chiral stationary phase (CSP). Structurally, CDs consist of 6, 7, or 8 (α, β, and γ respectively) D-glucopyranosyl units connected by α-(1,4)-glycosidic linkages. Each of the chiral glucose units of the cyclodextrins possesses a rigid $^4C_1$ chair conformation (Fig. 1.10).

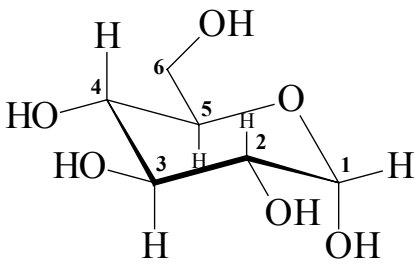



**Fig. 1.10: The natural α-D-glucopyranosyl unit in the rigid $^4C_1$ chair conformation is the base unit of any cyclodextrin (CD).**

The most stable three-dimensional macrocyclic configuration for these non-reducing cyclic oligosaccharides takes the form of a toroid in both solid state and solution. The height of the molecular cavity is constant, whereas its diameter varies by glycosidic units. The upper, larger and wider opening of the toroid is occupied exclusively by secondary hydroxyl groups (C2-OH and C3-OH). The opposite lower, narrower and smaller opening is occupied exclusively by a primary hydroxyl group (C6-OH). The interior of the toroid is hydrophobic

as a result of the absence of hydroxyl groups directed inwards. It has a Lewis base character because of the electron rich environment provided mainly by the glycosidic oxygen atoms. Hydrophobic interaction favours the formation of inclusion complexes. The outer hydroxyl groups at the cavity openings make the underivatized cyclodextrins hydrophilic and forms stable intarmolecular hydrogen bonds between secondary hydroxyl groups (Anonymous, 2003; Schurig and Nowotny, 1990). Fig. 1.11 shows the chemical structure and the geometrical orientation and Tab. 1.8 gives the molecular dimensions and physical data of α-, β- and γ-cyclodextrin.

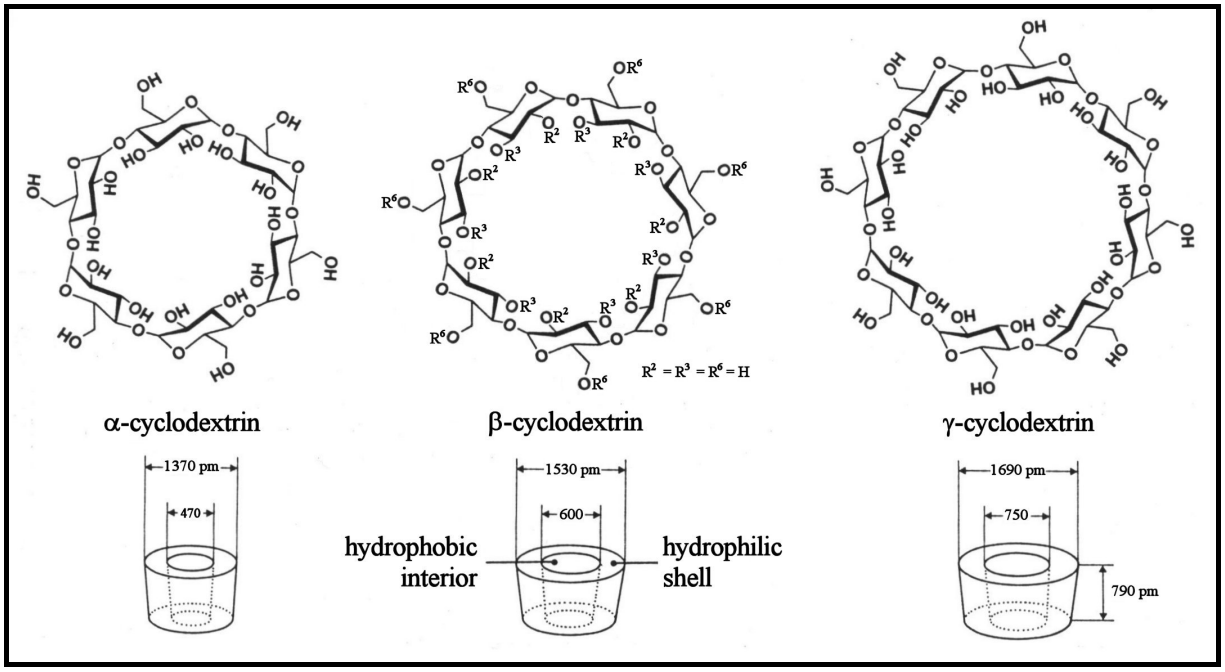



**Fig. 1.11: Chemical structure and the geometrical orientation of α-, β- and γ-cyclodextrin following (Cabrera and Schwinn, 1989)**

CDs have been modified with many different substituents. Derivatization of CDs, such as alkylations and acylations, have been carried out highly regioselectively at the hydroxyl groups in position 2, 3, and 6, but occasionally inhomogeneous and incompletely derivatization occurred. The C2 hydroxyl group is more acidic and the C6 hydroxyl group is less sterically hindered than the C3 hydroxyl group.

**Tab. 1.8: Molecular dimensions and physical data of α-, β- and γ-cyclodextrin following (Anonymous, 2003; König, 1992; Schurig and Nowotny, 1990)**

| cyclodextrin | α | β | γ |
|---|---|---|---|
| molecular weight (amu) | 972.86 | 1135.01 | 1297.15 |
| glucose monomers | 6 | 7 | 8 |
| number of chiral centers | 30 | 35 | 40 |
| external diameter (pm) | 1370…1460 | 1530…1540 | 1690…1750 |
| internal diameter (pm) | 470…520 | 600…650 | 750…850 |
| cavity height (pm) | 790 | 790 | 790 |
| volume of cavity ($nm^3$) | 0.174…0.176 | 0.262…0.346 | 0.472…0.510 |
| specific rotation $[\alpha]_D^{25}$ (in a saturated water solution) (grad $cm^2$ $g^{-1}$) | +150 ± 0.5 | +162.5 ± 0.5 | +177.4 ± 0.5 |
| melting and decomposition range (°C) | 255…260 | 255…265 | 240…245 |
| $pK_a$ of hydroxyl groups | 12.1…12.6 | 12.1…12.6 | 12.1…12.6 |

Consequently, derivatization is not always complete. Derivatized CDs show relatively low melting points, high thermal stability and good solubility in polysiloxanes. By diluting the chiral selector in polysiloxanes, it was possible to combine the GC properties of polysiloxanes with the enantioselectivity of the modified CDs (Schurig and Nowotny, 1988).

Enantiomer separation on CDs is not very well understood, both inclusion as well as exclusion interactions are discussed (Allenmark, 1991). Host-guest complexes are often favoured where the enantioselectivity is strongly influenced by the size of the macrocyclic cavity (König, 1993; König et al., 1989). However, enantiomer separation often cannot be explained by molecular shape, size and functionalities. Obviously, multimodal recognition processes take place, which may involve inter alia inclusion, hydrogen bonding, dipole-dipole interactions and other forces.

Additionally, small composition changes (due to completeness of derivatisation or the purity of the modified CDs) can influence the enantiomeric selectivity of cyclodextrins and therefore the reproducibility of chiral columns, thus separation characteristics of CD stationary phases can vary from column to column (Jaus and Oehme, 1999; Schreier et al., 1995).

Independently of the nature of the chiral selectors, the choice of appropriate polysiloxanes for the stationary phase allows to vary the polarity over a wide range that additionally influences the (enantiomeric) separation. The ratio between the derivatized CD and the dissolving stationary phase influences the enantiomeric separation. CD concentrations between 10 and 50 % (w/w) are optimal, dependent on the analyte and the solubility of the CD derivative in the chosen polysiloxane (Bicchi et al., 1991; Buda et al., 1995; Dietrich et al., 1994; Dietrich et al., 1995; Jung and Schurig, 1993).

# 2 Aim of the work

Identification of mould attacks in the indoor environment is mainly performed by sampling and counting spores and conidia. These procedures are highly dependent on seasonal and environmental parameters. Furthermore, in the absence of visible mould, spore samples will rarely confirm fungal contamination.

The aim of this work was to develop a method to detect mould within a building by tracing and quantifying selected microbial volatile organic compounds (MVOCs) in indoor air. This method should be applicable at any time independent of the fungal life cycle.

As first task, mould specific compounds should be selected to function as marker compounds.

Another objective was to choose and apply a time integrated sampling method for the selected MVOCs. A sufficient time resolution should be ensured as it was unreasonable to expect that air sampling over a short time period could prove fungal contamination. The sampling method should be robust, easy to handle, and inexpensive in contrast to the generally applied active sampling technique, where a trained operator and power supply are required.

Another major goal was to separate and detect the trace compounds by high resolution gas chromatography (HRGC) and mass spectrometry (MS). A non-enantioselective separation and trace detection of the marker compounds should be performed to identify possible mould contamination. Furthermore, an enantioselective separation for unequivocal identification of the biogenic source of origin should be explored.

Finally, the developed method should be applied to a real case site to detect and confirm mould growth.

# 3 Selection of microbial volatile organic compounds

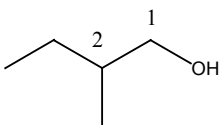
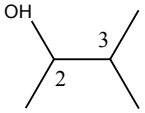
Mould and other microorganisms produce and spread a wide range of organic compounds throughout their life cycle (chapter 1.2). Most of these compounds are volatile, permeate materials, and diffuse into the surrounding air. Some of these microbial volatile organic compounds (MVOCs) have sufficiently low action thresholds and provoke an unpleasant smell or can act as irritants for human beings. The toxicological relevance of the MVOC is probably negligible as they occur at very low concentrations (Korpi et al., 1999; Kreja and Seidel, 2002; Pasanen et al., 1998), but these MVOCs may indicate microbial contamination in the indoor area even when other signs of microbial growth cannot be detected (Korpi et al., 1998). Certain substances are specifically emitted from microorganisms, while others are also released by intramural sources of a building, e.g. wall paint, untreated wood, or general building materials, ripened fruit, perfumed products, and chemicals introduced or emitted by humans.

Several studies have identified volatile metabolites from fungal cultures on various media (Bennett and Ciegler, 1983; Fiedler et al., 2001; Larsen and Frisvad, 1994; Wessén and Schoeps, 1996). The most commonly reported compounds were hydrocarbons (e.g. octane), alcohols (e.g. 2-methyl-1-butanol), aldehydes and ketones (e.g. octan-3-one), esters (e.g. ethyl acetate), ethers and furans (e.g. 2-methylfuran), terpenes and terpene derivatives (e.g. geosmin), nitrogen and sulphur compounds (e.g. pyridine and dimethyl disulfide) (Claeson et al., 2002). Both the microbial species and the substrates influence the MVOC profile. Nutrient rich synthetic media promote emissions of larger quantities and different types of metabolites compared to those produced on poor substrates like building materials (Bjurman, 1999; Bjurman and Kristensson, 1992; Pasanen et al., 1996; Wilkins and Larsen, 1995; Wilkins et

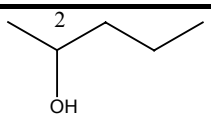
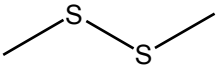
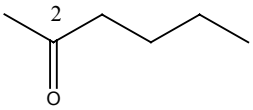
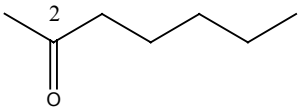
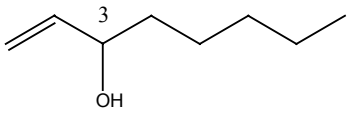
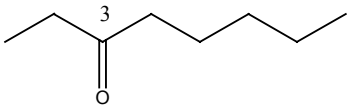
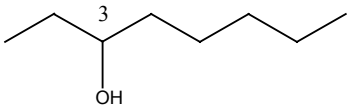
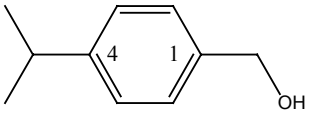
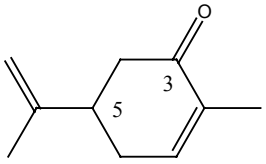
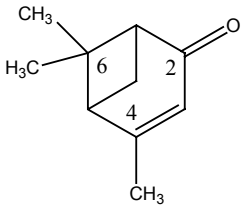
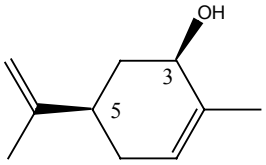
al., 2000). Metabolites found from growth on synthetic media can therefore not automatically be considered as possible markers of microbial growth in buildings. Additionally, the lack of knowledge of the MVOC composition during different growth conditions as well as of the regulation of MVOC biosynthesis limits the use of metabolites as marker compounds (chapter 1.2.1). Moreover, it is extremely difficult to relate MVOC patterns to particular mould, mould species, or to use them as taxonomic markers. Nevertheless, there are a few universal MVOCs acting as fungal signature compounds and as possible general markers of microbial growth.

Literature was screened to establish a list of volatile compounds reported as VOC emission from mould (compare Tab. 1.5). Compounds not only specific for some mould species but for most common indoor fungi were selected. Additionally, other possible compound sources were considered, e.g. food, furniture, chemicals, as well as different living and growing conditions. Attention was especially focussed on chiral compounds to allow an enantioselective screening to differentiate biogenic compounds from anthropogenic sources. Based on these considerations 22 MVOCs (Tab. 3.1) were chosen as target compounds in this study for a reliable mould detection.

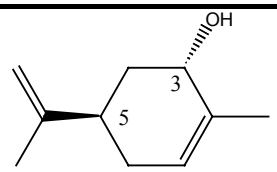
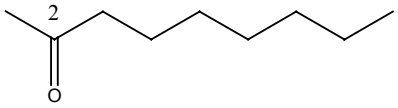
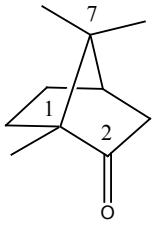
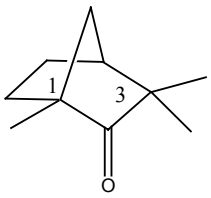
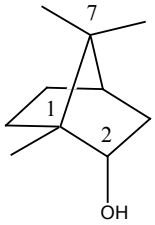
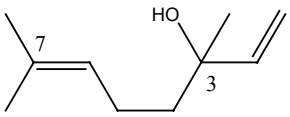
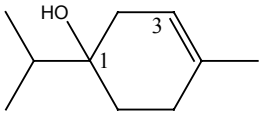
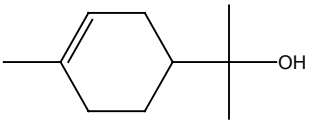
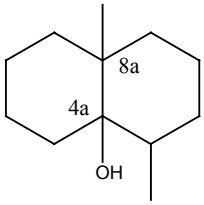
**Tab. 3.1:** **22 MVOCs were selected as reference compounds for mould identification in indoor air listed by molecular weight ($M_W$). Chiral compounds are marked with asterisk (*). (For further details about the compounds see appendix chapter 8.1, pg. 143)**

| substance (#) | molecular formula | structure | $M_W$ (amu) |
|---|---|---|---|
| 2-methyl-butan-1-ol* (5/6) | $C_5H_{12}O$ |   | 88.15 |
| 3-methyl-butan-2-ol* (1/3) | $C_5H_{12}O$ |   | 88.15 |

**Tab. 3.1: continuation**

| substance (#) | molecular formula | structure | $M_W$ (amu) |
|---|---|---|---|
| pentan-2-ol* (2/4) | $C_5H_{12}O$ |  | 88.15 |
| methyl disulfide (12) | $C_2H_6S_2$ |  | 94.20 |
| hexan-2-one (7) | $C_6H_{12}O$ |  | 100.16 |
| heptan-2-one (10) | $C_7H_{14}O$ |  | 114.10 |
| 1-octen-3-ol* (15/16) | $C_8H_{16}O$ |  | 128.21 |
| octan-3-one (11) | $C_8H_{16}O$ |  | 128.21 |
| octan-3-ol* (13/14) | $C_8H_{18}O$ |  | 130.23 |
| cuminol (37) | $C_{10}H_{14}O$ |  | 150.10 |
| carvone* (33/34) | $C_{10}H_{14}O$ |  | 150.10 |
| verbenone* (29/30) | $C_{10}H_{14}O$ |  | 150.10 |
| *cis*-(-)-carveol* (32) | $C_{10}H_{16}O$ |  | 152.12 |

**Tab. 3.1: continuation**

| substance (#) | molecular formula | structure | $M_W$ (amu) |
|---|---|---|---|
| *trans*-(-)-carveol* (31) | $C_{10}H_{16}O$ |  | 152.12 |
| nonan-2-one (23) | $C_9H_{18}O$ |  | 142.24 |
| camphor* (17/18) | $C_{10}H_{16}O$ |  | 152.24 |
| fenchone* (8/9) | $C_{10}H_{16}O$ |  | 152.24 |
| borneol* (19/20) | $C_{10}H_{18}O$ |  | 154.25 |
| linalool* (21/22) | $C_{10}H_{14}O$ |  | 154.25 |
| terpinen-4-ol* (24/25) | $C_{10}H_{18}O$ |  | 154.25 |
| terpineol; alpha-* (27/28) | $C_{10}H_{18}O$ |  | 154.25 |
| geosmin* (35/35) | $C_{12}H_{22}O$ |  | 182.17 |

# 4 Material and methods

## 4.1 Passive sampler

The organic vapour monitor (OVM) 3500 by the 3M Company (St. Paul, MN, USA) was selected. This passive sampler is produced for one-time throw-away use. It is typically purchased in packages of ten and in lots of fifty.

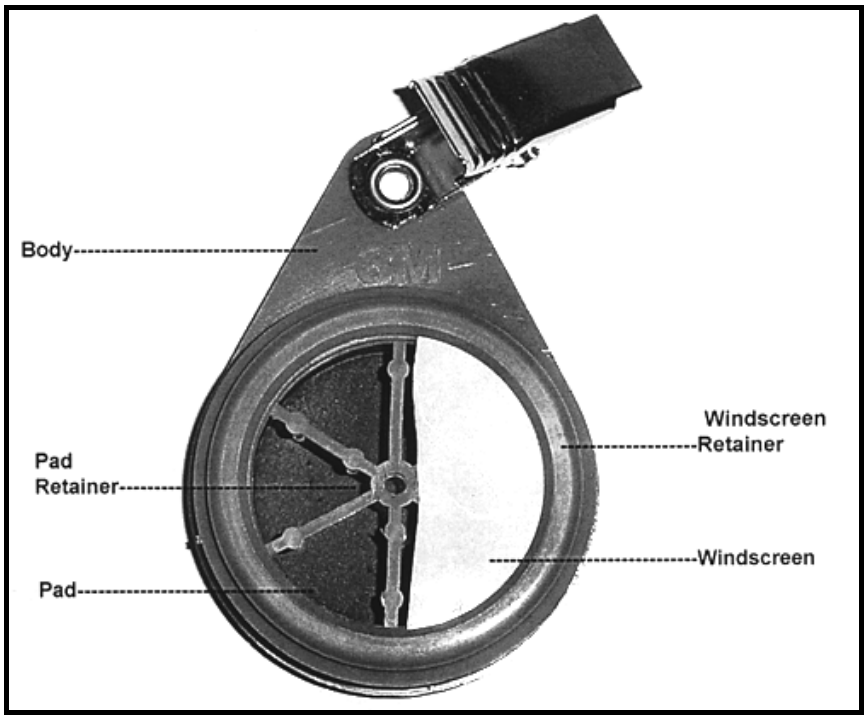



**Fig. 4.1: 3M organic vapour monitor (OVM) 3500 with part of the windscreen cut away to show the configuration (Cross, 2003).**

The OVM 3500 sampler is a combination of a diffusion and a permeation sampler with a badge configuration, where the inlet area is large relative to the diffusion path between the inlet and the adsorbent pad (A = 7.71 cm², L = 0.956 cm). The dosimeter consists of a draft shield (windscreen), a static diffusion layer and a adsorptive sink. The diffusion layer of air is between the inner face of a microporous polypropylene membrane and a plate of pressed charcoal as trapping medium. The windscreen prevents the inner diffusion gradient from

being disrupted by air movement in the vicinity of the inlet (see Fig. 4.1). Nevertheless, this air layer constitutes an additive diffusional resistance in series with the permeation resistance provided by the membrane. The sorbent out of 160 mg of activated coconut charcoal ensures that adsorbed target chemicals do not reverse diffuse once trapped onto the collection media. The sorbent of the OVM 3500 has a relative large surface area and, therefore, provides a high capacity for the analytes (see chapter 1.3.2, pg. 26).

## 4.2 Chemicals

The solvent diethyl ether anhydrous (> 99.5 % purity) was obtained from J.T. Baker (Deventer, The Netherlands). It was stabilized by the manufacturer with approximately 7 ppm butylated hydroxytoluene (BHT) as an antioxidant agent. Other solvents tested were: diethyl ether ACS reagent (≥ 99.5 %, BHT stabilized) and diethyl ether for HPLC (≥ 99.8 %, BHT stabilized), as well as diethyl ether reagent for residue analysis (≥ 99.8 %, ethanol stabilized) by Fluka Chemie GmbH (Buchs, Switzerland) and multisolvent grade diethyl ether (≥ 99.5 %, BHT stabilized) by Scharlau (Barcelona, Spain).

Solvents used for cleaning purposes were of pesticide residue analysis quality obtained from Scharlau (Barcelona, Spain) or of puriss. / p.a. grade from Fluka Chemie GmbH (Buchs, Switzerland). Ultra pure water was prepared by a two-stage water purification unit (Elgastat MAXIMA HPLC, Elga Ltd., Bucks, United Kingdom).

The following MVOC standard compounds were obtained from Fluka Chemie GmbH (Buchs, Switzerland): (-)-borneol (≥ 99.0 % GC, p.a. terpene standard for GC), (+)-borneol (≥ 98.0 % GC), 3-methyl butan-2-ol (≥ 98.0 % GC), S-(-)-2-methyl-1-butanol (≥ 95.0 % GC), (-)-camphor (≥ 99.0 % GC, p.a. standard for GC), (±)-camphor (≥ 95.0 % GC, synthetic), (-)-carvone (≥ 99.0 % GC, p.a. terpene standard for GC), (+)-carvone (≥ 99.0 % GC, p.a.

terpene standard for GC), (+)-fenchone (≥ 99.5 % GC, p.a. terpene standard for GC), 2-heptanone (≥ 98.0 % GC), 2-hexanone (≥ 99.5 % GC, p.a. standard for GC), (-)-linalool (≥ 95.0 % GC), methyl disulfide (≥ 98.0 % GC), 2-nonanone (≥ 99.5 % GC), 3-octanone (≥ 97.0 % GC), (±)-2-pentanol (≥ 99.5 % GC), (+)-terpinen-4-ol (≥ 99.0 % GC, terpene standard for GC), (-)-verbenone (≥ 99.0 % GC, terpene standard for GC), 3-methyl-butan-1-ol (≥ 99.8 % GC, p.a. standard for GC), 1-butanol (≥ 99.5 % GC, ACS reagent), 1-hexanol (≥ 99.0 % GC), ethyl isobutyrate (≥ 98.0 % GC), 2-pentanone (≥ 99.0 % GC), 2-methyl propan-1-ol (≥ 99.5 % GC, ACS reagent). DL-3-octanol (≥ 99 %), (-)-terpinen-4-ol (≥ 97 % mixture of isomers), 1-octen-3-ol (≥ 98 %), α-terpineol (≥ 97 %, mixture of α- and γ-terpineol), cuminol (≥ 97 %) and 2,5-dimethyl-pyrazine (≥ 98.5 %) were purchased from Acros Organics (Geel, Belgium). The (±)-geosmin (≥ 98 %) stock solution in methanol (≈ 2 mg $mL^{-1}$) as well as (1R)-(+)-camphor (≥ 98 %) were obtained from Sigma Chemical Corp. (St. Louis, MO, USA). (-)-Carveol (97 %; mixture of isomers), (-)-fenchone (≥ 98 %) and camphene (≥ 95 %) were from Aldrich Chemical Corp. (Milwaukee, WI, USA). More detailed information about the applied reference compounds is given in appendix 8.1 (pg. 143).

For GC separation and detection helium of 99.999 % purity, hydrogen of 99.995 % purity, nitrogen of 99.995 % purity, and synthetic air were purchased from Carbagas (Basel, Switzerland). Compressed air for general use was supplied by the in-house installation. All gases were further purified by oxygen (CP17970), moisture (CP17971) and charcoal (CP17972) gas cleaning filters (Varian-Chrompack International, Middelburg, The Netherlands).

## 4.3 Instrumentation

Laboratory glassware including volumetric flasks were obtained from VWR International (Dietikon, Switzerland) and Huber&Co AG (Reinach, Switzerland). Samples were injected into the GC-systems with 10 µL glass syringe of the 700 MICROLITER® Series of Hamilton Bonaduz AG (Bonaduz, Switzerland). 100, 250, and 500 µL glass syringes of the 1700 series as well as 1 and 5 mL glass syringes of the 1000 series from the same manufacturer were applied for solution handling and dilution. The higher volume syringes were equipped with a gastight TEFLON® PTFE-tipped plunger. Most of the lower volume syringes had a stainless steel plunger. All syringes had a glued stainless steel needle. Disposable gloves made from latex or nitrile were obtained from SafeMed E.D. Ltd (Zimmerwald, Switzerland).

A Mettler M 3 and a Mettler AE 100 balance (Mettler-Toledo (Schweiz) AG, Greifensee, Switzerland) were used for the preparation of reference solutions. Weighing precision was ± 0.1 mg over a range 0…100 g.

Three different GC-systems were employed: Two GC-systems equipped with a split/splitless injector and a flame ionization detector (FID) were used for method development, column conditioning, test of columns and compounds, and sample screening: a) HP 5890 Series II (Hewlett Packard, Palo Alto, CA, USA) and b) a Varian CP-3800 GC (Varian Analytical Instruments, Walnut Creek, CA, USA). The HP 5890 was operated with hydrogen as carrier gas and used for column conditioning. Data acquisition and handling for both systems was carried out on a Star GC workstation software (version 5.31, Varian Associates, Inc., Walnut Creek, CA, USA).

Most of the work was carried out on a third GC-system: Fisons MS8060CB (Fisons Instruments S.p.A., Rodano–Milan, Italy) equipped with a split/splitless injector (SSL 71) and a manual cold on-column injector (OCV 71). This GC-system was combined with a MD800 (Fisons Instruments, Manchester, United Kingdom) mass spectrometer equipped with a quadrupole mass analyser and a dynolite ion detector. Data acquisition and handling was predominantly performed by the Xcalibur$^{TM}$ software (version 1.2, Finnigan Corp., San Jose, CA, USA).

## 4.4 Procedures

### 4.4.1 Standard cleaning procedure

In order to minimize the risk of contamination all glassware and other small laboratory tools were treated with a standard cleaning procedure: The glassware was cleaned in a clean water laboratory dishwasher (Desinfektor by Miele AG, Spreitenbach, Switzerland), followed by a 24 hour soaking with alkaline detergent (Deconex by VWR International, Dietikon, Switzerland) in a dipping bath followed by several risings with ultra pure water. The cleaned material was heated in an oven at 375 °C for 3 hours. This practice has been shown to be sufficient for elimination contaminants (Oehme, 1998).

All syringes were cleaned by a special washing procedure to exclude any carry-over of substances and solutions. A home-made cleaning apparatus was employed (see Fig. 4.2). 12 mL of solvent were pushed through the syringe with a nitrogen pressure of 200 to 400 kPa (depending on the viscosity of the solvent). Then, the syringe was dried with nitrogen (Oehme, 1998).

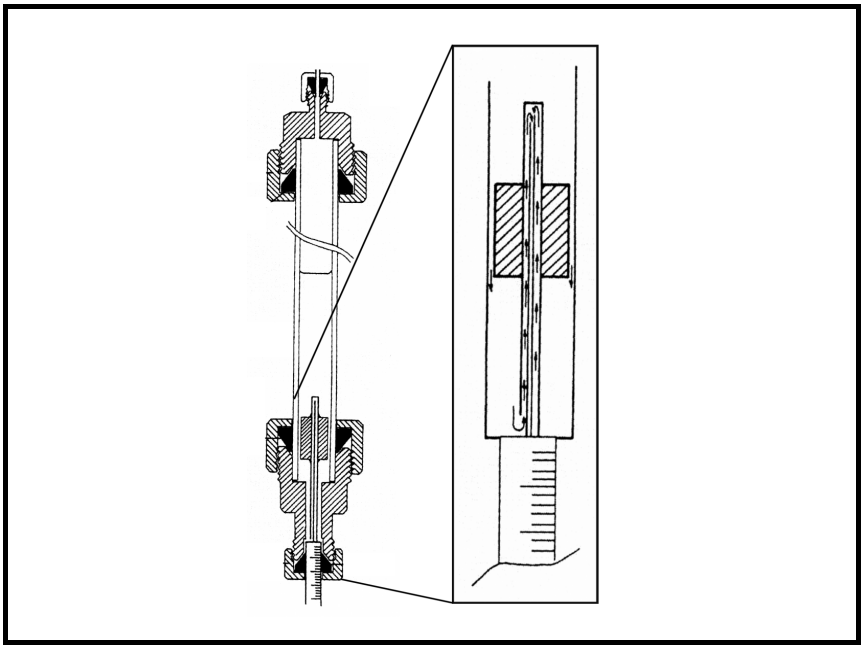


**Fig. 4.2: Home-made cleaning apparatus for syringe cleaning with magnification of the solvent flow path through the syringe needle (Oehme, 1998)**

### 4.4.2 Internal standard and recovery standard

An internal standard method was used for quantification. As a result of preliminary tests 1-chlorohexane (≥ 99.5 % p.a. standard for GC; GC) was selected as internal standard (ISTD) and chlorocyclohexane (≥ 98.0 % ACS reagent; GC) was selected as recovery standard (RSTD). Both compounds were obtained from Fluka Chemie GmbH (Buchs, Switzerland).

The ISTD 1-chlorohexane was added to each sample and the calibration solution. A concentration of 5 ng $\mu L^{-1}$ ISTD was found to be suitable for identification and quantification. For easier handling, the desorption solvent diethyl ether was laced with 1-chlorohexane before sampler desorption. The RSTD was added to the desorption extract of the sampler. 10 µL of a RSTD solution containing 50 ng $\mu L^{-1}$ chlorocyclohexane in diethyl ether were added to 100 µL of the desorption solution. This resulted in a concentration of 5 ng $\mu L^{-1}$ RSTD.

### 4.4.3 Preparation of reference and quantification standards

Stock solutions were prepared with diethyl ether. Most substances and solutions were highly volatile and difficult to dilute. Therefore, all stock solutions were prepared gravimetrically with volumes of 100 mL and concentrations of 1 μg $\mu L^{-1}$ following the recommendation of the Commission on Air Pollution Prevention of VDI and DIN – Standards Committee (KRdL) (Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 2001).

Graduated measuring flasks were used. 100 mg of compound was weighted into a 20 mL borosilicate glass screw vial (InfoChroma AG, Zug, Switzerland). For the complete transfer of the pre-diluted compound each of the weighing vials was flushed several times with small amounts of solvent. The transfer of both the pure reference compound into the vial as well as the pre-diluted compound into the flask was carried out by Pasteur pipettes.

For calibration the single stock solutions were combined as followed: one mixture contained 11 MVOCs the other one 10 MVOCs each at concentrations of 100 ng $\mu L^{-1}$ (Tab. 4.2). In a second step these pre-diluted MVOC solutions were combined together with a geosmin stock solution to one single mixture with a concentration of 10 ng $\mu L^{-1}$. These working standards as well as all following dilutions were prepared in diethyl ether. The dilutions were prepared with microsyringes adding the MVOC mixture to vials partially filled with diethyl ether to obtain total volumes of 1000 and 100 μL, respectively.

The concentration of each compound in the standard solutions ranged from 0.01 ng $\mu L^{-1}$ to 5 ng $\mu L^{-1}$. A calibration curve was generated, with concentrations of 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025, and 0.01 ng $\mu L^{-1}$.

**Tab. 4.2: MVOC calibration mixtures**

| calibration mixture | substance | |
|---|---|---|
| MVOC 1<br>(very low boiling points) | methyl disulfide<br>3-methyl-butan-2-ol*<br>pentan-2-ol*<br>hexan-2-one<br>2-methyl-butan-1-ol*<br>heptan-2-one | octan-3-one<br>octan-3-ol*<br>1-octen-3-ol*<br>nonan-2-one<br>fenchone* [2] |
| MVOC 2<br>(low boiling points) | linalool*<br>camphor*<br>terpinen-4-ol* [2]<br>borneol* [2]<br>α-terpineol*<br>(incl. + γ-terpineol) | verbenone*<br>*cis*-(-)-carveol*<br>*trans*-(-)-carveol*<br>cuminol<br>carvone*<br>geosmin* |

*chiral compound [2] self-prepared racemic mixtures

### 4.4.4 Handling and storage of ether solutions

Working solutions were placed in 1.1 or 2 mL Chromacol narrow opening borosilicate glass screw vials (Chromacol Ltd., Herts, United Kingdom) with green thermoset polytetrafluoroethylene (PTFE) lined screw caps (Qorpak, Bridgeville, PA, USA).

For long term storage of reference and stock solutions CERTAN® capillary bottles were purchased from LGC Promochem GmbH (Wesel, Germany) (Oehme, 1998). All standard compounds and stock solutions were stored in a freezer at –18 °C. Working solutions were ice-cooled and kept in a refrigerator at +4 °C overnight.

## 4.5 Sampling

The OVM 3500s (chapter 4.1, pg. 49) by the 3M Company (St. Paul, MN, USA) for the passive sampling were delivered in protective double-sealed aluminium cans. After removing a white plastic cap, the cans were opened with an attached pull flap just prior to sampling. Each can contained a ready to use OVM 3500 and a clear plastic closure cap (with two plugs)

for later use (see below). The samplers were exposed directly from the can without further action (see Fig. 4.3)

### 4.5.1 Simulation of passive sampling

During method development sampling of MVOCs with the passive sampler was simulated. Experiments were carried out in a fume hood of a rarely frequented lab room with a not directly exposed atmosphere (especially belonging any VOCs). The snap-on elution cap was installed instead of the permeation membrane on the 3M OVM 3500 sampler (chapter 4.5.2.1 and Fig. 4.3). Then, the plug of the elution cap was removed, the calibration solution injected and the sampler immediately resealed. A volume of 25 µL of MVOC standards of different concentrations (0...40 ng $\mu L^{-1}$) was brought directly onto the active charcoal. The duration of exposure was 60 minutes minimum. For each batch at least one untreated sampler was analyzed as a blank.

### 4.5.2 Passive sampler and real case sampling

#### 4.5.2.1 Application steps of the 3M OVM 3500 passive sampler

For indoor air sampling the passive sampler was placed ideally in the middle of the room in the human breathing zone at a height of about 1.5 to 2.0 m with a minimum distance of 50 cm to the next furniture, wall, window, or door. Special care was taken that the sampler was freely opened to the atmosphere and that its face was not obscured by anything. Generally, the monitors were exposed under normal living conditions for a sampling period of 28 days.

In parallel to indoor air measurements of the examined room a reference sample was obtained under the same conditions from a distant room of the same building or by sampling the outdoor air. If outdoor measurements were performed, the passive sampler was placed at a

rain-protected position next to a window. For direct sampling of a suspected mould contamination the sampler was place next to or nearby the spot in question. Sampling locations were always checked for possible interferences (e.g. air draft) or contaminants (e.g. fruity odours) to identify possible and exclude any cross-contaminations. All measurements refer to normal conditions of assumed 25 °C and 101.32 kPa.

Each sampler had a serial number given by the manufacturer and printed on the back of the sampler body for unequivocal identification. This number (e.g. RF 9036) made a clear assignment of sampler (including sampler lot), sampling site, protocol, and analysis during the whole sampling and analysis process possible.

At the end of the sampling period sampling was stopped by removing the white microporous polypropylene membrane and the retaining plastic O-ring from the sampler body. The monitor was immediately sealed according to the manufacturers instructions (Fig. 4.3).

In parallel to each indoor air sampling, blanks from one or two samplers of the same batch were evaluated in addition to the exposed samplers. All sampling blanks were subjected to the same handling as the loaded samplers except for the actual period of exposure.

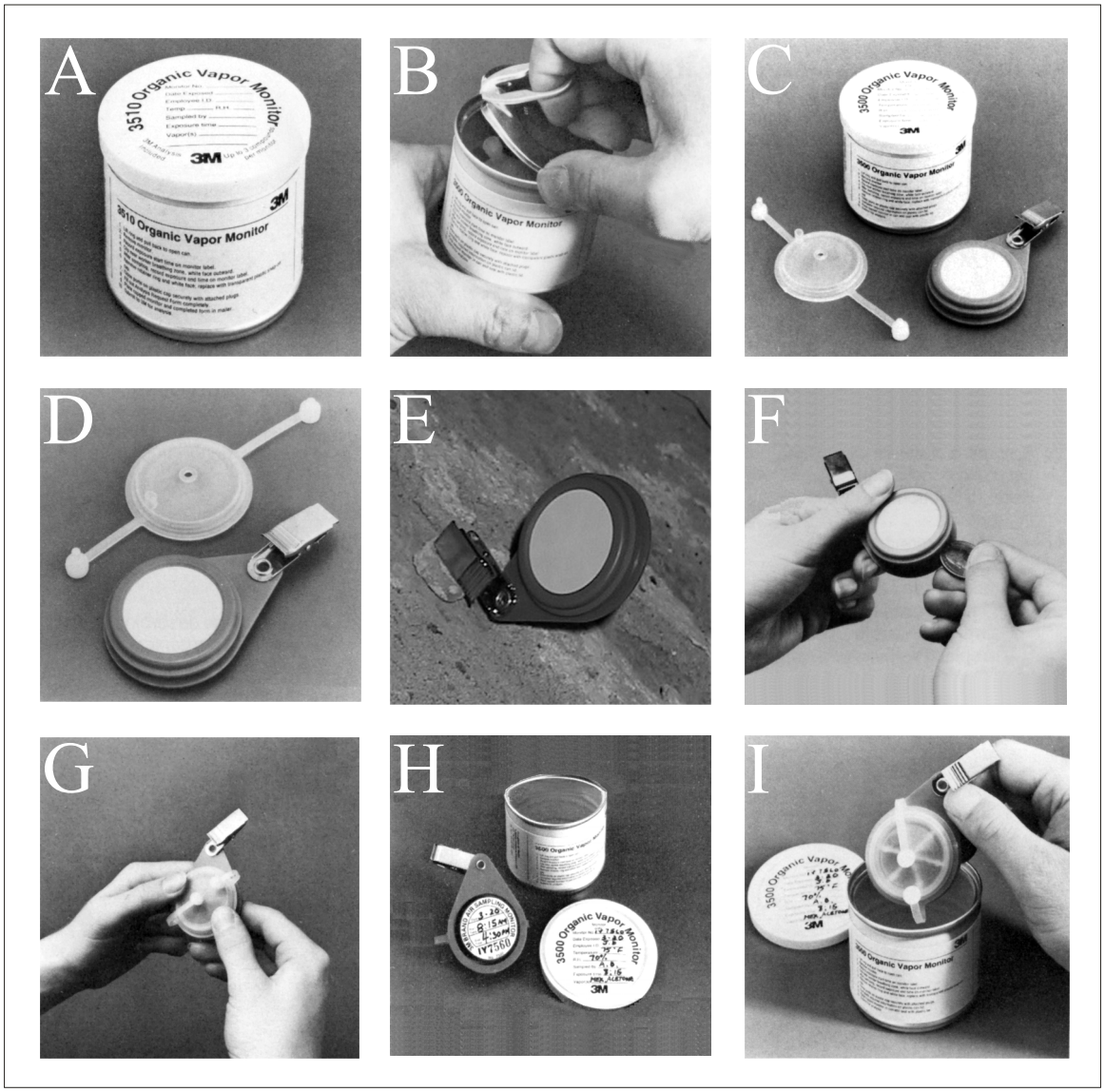



**Fig. 4.3: Application steps of the 3M OVM 3500 passive sampler:** (A) **Monitor and snap-on closure cap were packed and originally shipped in an aluminium can.** (B) **After removing the white plastic can lid, the can is opened by pulling up the airtight metal lid.** (C)/(D) **The ready to use sampler was removed from the can and the closure cap was kept for later use.** (E) **The sampler was installed for a 28 days sampling period to monitor the indoor air under normal living conditions.** (F) **After the sampling period the plastic ring and the white microporous polypropylene membrane were removed to stop sampling.** (G) **Immediately afterwards, the closure cap was firmly snapped onto the monitor body to definitively terminate the sampling.** (H) **For documentation monitor serial number and sampling data were noted.** (I) **The loaded monitor was placed into the can, sealed with the plastic lid, and shipped to the laboratory for analysis. (3M - Occupational Health and Environmental Safety Division, 1986)**

#### 4.5.2.2 Situation of an exemplary real case sampling site

Mould contamination was suspected in several apartments of a multi-story building in southern Germany. A few inhabitants showed non-specific health problems such as blocked and running nose, itching eyes, skin irritation, headache and mental fatigue that are typically

correlated to SBS. Preliminary examinations could not detect any microbial growth above the typical concentration. The number of airborne microorganisms did not differ from levels detected in unaffected buildings in the direct surrounding, both with respect to the total count determined by direct quantification of fungal spores in the air and concerning viable (cultivatable) microorganisms.

The relative humidity in the building was in a normal range of 38…42 % at a room temperature of 20 °C. Measurements showed that the material hydration of some apartment walls was increased ($a_w$ = 0.8…0.95) and several thermal bridges were found (surface temperature range between 9...14 °C). The investigation took place during February and March. The outside temperature averaged 12 °C with 70 % relative humidity.

Because visible signs of microbial growth could not be found and no significant amount of fungal or bacterial cells in the indoor environment could be detected, hidden mould growth was suspected within the building construction (e.g. in the insulation material, studding, underneath the wallpaper) or behind fitted furniture especially in the kitchen and bedroom. MVOC passive sampling was performed in the bedroom of one apartment. In parallel, a second passive sampler was installed in the staircase at the main entrance of the apartment building for background sampling.

### 4.5.3 Handling and storage of the loaded OVM 3500 samplers

After sampling, exposed samplers and blanks were kept cold and transferred as fast as possible to the laboratory for analysis. If analysis was delayed, the samplers were stored in an organics-free freezer at –18 °C to minimize the possible loss of sampled substances (3M -

Occupational Health and Environmental Safety Division, 1996; Pekar, 2000). However, none of the loaded OVM 3500 were stored for more than 14 days prior analysis.

### 4.5.4 Solvent desorption and recovery determination

For desorption, the plastic cap plug of the OVM 3500 was removed, and 1 mL of the desorption solvent diethyl ether containing 5 µg of the ISTD 1-chlorohexane was injected. The sampler was immediately resealed and placed on a cooling unit for 30 minutes with occasional gentle agitation to ensure maximum desorption. In general, a solvent extract of 400…600 µL could be recovered. For analysis 10 µL RSTD solution (50 ng $\mu L^{-1}$ chlorocyclohexane) were added to 100 µL of the desorption extract. The prepared extract was mixed and stored in an ether-tight vial, and immediately analyzed or stored cold for later analysis (3M - Occupational Health and Environmental Safety Division, 2001; Pekar, 2000; Pekar, 1996; Rehwagen et al., 2003).

The following procedure was applied to determine the amount of substance recovered from the OVM 3500 passive sampler by solvent desorption:

Unexposed 3M OVM 3500 monitors were spiked with 25 µL of the calibration mixtures containing between 0.4 ng $mL^{-1}$ and 200 ng $mL^{-1}$ of each of the monitored substances in diethyl ether. The samplers were then desorbed with 1 mL of diethyl ether containing 5 µg of the ISTD 1-chlorohexane. 10 µL of a solution containing 50 ng $mL^{-1}$ chlorocyclohexane in diethyl ether was added as RSTD to 100 µL of the desorption solution prior analysis by GC-MS.

## 4.6 Chromatographic separation and detection

Separation of the selected MVOCs was carried out by conventional and enantioselective high resolution gas chromatographic (HRGC) separation. The detection was mainly performed by mass spectrometry (MS) but also a FID was applied.

### 4.6.1 Non-enantioselective determination

Initially, the split/splitless injector was employed with all GC-systems. After evaluating several septa for the split/splitless injection, only Supelco Thermogreen LB-2 (Supleco, Buchs, Switzerland) and Agilent BTO (Bleed and Temperature Optimized) (Agilent Technologies (Schweiz) AG, Basel, Switzerland) septa were used. A deactivated glass liner of simple straight geometry without glass wool was applied. The split ratio (typically 10:1) of all three applied systems was regulated manually with a mechanical valve. For trace amounts splitless injection was applied. A splitless time of 2 minutes was selected. Injector temperature for split as well as splitless injections was set to 220 °C.

All samples were injected manually. An injection procedure consisted of a combination of the air plug method (Gottwald, 1994) and the hot needle injection technique (Rood, 1999). 1 to 2 µL of air were pulled into the syringe prior the sample. Additional air was introduced afterwards. The needle of the syringe was inserted into the injector and the injection was delayed for 2 to 3 seconds. This allowed heating of the needle by the hot injector and ensured a complete vaporization of all compounds.

In addition, cold on-column injection was applied at the Fisons MS8060CB GC. The required 10 µL glass syringes with a 70 mm stainless steel needle were custom-made from the 700 MICROLITER® Series of Hamilton Bonaduz AG (Bonaduz, Switzerland). The phenyl-methyl-polysiloxane deactivated retention gap (BGB Analytik AG, Anwil, Switzerland) was a

5 m long silica capillary tubing with an inner diameter (ID) of 0.32 mm. Retention gaps were mounted in front of the separation capillary by deactivated universal press-tight glass connectors (BGB Analytik AG, Anwil, Switzerland).

Three different stationary phases were used: 100 %-methylpolysiloxane (HP-1), 5 %-phenyl-95 %-methylpolysiloxane (HP-5 MS), and polyethylene glycol (PEG) (HP-INNOWax, and DB-Wax). The columns were obtained from Agilent Technologies (Schweiz) AG (Basel, Switzerland). All selected columns had the same dimensions of 30 m length, 0.25 mm ID and 0.25 µm film thickness of the stationary phase (compare appendix 8.3, Tab. 8.3, pg. 163).

Column conditioning was performed with a HP 5890 GC with hydrogen as carrier gas. All columns were evaluated with a modified Grob-test (appendix 8.2, pg. 159). Conditioned capillary columns were kept at 100 °C overnight and when not used for short periods of time.

First, method development and separation tests of the selected capillaries were performed with the GC-FID systems before the capillary were transferred to the GC-MS system. Depending on the stationary phases, different temperature programs provided best possible separation:

For the HP-1 and HP-5 MS capillary columns the oven temperature was held at an initial temperature of 40 °C for 2 min, then raised to 160 °C at a rate of 5 °C $min^{-1}$, held for 3 min, then raised to 250 °C at a rate of 30 °C $min^{-1}$, and finally held isothermally at 250 °C for 5 min (total run time: 37 min; temperature program 1).

For the HP-INNOWax capillary column the oven temperature was held at an initial temperature of 40 °C for 2 min, then raised to 130 °C at a rate of 5 °C $min^{-1}$, then further raised at 12 °C $min^{-1}$ to 170 °C, and finally raised to 250 °C at a rate of 30 °C $min^{-1}$, and held isothermally for 3 min (total run time: 29 min; temperature program 2).

For the DB-Wax capillary columns the oven temperature was held at an initial oven temperature of 35 °C for 5 min, followed by a raise to 175 °C at a rate of 9 °C $min^{-1}$, and then with a heating rate of 30 °C $min^{-1}$ to a final temperature of 250 °C isothermal for 7 min (total run time: 30 min; temperature program 3).

The analysis cycle from the start of one sample to the start of the next sample ranged from 35 to 60 minutes depending on the ambient temperature in the lab and resulting from the low starting temperature (35 °C). To shorten this cycle, an additional external cooling of the GC oven for faster temperature re-equilibration was provided by a mobile air conditioning system.

The column head pressure of the Fisons MS8060CB GC equivalent to the optimum performance of a linear gas velocity of 35 cm $s^{-1}$ He was determined to 79.3 kPa at 100 °C following the van Deemter equation (Rood, 1999). The automatically controlled inlet pressure for helium at the Varian CP-3800 GC yielded similar results at 68.9 kPa. This system was always used in constant pressure mode for reasons of comparison. The HP 5890 GC employed hydrogen as carrier gas. The optimum linear gas velocity of 50 cm $s^{-1}$ was achieved at 72.5 kPa for 100 °C.

Sample volumes of 1 µL were injected in the splitless mode whereas volumes of 2 µL in the split mode (split ratio 10:1) or by on-column injector. The HP 5890 and the CP-3800 GC gas chromatographs were equipped with a FID. Hydrogen at a flow rate of 30 mL $min^{-1}$ and synthetic air at a flow of 300 mL $min^{-1}$ were applied as combustion gases. The FID was run without make-up gas to achieve a lower background. The FID temperature was 250 °C.

Mass selective detection (MSD) was performed with a Fisons MD800 mass spectrometer coupled to the Fisons MS8060CB GC. The MSD was operated in the electron ionization (EI) mode at 70 eV. The temperature of the ion source was 200 °C and of the GC-MS transfer line 250 °C.

The instrument was tuned and calibrated regularly with heptacosafluorotributylamine (Fluka Chemie GmbH, Buchs, Switzerland)) that was performed automatically by the auto diagnosis routine of the Xcalibur™ software.

**Tab. 4.3: Summary of the non-enantioselective HRGC-MS method on the DB-WAX capillary column (for compound details refer to Tab. 5.2 and Fig. 5.7).**

| | | |
|---|---|---|
| Instrument | : | Fisons Instruments GC8060 with MD800 |
| GC-Column | : | DB-Wax: 30 m x 0.25 mm ID x 0.25 µm film thickness (J&W#:122-7032) |
| Retention gap | : | 5 m x 0.32 mm ID phenyl/methyl-deactivated (BGB: TSP-320450-D-10) |
| Carrier gas | : | He (50) at 40 cm $s^{-1}$; head pressure: 79.3 kPa |
| Injector | : | cold on-column (OVC 71) |
| Temperature program 3 | : | 35 °C (5 min) / 9 °C $min^{-1}$ / 175 °C (0 min) / 30 °C $min^{-1}$ / 250 °C (7 min): total run time 30 min |
| Detector | : | operated in electron impact mode with electron energy of 70 eV |
| Full Scan | : | scanning from *m/z* 35 to 275 at 2.5 scan $s^{-1}$ at a temperature of 200 °C; photo multiplier 380 V |
| SIM | : | 14 retention windows scanning between 4 and 10 points per second with a fixed dwell time of 0.05 s and a mass span of *m/z* 0.10 at a temperature of 200 °C; photo multiplier 380 V |

Samples were analyzed both in the scan and in the selected ion monitoring (SIM) mode. Scan delay time was 4 min. The scan range was *m/z* 35 to 275 with a scan frequency of 4 scans per second. In SIM mode one target ion together with one or two qualifier ions were selected for each compound (Tab. 5.2). The data from the MS analysis were recorded, integrated and quantified by the Xcalibur$^{TM}$ software. Tab. 4.3 summarizes the run parameters of the final non-enantioselective method.

In addition, a tandem column was tested consisting of a 5 %-phenyl-95 %-methylpolysiloxane phase and a PEG phase. The tandem column was constructed from a 30 m long capillary with an ID of 0.25 mm coated with a film thickness of 1 µm (HP–5: Agilent Technologies (Schweiz) AG, Basel, Switzerland) followed by a second capillary of 30 m length, 0.25 mm ID and 0.4 µm film thickness (HP-INNOWax: Agilent Technologies (Schweiz) AG, Basel, Switzerland).

The analysis of the selected 22 MVOCs by the tandem capillary column was performed on a second GC–MS system made available by Carbotech AG (Basel, Switzerland). The GC–MS set-up at Carbotech AG consisted of a HRGC-5300 Carlo Erba (Rodano – Milan, Italy) gas chromatograph equipped with an AS200 Carlo Erba (Rodano – Milan, Italy) auto sampler. A Finnigan MAT ion trap detector (ITD) (Finnigan Corp., San Jose, CA, USA) was used in the scan mode (*m/z* 48 to 201) with an ion trap temperature of 190 °C. Helium with a column pressure of 200 kPa was used as carrier gas, and 2 µL solvent was injected in the splitless mode at an injector temperature of 240 °C. The splitless injection time was set to 30 s and the applied temperature program was as follows: 35 °C for 13 min, then with 3 °C min$^{-1}$ to 65 °C, with 4 °C min$^{-1}$ to 120 °C, and then isothermal for 10 min. This was followed by a final raise of 49 °C min$^{-1}$ to 250 °C and a final time of 15 minutes (total time 59 min).

### 4.6.2 Quantification and method validation

Quantification and method validation of the non-enantioselective separation and detection was carried out with the internal standard method. The collected data were processed with the Xcalibur™ module Quan Browser and transferred to Microsoft® Excel 2000 for further evaluation. The compound amount was calculated via the signal to area ratio ($P_A$) of the analyte ($A_{analyte}$) and the ISTD ($A_{ISTD}$):

$$P_A = \frac{A_{analyte}}{A_{ISTD}} \tag{6}$$

Linear regression of a concentration range of 0.01…5 ng $\mu L^{-1}$ (pg. 55) was performed. Each standard was injected at least 3 times (n = 27). The dependence of linear relationship was proven for each compound by the linear least-squares statistics:

$$y = a + bx \tag{7}$$

where **y** is the estimated response/dependent variable (concentration), **b** is the slope (gradient) of the regression line, **a** is the intercept, and **x** is the independent variable (signal to area ratio).

Correlation analysis was performed by the more precise coefficient of determination ("Bestimmtheitsmaß"), $r^2$, and not by the Pearson correlation coefficient, r, which is misleading in suggesting the existence of more co-variation than exists.

The coefficient of determination gives the proportion of variation in y that can be attributed to an approximate linear relationship between x and y. The term $r^2$ takes two kinds of variation into account: First, the variation of y values around the regression line, i.e. around their predicted values, and second, the variation of y values around their own mean. Therefore, the

coefficient of determination describes the ratio of the explained variation versus the total variation:

$$r^2 = \left( \frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)}{\underbrace{\sqrt{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2 \sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2}}_{=r}} \right)^2 = \frac{\sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2 - \sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)^2}{\sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2} = 1 - \frac{\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)^2}{\sum_{i=1}^{n}\left(y_i - \bar{y}\right)^2} \qquad (8)$$

where $\bar{y}$ is the mean value of y, $\hat{y}$ is the predicted value of y, and n is the number of samples.

The precision of the results was evaluated by three experimental replication at different times for the full concentration range (0.01 to 5 ng $\mu L^{-1}$) of the MVOC standards including the passive sampler matrix. Each sample was analysed twice, a few extra repetitions of single solutions were performed.

The S/N-ratio to establish the limit of detection (LOD) and limit of quantification (LOQ), was determined by the Xcalibur™ module "signal-to-noise" from the chromatographic runs of the 50 and 100 pg $\mu L^{-1}$ calibration standards, respectively.

### 4.6.3 Enantioselective determination

Enantiomers possess identical properties in a symmetrical environments, but their properties may differ in unsymmetrical environments. 16 of the selected 22 MVOCs in this study are chiral (Tab. 3.1). The enantioselective separation was generally performed under the same conditions as the non-enantioselective separation of the compounds (chapter 4.6.1). The same

analytical systems were used and nearly all the procedures applied were identical. Only a few specific parameters were changed and optimized for the enantioselective methodology.

Enantioselective separation of the MVOCs was evaluated by the prepared standard mixtures (Tab. 4.2, pg. 56) including racemates of each chiral compound. The racemic mixtures of (±)-borneol, (±)-fenchone and (±)-terpinen-4-ol were not commercially available and were composed manually. Verbenone was only available as (-)-verbenone and also *trans*- and *cis*-carveol was only obtained as (-)-enantiomer. However, the (-)-verbenone standard obtained from Fluka Chemie GmbH (Buchs, Switzerland) contained trace quantities of the (+)-enantiomer (ee ≈ 70 % determined by the developed method) and was therefore suitable for further separation tests. Additionally, γ-terpineol was present in traces in the α-terpineol standard obtained from Acros Organics (Geel, Belgium).

Column conditioning, pre-tests, and first method developments were performed with the HP as well as the Varian GC-FID systems. The main work was carried out on a Fisons MS8060CB GC with the manual cold on-column injector (OCV 71) combined with a MD800 quadrupole mass spectrometer.

Enantioselective separation of the chiral MVOCs was achieved by capillary columns with stationary phases of derivatized cyclodextrins (see chapter 1.4.2). It was difficult to predict which stationary phase would solve the given separation problems and which would best separate all 14 chiral MVOCs (borneol, 2-methyl-butan-1-ol, 3-methyl-butan-2-ol, camphor, carvone, fenchone, geosmin, linalool, octan-3-ol, 1-octen-3-ol, pentan-2-ol, terpinen-4-ol, α-terpineol, verbenone). Therefore, an empirical process in combination with application experiences was applied for column selection and parameter optimization (Mosandl, 2002;

Restek Corporation, 1997). 15 different columns by several manufacturers were examined and are summarized in Tab. 4.4, Tab. 4.5, Tab. 4.6, Tab. 4.7, and Tab. 4.8.

**Tab. 4.4: Overview of selected and tested column for chiral MVOC separation manufactured by** BGB Analytik AG **(Zurich, Switzerland) (TBDMS = *tert.*-butyldimethylsilyl)**

| column notation | stationary phase | characteristic: length x ID x film thickness and temperature limits |
|---|---|---|
| BGB-172 | 20 % heptakis-(2,3,6-per-O-TBDMS)-β-cyclodextrin dissolved in BGB-15 | 20 m x 0.25 mm x 0.15 µm 250/270 °C |
| BGB-174 | 50 % heptakis-(2,3-diacetyl-6-O-TBDMS)-β-cyclodextrin dissolved in BGB-1701 | 30 m x 0.25 mm x 0.25 µm 220/240 °C |

**Tab. 4.5: Overview of selected and tested column for chiral MVOC separation manufactured by** Macherey-Nagel GmbH & Co. KG **(Düren, Germany) (TBDMS = *tert.*-butyldimethylsilyl)**

| column notation | stationary phase | characteristic: length x ID x film thickness and temperature limits |
|---|---|---|
| FS-LIPODEX® E | octakis-(2,6-di-O-pentyl-3-O-butyryl)-γ-cyclodextrin undissolved | 30 m x 0.25 mm x 0.25 µm 30 °C to 220/240 °C |
| FS-HYDRODEX® β-6TBDM | heptakis-(2,3-di-O-metyl-6-O-TBDMS)-β-cyclodextrin dissolved in OV-1701 | 30 m x 0.25 mm x 0.25 µm 230/250 °C |

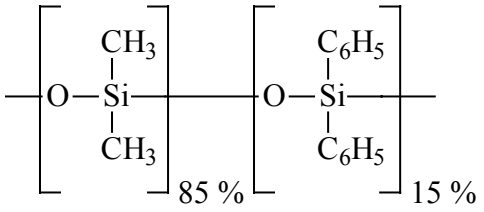
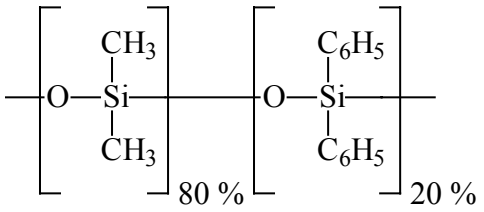
**Tab. 4.6: Overview of selected and tested column for chiral MVOC separation manufactured by** Swiss Federal Research Station **(Wädenswil, Switzerland) (TBDMS = *tert.*-butyldimethylsilyl)**

| column notation | stationary phase | characteristic: length x ID x film thickness and temperature limits |
|---|---|---|
| 23 x 0.25 x 0.14 | heptakis-(2,3-di-O-metyl-6-O-TBDMS)-β-cyclodextrin dissolved in OV-1701 | 23 m x 0.25 mm x 0.14 µm n.d. |

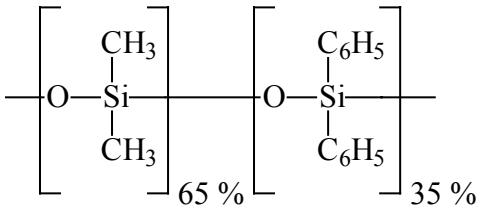
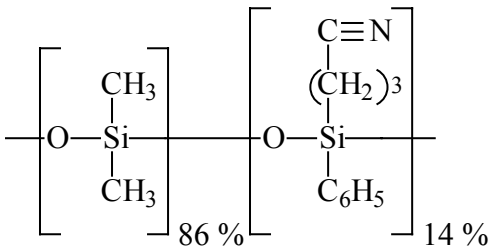
**Tab. 4.7: Overview of selected and tested column for chiral MVOC separation manufactured by** Supleco **(Buchs, Switzerland)**

| column notation | stationary phase | characteristic: length x ID x film thickness and temperature limits |
|---|---|---|
| SUPELCO cyclodextrin column selection kit I | | |
| α-DEX 120 | 20 % hexakis-(2,3,6-per-O-methyl)-α-cyclodextrin dissolved in SPB-35 | 30 m x 0.25 mm x 0.25 µm 30 °C to 240/250 °C |
| β-DEX 120 | 20 % heptakis-(2,3,6-per-O-methyl)-β-cyclodextrin dissolved in SPB-35 | 30 m x 0.25 mm x 0.25 µm 30 °C to 240/250 °C |
| γ-DEX 120 | 20 % octakis-(2,3,6-per-O-methyl)-γ-cyclodextrin dissolved in SPB-35 | 30 m x 0.25 mm x 0.25 µm 30 °C to 240/250 °C |
| SUPELCO cyclodextrin column selection kit II | | |
| β-DEX 225 | 25 % heptakis-(2,3-di-O-acetyl-6-O-TBDMS)-β-cyclodextrin dissolved in SPB-20 | 30 m x 0.25 mm x 0.25 µm 30 °C to 240/250 °C |
| γ-DEX 225 | 25 % octakis-(2,3-di-O-acetyl-6-O-TBDMS)-γ-cyclodextrin dissolved in SPB-20 | 30 m x 0.25 mm x 0.25 µm 30 °C to 240/250 °C |
| β-DEX 325 | 25 % heptakis-(2,3-di-O-methyl-6-O-TBDMS)-β-cyclodextrin dissolved in SPB-20 | 30 m x 0.25 mm x 0.25 µm 30 °C to 240/250 °C |

**Tab. 4.8: Assignment of the non-enantioselective stationary phases to dissolve the CDs according to the manufacturers (BGB:** BGB Analytik AG **(Zurich, Switzerland); OV:** Ohio Valley Specialty Chem. **(Marietta, OH, USA); PS:** Petrarch Systems **(Bristol, PA, USA); SPE:** Supleco **(Bellefonte, PA, USA)) – Phases are listed in order of increasing polarity.**

| phase notation | stationary phase | structure |
|---|---|---|
| BGB-15<br>PS-086 | 15 % diphenyl<br>85 % dimethyl polysiloxane |  |
| SPB-20<br>(OV-7) | 20 % diphenyl<br>80 % dimethyl polysiloxane |  |

**Tab. 4.8: continuation**

| phase notation | stationary phase | structure |
|---|---|---|
| SPB-35 | 35 % diphenyl<br>65 % dimethyl polysiloxane |  |
| BGB-1701<br>OV-1701<br>(CP-Sil 19) | 14 % cyanopropylphenyl<br>86 % dimethyl polysiloxane |  |

The procedure for selecting the optimum chiral column and for determining instrument parameters for separating chiral compounds was subdivided into several steps. First, separation abilities of the columns were tested with a few single compounds on the GC-FID systems and some isothermal chromatographic runs were performed. In the second step, the MVOC standard mixture (see Tab. 4.2) was used in combination with several different temperature programs, depending on the non-enantioselective chromatographic separation (chapter 4.6.1, pg. 63). In combination with the different temperature programs, the linear gas velocity was also varied and almost doubled for maximum enantiomeric resolution ($cR_s$). The head pressure of the Fisons MS8060CB GC was adjusted to 172.4 kPa at 100 °C resulting in a helium carrier gas velocity of 60 cm $s^{-1}$. The automatically controlled inlet pressure of the helium carrier gas at the Varian CP-3800 GC was set to 134.4 kPa. For the HP 5890 GC with hydrogen as carrier gas the head pressure was determined to 152 kPa at 100 °C resulting in a linear gas velocity of 75 cm $s^{-1}$.

All tested and applied enantioselective capillary columns were routinely equipped with the standard phenyl-methyl-polysiloxane deactivated retention gap (5 m tubing with an ID of 0.32 mm; see retention gap pg. 63).

The conditioned and used enantioselective capillary columns were kept at 80 °C with all applied systems overnight and when not used for short periods of time. In order to safeguard the enantioselective columns their upper temperature limits were never exceeded and no unnecessary high temperature runs were ever made on these columns. One exception was made; the upper temperature limit was run once for column conditioning following the recommendation of Bicchi et al. (Bicchi et al., 1991).

Thermal stability of the non-bonded CSPs was tested by 3 different temperature programs. The bleeding tests were distinguished by high temperature rates and isothermal sequences at different moments during the GC program as summarized in Tab. 4.9.

**Tab. 4.9: Selected conditions for bleeding tests of the enantioselective capillary columns**

| **Gas chromatograph** | **HP 5890 Series II** (Hewlett Packard, Palo Alto, CA, USA) | **Fisons MS8060CB** (Fison Instruments S.p.A., Rodano – Milan, Italy) |
|---|---|---|
| Injector | split-splitless | on-column (OV 71) |
| Split | splitless for 2 min<br>split: split ratio 10:1 | --- |
| Injector temperature | 220 °C | --- |
| Carrier gas | hydrogen (99.995 %) | helium (99.999 %) |
| Head pressure | 152 kPa | 172.4 kPa |
| Temperature of GC-MS transfer line | --- | 250 °C |
| Bleed test 1 | 40 °C (10 min) / 15 °C $min^{-1}$ / 220 °C (20 min): total time 42 min | |
| Bleed test 2 | 40 °C (10 min) / 10 °C $min^{-1}$ / 80 °C (5 min) / 10 °C $min^{-1}$ /<br>100 °C (5 min) / 10 °C $min^{-1}$ / 220 °C (22 min): total time 60 min | |
| Bleed test 3 | 40 °C (10 min) / 20 °C $min^{-1}$ / 220 °C (21 min): total time 40 min | |
| Detector | FID<br>hydrogen flow rate 30 mL $min^{-1}$<br>synthetic air flow rate<br>300 mL $min^{-1}$ | MD800 mass spectrometer<br>m/z 35…275 |
| Detector temperature | 250 °C | 200 °C |
| Software | Star GC workstation software<br>(Varian Associates, Inc., Walnut Creek, CA, USA) version 5.31 | Xcalibur™<br>(Finnigan Corp., San Jose, CA, USA)<br>version 1.2 |

Mass selective detection (MSD) was performed with the MD800 in the scan and selected ion monitoring (SIM) mode analogous to the non-enantioselective methodology (see pg. 65). Recording of spectra in the full scan mode was carried out with a mass range of *m/z* 35 to 275. The solvent delay was set to 4 min at a linear gas velocity of 35 cm $s^{-1}$ and to 2.5 min with the increased linear gas velocity before acquiring data. The SIM was based on one target ion together with one or two qualifier ions that were selected for each compound. The data from the MS analysis were recorded and identified by the Xcalibur™ software. Tab. 4.10 summarizes the run parameters of the final enantioselective method.

**Tab. 4.10: Summary of the enantioselective HRGC-MS method on the BGB-174 capillary column (for compound details refer to Tab. 5.8 and Fig. 5.17).**

| | | |
|---|---|---|
| Instrument | : | Fisons Instruments GC8060 with MD800 |
| GC-Column | : | BGB-174: 30 m x 0.25 mm ID x 0.25 µm film thickness (BGB#:27430-025) |
| Retention gap | : | 5 m x 0.32 mm ID phenyl/methyl-deactivated (BGB: TSP-320450-D-10) |
| Carrier gas | : | He (50) at 60 cm $s^{-1}$ at 100 °C; head pressure: 172.4 kPa |
| Injector | : | cold on-column (OVC 71) |
| Temperature program 4 | : | 35°C (8 min) / 2 °C $min^{-1}$ / 60°C (0 min) / 8 °C $min^{-1}$ / 170°C (0 min) / 30 °C $min^{-1}$ / 210°C (4 min): total run time 40 min |
| Detector | : | operated in electron impact mode with electron energy of 70 eV |
| Full Scan | : | scanning from *m/z* 35 to 275 at 2.5 scan $s^{-1}$ at a temperature of 200 °C; photo multiplier 380 V |
| SIM | : | 15 retention windows scanning between 3 and 10 points per second with a fixed dwell time of 0.05 s and a mass span of *m/z* 0.10 at a temperature of 200 °C; photo multiplier 380 V |

## 4.6.4 MVOC detection of real case sites

### 4.6.4.1 Compound identification

MVOC identification was based on the retention times of the compounds determined during method development (non-enantioselective methodology: Fig. 5.7 (pg. 95), enantioselective methodology: Fig. 5.17 (pg. 117)) and reference measurements during the analysis sequence (Tab. 5.9). Compounds were rejected and regarded as volatile impurities or general contaminants rather than MVOCs, if the retention times deviated by more than 0.05 to 0.08 min (3 to 5 s). Deviations of the RT of ± 0.25 % were supposed acceptable especially because of manual injection. Additionally, shifts in RT were compensated by the ISTD through the relative retention time (RRT, definition see appendix 8.5, pg. 168). Extra reference points in the chromatogram were given by the RSTD and the ether stabilizer BHT.

For identification by MS the chromatographic signal had to exceed the *S/N*-ratio of 3:1 (LOD). In scan mode (*m/z* 35 to 275) the complete mass spectrum was compared with the

recorded reference spectra. In SIM mode the MS identification criteria was the abundances ratio of the selected fragment ions. A derivation of more than 15 % of the observed abundance ratio compared to the determined ratio of the reference compound was not accepted (Oehme, 1996).

Additionally, the "Library Browser" in the Xcalibur™ data system, including the NIST/EPA/NIH Mass Spectral Database (version 1.7 ©1999, National Institute of Standard an Technology, Gaithersburg, MD, USA) and the Wiley Registry of Mass Spectral Data (7th edition ©2000, Wiley Intersicence, New York, NY, USA) containing about 392'000 spectra, were available for compound identification. In agreement of at least 80 % between the recorded and the library mass spectra was set as threshold for positive identification. Spiking experiments were carried out in a few cases, if matrix interference effects were assumed and peak purity had to be proven.

#### 4.6.4.2 Calculation of MVOC concentration in air

The amount of the MVOCs adsorbed on the active charcoal of the passive sampler was determined by the signal/area-ratio $P_A$ (Eq. (6), pg. 67), if the peak signal exceeded the LOQ (*S/N*-ratio of 10:1). The calculation was based on the calibration function (Tab. 5.3) under consideration of the blank values. The absolute mass amount **m** (pg) of each adsorbed component extracted from the charcoal pad was obtained by equation (9):

$$m = \left( \frac{m_D}{V_{Inj.}} * V_{Desorp.} \right) - m_{blank} \qquad (9)$$

where $\mathbf{m_D}$ is the gas chromatographic detected amount of the pollutant (pg) in the injected volume $\mathbf{V_{Inj.}}$ (in general 2 µL) of the desorption extract, $\mathbf{V_{Desorp.}}$ is the total volume of solvent used for the solvent desorption of the passive sampler (in general 1000 µL), and $\mathbf{m_{blank}}$ (pg) is

the total determined blank amount of the target analyte in the dosimeter. For the calculations of the true amounts the calculated values of the absolute mass amounts were corrected by the absolute recovery coefficients (RC(%)*$10^{-2}$) of the selected MVOCs according to the method validation (see Tab. 5.5).

Following Fick's first law of diffusion (Eq.(1), pg. 32) under the consideration of the OVM 3500 characterizing parameters the determined value of **m** could be converted into the mean mass concentration $\mathbf{c_m}$ (ng $m^{-3}$) of the pollutant in the (indoor) air during the exposure period:

$$c_m = \frac{m}{t * \left( \frac{L}{D * A} * \frac{1}{60} \right)} * 10^3 = \frac{m}{t * SR} * 10^3 \qquad (10)$$

where **t** is the time of exposure (in general 40320 min = 28 days), **SR** is the sampling rate of the compound in question for the regarded passive sampler ($cm^3$ $min^{-1}$; Tab. 4.11), **L** is the length of diffusion path (cm), **A** is the cross-sectional area of the diffusion path ($cm^2$), and **D** is the molecular diffusion coefficient of a particular gas or vapour in air at normal temperature of 25 °C and standard pressure of 101.325 kPa ($cm^2$ $s^{-1}$).

Often, the mean molar concentration instead of the mean mass concentration was denoted. To convert the value of **m** (pg) into the mean molar concentration **c** (mol $m^{-3}$) the molecular weight $\mathbf{M_W}$ of a particular gas or vapour (g $mol^{-1}$) (see Tab. 4.11) has to be taken into consideration:

$$c = \frac{\frac{m}{M_W}}{t * SR} * 10^{-6} = \frac{n}{t * SR} * 10^{-6} \qquad (11)$$

where **n** is the molar amount of detected substance (mol).

Alternatively, the concentration of organic vapour in the sampled air may be expressed in parts per billion (ppb):

$$c(ppb) = \frac{c_m * V_{Mol}}{M_W * 10^9} \quad (12)$$

where $\mathbf{V_{Mol}}$ is the molar volume of dry air at normal temperature of 25 °C and standard pressure of 101.325 kPa with a value of 0.02445 $m^3\ mol^{-1}$.

For the principal share of the 22 MVOCs the SRs were obtained from the 3M company by personal communication (see Tab. 4.11). Most of the values were calculated based on the diffusion constants according to a procedures of Hirschfeld (Friz and Freise, 1951) and Pannwitz (Pannwitz, 1983; Pannwitz, 1991). Pannwitz proved that the general deviation between the calculated and empirical determined values of D for most compounds was not greater than 5 % (Pannwitz, 1983).

**Tab. 4.11: Molecular weight ($M_W$) and sampling rates (SR) of the monitored 22 MVOCs for the 3M OVM 3500 passive sampler (3M - Occupational Health and Environmental Safety Division, 2002; 3M - Occupational Health and Environmental Safety Division, 1999).**

| substance | $M_W$ (amu) | SR ($cm^3\ min^{-1}$) | substance | $M_W$ (amu) | SR ($cm^3\ min^{-1}$) |
|---|---|---|---|---|---|
| 3-methyl-butan-2-ol* | 88.15 | 32.7 | verbenone* | 150.22 | 21.7 |
| pentan-2-ol* | 88.15 | 31.2 | carvone* | 150.22 | 20.5 |
| 2-methyl-butan-1-ol* | 88.15 | 31.1 | fenchone* | 152.23 | 21.6 |
| methyl disulfide | 94.2 | 33.9 | camphor* | 152.23 | 21.4 |
| hexan-2-one | 100.16 | 29.7 | *trans*-(-)-carveol* | 152.23 | 20.3 |
| heptan-2-one | 114.19 | 27.7 | *cis*-(-)-carveol* | 152.23 | 20.3 |
| octan-3-one | 128.21 | 26 | terpinen-4-ol* | 154.25 | 20.1 |
| 1-octen-3-ol* | 128.21 | 25.6 | alpha-terpineol* | 154.25 | 20 |
| nonan-2-one | 142.24 | 24.4 | borneol* | 154.25 | 20.7 |
| linalool* | 150.22 | 23.5 | geosmin* | 182.3 | 17.8 |
| *1-chlorohexane (ISTD)* | 120.62 | 27.4 | *chlorocyclohexane (RSTD)* | 118.6 | 26.3 |

Although, by definition all measurements and calculation in this study referred to the normal conditions (T = 25 °C, p = 101.325 kPa), it cannot be excluded that the calculated values of

the time-weighted average (TWA) concentrations were to some extend influenced by temperature (**T** (°C)) and pressure (**p** (kPa)) deviation:

$$c_{corr} = c_m * \frac{101.325}{p} * \frac{(T + 273)}{298} \tag{13}$$

However, the temperature correction factors for samples collected at temperatures other than 25 °C were determined to 0.99 for 31 °C and 1.01 for 19 °C (3M - Occupational Health and Environmental Safety Division, 1999) and was therefore suggested negligible for the indoor sampling and the calculations.

# 5 Results and Discussion

## 5.1 Method evaluation by simulated sampling

### 5.1.1 Passive sampler

The organic vapour monitor (OVM) 3500 by the 3M Company (St. Paul, MN, USA) was selected out of several commercially available samplers. All the pre-work, the extensive validation, the good experience and especially the ideal sampling rate (SR) for the indoor environment favoured the 3M OVM 3500 badge-type sampler above others e.g. the Dräger ORSA 5 tube-type monitor or the Radiello 3310 radial-type sampler.

The selected passive sampler has been commercially available since 1978 and used for both occupational and environmental exposure assessments. It meets validation requirements of several authorities in Europe and North America, and is included in ISO 16200-2 (CEN/TC 264 "Luftbeschaffenheit", 2000). Additionally, several applications of the OVM 3500 for the determination of VOC concentrations in indoor and outdoor air at environmental trace conditions proved the suitability down to the ng $m^{-3}$-range even with long sampling periods of several weeks (Begerow et al., 1995; Blome, 1988; Blome and Hennig, 1985; Blome and Hennig, 1985; Blome and Hennig, 1986; Pekar, 2000; Pekar, 1996). Furthermore, the precision of the 3M passive sampler at trace level was studied over a 31-day period based on triplicate sampling. For those compounds whose mean concentration was > 0.2 µg $m^{-3}$ the precision was about 13 % of the mean concentration (25 % at a 95 % confidence level) (Rehwagen et al., 2003; Shields and Weschler, 1987).

SRs of the OVM 3500 have been (empirically) determined in a range of 15 to 50 mL $min^{-1}$ for at least 200 VOCs (Health and Safety Executive Committee on Analytical Requirements (CAR) - Working Group 5, 2001). However, Cross showed within the scope of a re-evaluation of the OVM 3500 dosimeter that the SRs measured from the position of the adsorbent pad were lower than those reported by the manufacturer (Cross, 2003).

## 5.1.2 Reference standards

### 5.1.2.1 Internal standard and recovery standard

The internal standard method was applied to compensate variability of experimental conditions (e.g. sample preparation, matrix interference, GC injection and compound vaporization) that can adversely affect the precision of the data. Compounds suitable as standards were identified by preliminary tests. Several candidate compounds were selected including 1-chloropentane, 1-chlorohexane, 3-chlorotoluene, chlorocyclohexane as well as some mono-chlorinated alcohols. None of them were present in indoor air samples or reported as indoor contaminants. All of them matched the volatility range of the monitored compounds and could be obtained in high analytical quality. Finally, 1-chlorohexane was chosen as ISTD and chlorocyclohexane was chosen as RSTD.

Even though these two reference compounds did not have the same properties for detection as the MVOCs (such as similar physicochemical properties (Brodacz, 1997; Herbold and Schmitt, 1998)), their suitability was confirmed during the MVOC method development. Both compounds did not interfere with other analytes during chromatographic separation and showed good detection properties. 1-chlorohexane was also suggested for VOC analytics by the VDI 2100 Blatt 2 Part 2 (Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 2001) as ISTD. In addition, both compounds have been used as reliable standards for VOC

analysis before (Carbotech AG, 2003; Pekar, 2000). They were added to the samples at concentrations of 5 ng $\mu L^{-1}$ to ensure detectability even at higher background levels.

Additionally, the diethyl ether stabilizer 2,6-bis(1,1-dimethylethyl)-1-hydroxy-4-methyl benzene (BHT; see appendix 8.1.3, pg. 157 for details) could be used as a semiquantitative standard for controlling retention times. This compound was added by the manufacturer and was thus present in all solutions. However, it could not be used for quantifications, since the added concentration was not exactly known (Baker labelled the ether with the remark "about 7 ppm") and a part of BHT was adsorbed on the active charcoal of the passive sampler during desorption.

#### 5.1.2.2 Preparation of reference and quantification standard solutions

It is common practice to use some combination of gravimetry and volumetric measurements for the preparation of standard solutions even of very volatile compounds. Microsyringes and pipettes can introduce errors of up to 3 % (Health and Safety Laboratory, 1997).

The stepwise preparation of the calibration and reference solutions of the 22 MVOCs was necessary because geosmin could only be obtained as stock solution of 2 mg $mL^{-1}$ in methanol. The high-concentration standards with only 11 MVOCs were divided into a group of very high volatility and therefore early elution compounds and a later eluting group consisting of mainly terpenes (Tab. 4.2).

The stability of the standard solutions was controlled by comparison of selected compounds with freshly prepared solutions. No changes were observed during 18 months although randomized samples were taken regularly. Racemization of the pure enantiomer compounds

in mixtures and during storage or analysis (Ahuja, 2000; Mosandl et al., 2000) was not observed. Also condensation reactions between alcohols and ketones were not observed as mentioned in EN ISO 16017-2 (Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 2003).

#### 5.1.2.3 Handling and storage of ether solutions

Handling of diethyl ether solution was problematic due to the high volatility of the solvent. Only the combination of the Chromacol narrow opening screw vials (Chromacol Ltd., Herts, United Kingdom) and Qorpak Teflon® lined screw caps (Qorpak, Bridgeville, USA) was ether-tight for a period of 8…12 weeks. The solutions were always ice-cooled during handling to minimize vaporization. Refrigeration of the solutions at +4 °C overnight and when not in use improved their life span to 6 months.

Long-term storage losses were prevented by using CERTAN® capillary bottles (Oehme, 1998; Promochem, 2001) stored in a freezer at -18 °C. With these capillary bottles no losses of solvent or compounds were noticed during 18 months.

### 5.1.3 Sampling

Passive sampling is preferably calibrated by loading the absorption material with appropriately prepared gas mixtures in a standardized room, since this procedure agrees best with the sampling procedure (Rodriguez et al., 1982). Unfortunately, this calibration could not be applied due to lack of gas chambers and exact gas flow measurements in the laboratory.

The alternative and selected procedure for sampling simulation and calibration involved direct injection of a known amount of dissolved MVOCs on the activated charcoal using a microliter

syringe. After keeping the sealed sampler at room temperature overnight, the sample was eluted with diethyl ether and analysed. Experiments showed that the duration of exposure for the simulated sampling of the MVOC onto the passive sampler did no influence the adsorption as long as the exposure exceeded 1 h. Concentration variations were observed due to the missing phase equilibrium (Tab. 5.1).

**Tab. 5.1: Time dependent recovery of some MVOCs from the 3M passive sampler**

| compound | recovered amount (%) | | |
|---|---|---|---|
| | after 30 min | after 1 h | after 24 h |
| hexan-2-one | 83 | 121 | 122 |
| octan-3-ol | 56 | 73 | 73 |
| camphor | 88 | 97 | 105 |
| geosmin | 95 | 123 | 124 |

### 5.1.4 Solvent desorption

Atmospheric humidity and temperature had a great influence on the formation of by-products from the diethyl ether. Especially during summertime the solvent chromatogram showed an additional peak that was identified by the spectra library as acetic acid or acetic acid anhydride (Fig. 5.1 and Fig. 5.2). This phenomenon was not known by the manufacturer and could not be dealt with by different solvent handling such as refrigerating or protective gas. Even though diethyl ether was kept protected from light and was stored in the original amber glass bottles, and purity was checked regularly including peroxide contents (analytical peroxide test strips Merckoquant®: Merck KGaA, Darmstadt, Germany). However, these compounds did not interfere with the MVOC analysis. Peroxides were never detected.

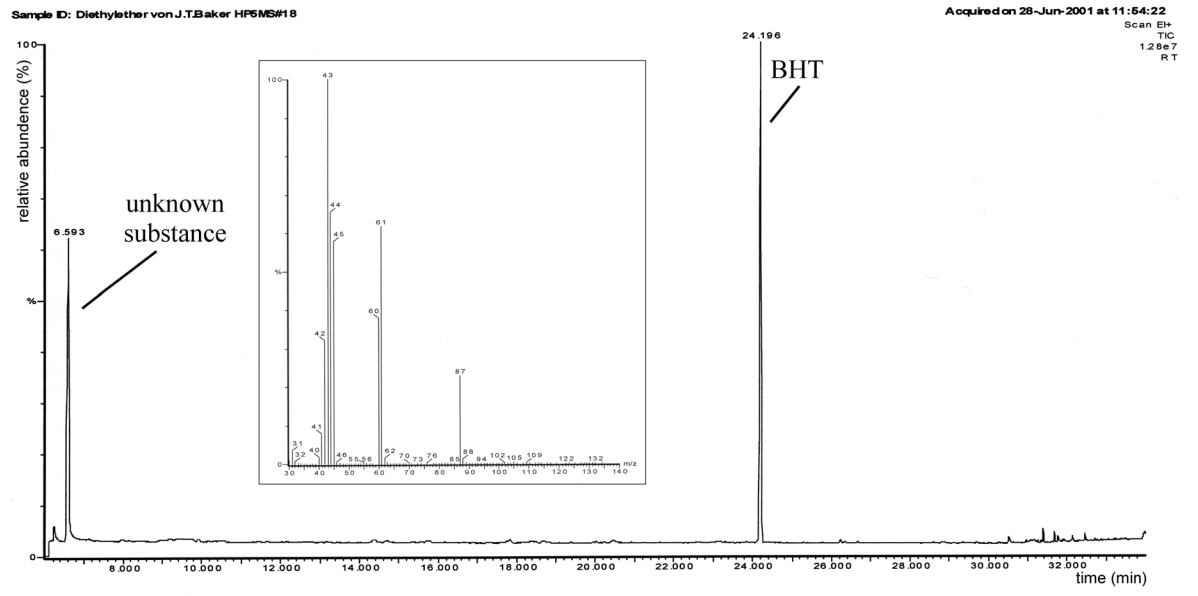



**Fig. 5.1: By-product of the diethyl ether identified by the mass spectral reference library as acetic acid or acetic acid anhydride. Total ion chromatogram was recorded by the Fisons GC-MS system (splitless mode).**

### 5.1.5 Blanks

The blank values of the passive sampler varied with the sampler batch. Their fluctuation was checked by desorbing unexposed and untreated samplers in the same way as loaded samplers. Main contaminants in the blank could be assigned to the plastic housing of the OVM 3500, particularly caprolactam (6-amino hexanoic acid lactam, Fig. 5.2 compound B6). These observations were in agreement with those of the 3M company as well as the work of Pekar (Pekar, 2000). The higher concentrated impurities did not interfere with the chromatographic separation of the target compounds. The overall blank was acceptable for indoor air monitoring.

To obtain a zero reading of the adsorbent matrix and to mask out interfering blank compounds of the chromatograms the analysis of the blanks was carried out in parallel to real case

samplers using the same conditions. One or two blank and unexposed cartridges were taken from the same package and lot.

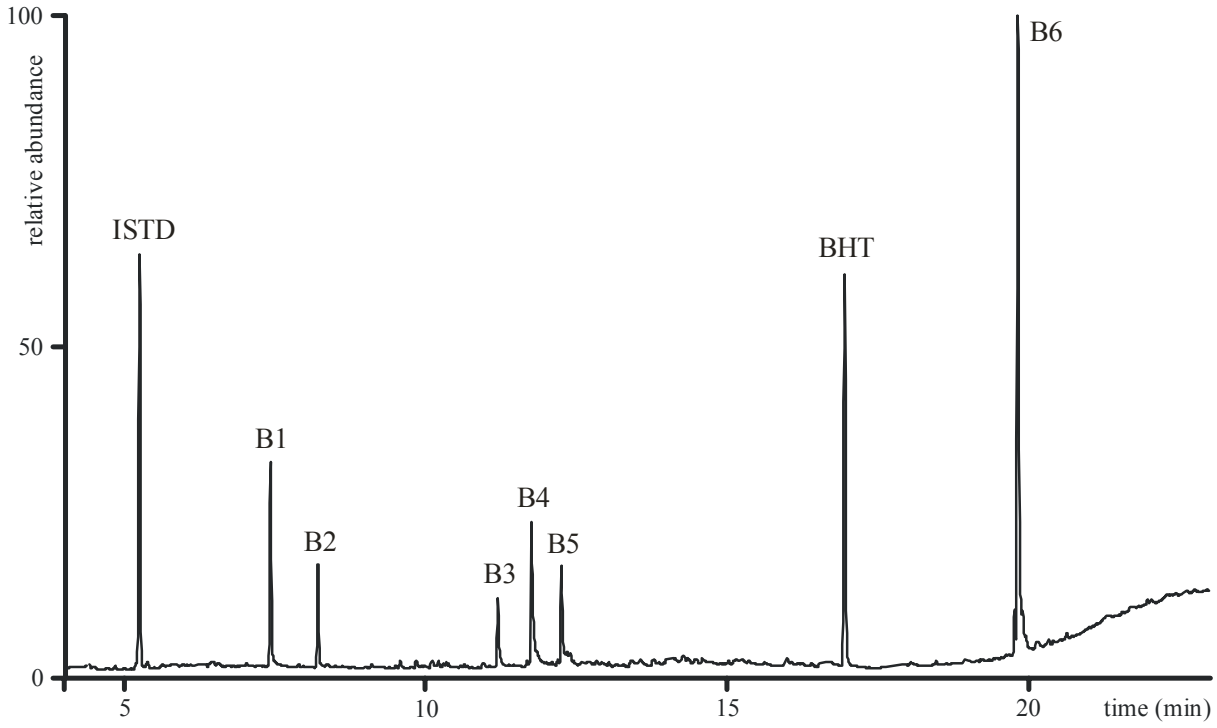



**Fig. 5.2: Blank of an unloaded OVM 3500 passive sampler desorbed with diethyl ether. Separation was carried out on a DB-Wax capillary column (temperature program 3), and detection with the Fisons GC-MS system in the scan mode: B1: butan-1-ol, B2: dimethylsulfone, B3: but-2-oxy-ethan-1-ol, B4: acetic acid (from the solvent), B5: ethyl-2-hexan-1-ol, B6: caprolactam, ISTD: 1-chlorhexane, BHT: butylated hydroxytoluene (solvent stabilizer).**

### 5.1.6 Chromatographic separation and detection

Method development had two objectives: First, to develop a robust quantitative method for the detection of the selected 22 MVOCs. Second, to determine the natural enantiomer ratios of the selected chiral analytes produced by mould. Both objectives were handled separately, whereas the enantioselective methodology was based on experience from the non-enantioselective methodology.

#### 5.1.6.1 Non-enantioselective determination

At first split and splitless injection techniques were applied. Both vaporizing inlets had a column-protecting function but caused high thermal stress to the analytes during the transfer to the column. Typical problems of such vaporizing inlets as solute discrimination and decomposition were not observed. However, problems were observed with the larger SSL 71 injector of the Fisons GC, particularly for compounds containing hydroxyl groups. Peak shapes were distorted (i.e. tailing, rounded, broad or split). These effects increased after repeated injections, suggesting that an altered liner activity may have played a complementary role in generating distorted peaks. Injector temperature had no significant influence. Best results were obtained for 220 °C injector temperature using simple straight deactivated glass tubes.

The selected split ratio of 10:1 for split injection was sufficient to avoid peak broading. For trace analysis splitless injection was applied. However, this caused more stress to the solutes. The splitless time of 2 min was sufficient to transfer the compounds completely. Nevertheless, mainly the Fisons system and to less extend the other two GC systems showed slight peak distortion (i.e. tailing, rounded, broad or split).

Occasionally, some additional peaks were observed in the chromatogram. These ghost peaks accumulated generally during system standby (especially overnight) and were caused by septa bleeding. They were identified mainly as phthalates by MS detection. These peaks did not interfere with the analysis and disappeared after a couple of blank injections.

During routine operation GC oven-temperatures below 35 °C were difficult to maintain, making it difficult or impossible to focus the sample in a short band at the front of the column to make full benefit of the solvent effect. Cold trapping did occur for about 13 compounds

with boiling points of 150 °C above the initial column temperature. Unfortunately, the Fisons split/splitless GC appeared to be unsuitable for MVOCs. Problems due to discrimination as well as peak shape could not be eliminated by method optimization (Fig. 5.3).

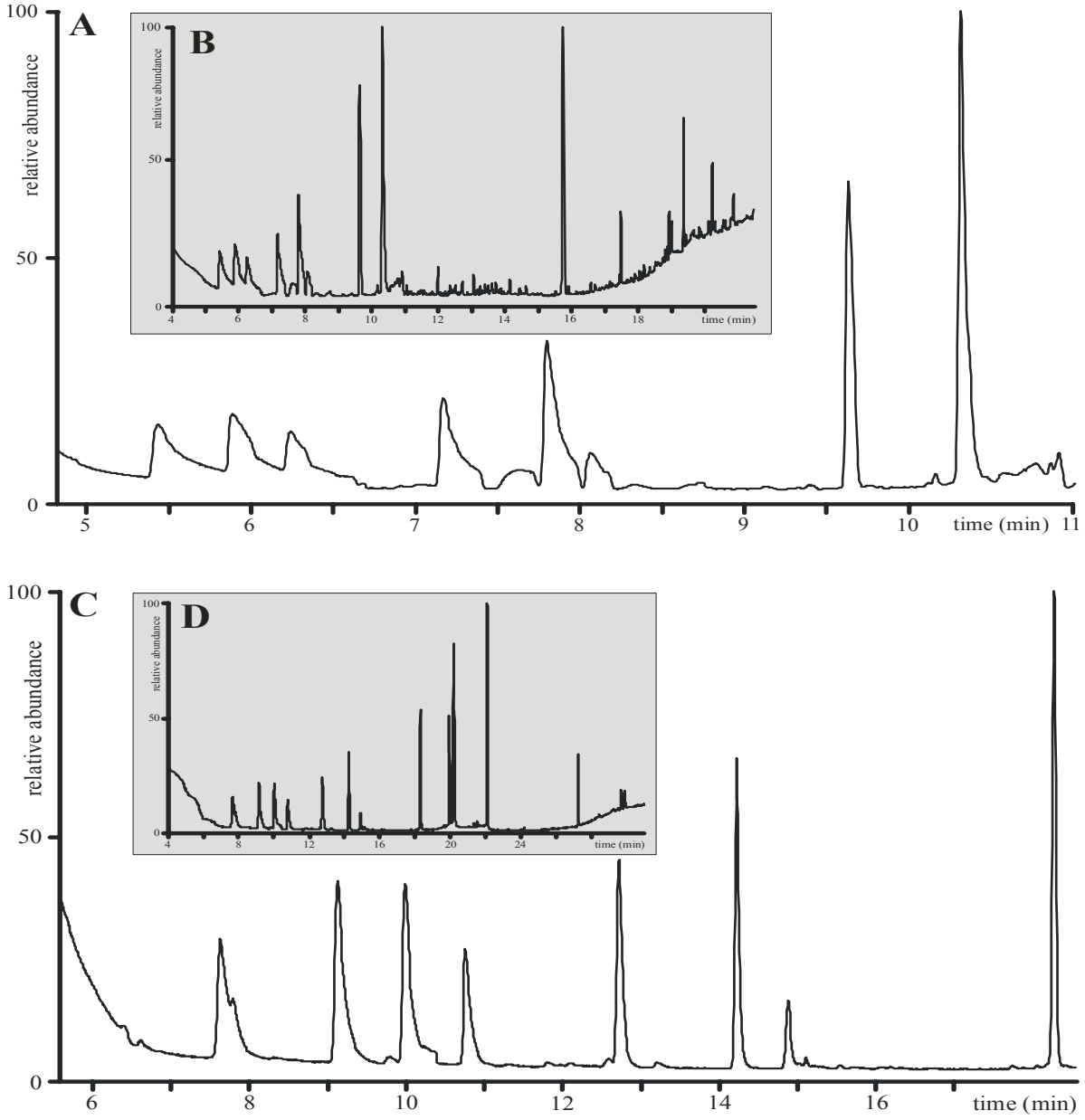



**Fig. 5.3: Typical examples of distorted peaks during the chromatographic separation of the MVOCs after splitless injection with the Fisons GC-MS system on (A)/(B) the HP-INNOWax capillary column and (C)/(D) the DB-Wax capillary column. (B) and (D) show the complete chromatograms.**

Consequently, cold on-column injection as a non-vaporizing injection technique was applied. It guaranteed that the composition of the sample introduced into the column was not altered. All sample components were quantitatively deposited directly in the cold column, eliminating thermal stress. No peak disturbances were observed with this technique. However, it was important to inject the sample with the right speed. Slow enough to eliminate aerosol formation to avoid relatively broad peaks, but fast enough to prevent the sample from adhering to the needle, which would have resulted in lower peak height and sensitivity.

A pre-column was installed to adapt the capillary diameter and to prevent column contamination from non-volatile sample components. This so-called retention gap also reduced the length of the zone flooded by solvent and helped to focus the analytes. In general, a retention gap was usable for 3 to 6 months before peak broadening and tailing were observed (similar to peak appearance in Fig. 5.5, pg. 93).

### Column and column dimension

In order to keep the analysis time short, a medium column length of 30 m was selected for the achiral separation of MVOCs. The selected narrow bore capillary column with an ID of 0.25 mm represented the best compromise between resolution, speed, sample capacity and ease of operation. A standard film thickness of 0.25 µm was chosen to achieve higher resolution and faster analysis instead of the commonly used thick film columns for very volatile compounds (Restek Corporation, 2003). Since column bleeding is proportional to the amount of liquid phase in the column, thick films do bleed more resulting in more noise. High sample capacities for the trace analysis were not needed due to the lower noise level.

Because of the large variability in functionality, volatility, and polarity of the MVOCs the non-enantioselective chromatographic separation was tested on 3 different stationary phases: 100 % dimethylpolysiloxane, 5 % diphenyl 95 % dimethylpolysiloxane, and polyethylene glycol (PEG).

In a first step, the two non-polar stationary phases were applied in terms of a HP-1 and HP-5MS capillary column. Generally, these phases can be used over a wide temperature range (-60…325/350 °C). They do not generate high levels of background noise, are relatively robust, and have long life-times. Both non-polar columns separated the analytes according to their boiling points and compound vapour pressures. Several compounds co-eluted on both stationary phases. The low phenyl content of the HP-5MS, which should slightly increase selectivity, was only marginally notable. Both non-polar columns showed peak broading and poor performance for the polar analytes, particularly the alcohols. In summary, none of the non-polar stationary phases could provide the desired separations.

In a second step the polar PEG stationary phase was tested. The three major interactions between the stationary phase and the target molecules are dispersion, dipole interaction, and hydrogen bonding. PEG stationary phases have particularly strong dipole interactions and therefore a large influence on the retention of compounds with (permanent or induced) dipole moments. As the selected MVOCs are compounds with heteroatoms like oxygen and sulphur, they possess a dipole momentum. Hydroxyl groups have large dipoles and ketones have moderate dipole momentums. Therefore, the stationary phase could distinguish well between such structures. PEG stationary phases also showed strong hydrogen bond interactions that made the separation of compounds with even small hydrogen bond differences possible. However, hydrogen bond interaction is determined by the difference in the hydrogen bond

strength of the compounds and not by their absolute strength. It is also more dependent on present functional groups than on the overall structure (Jennings et al., 1997). In summary, the influence of each interaction on the separations is difficult to predict.

The separation characteristics of the two tested PEG capillary columns of different brands varied not much. The main difference was the lower temperature limit. The DB-Wax capillary column had a lower limit of 20 °C and the HP-INNOWax of 40 °C. At a temperature of 35 °C the HP stationary phases showed broad and rounded peaks. In addition, the chromatography of early elution compounds was not satisfactory (Fig. 5.3).

After several optimization steps the best separation was achieved with the DB-Wax in combination with the 30 min temperature program (Tab. 4.3, Tab. 5.2, and Tab. 5.4). During the optimization the elution order of certain compounds changed when the temperature program was altered. Separation of the MVOC mixture with the PEG stationary phase was satisfactory concerning compound detection and quantification. Early eluting compounds showed good peak symmetry. Nearly all compounds were baseline resolved with the exception of nonan-2-on (23) and fenchone (8/9) ($R_S = 0.7$), geosmin (35/36) and *trans*-(-)-carveol (31) ($R_S = 1.1$), as well as α-terpineol (27/28), borneol (19/20) ($R_S = 1.1$), and verbenone (30/31) ($R_S = 1.2$) (Fig. 5.4 and Fig. 5.7). (For further details on peak resolution $R_S$ refer to appendix 0 (pg. 165).)

The major disadvantage of the PEG stationary phases is their extreme sensitivity to oxygen, especially at high temperatures. The presence of oxygen in the carrier gas causes a rapid decomposition of the PEG phase (Fig. 5.3). It was not possible to prevent small amounts of oxygen from leaking into the GC-system during injection and separation. After 18 months of

use, a PEG column showed typical aging symptoms and oxygen damages such as signal broadening and tailing and had to be exchanged.

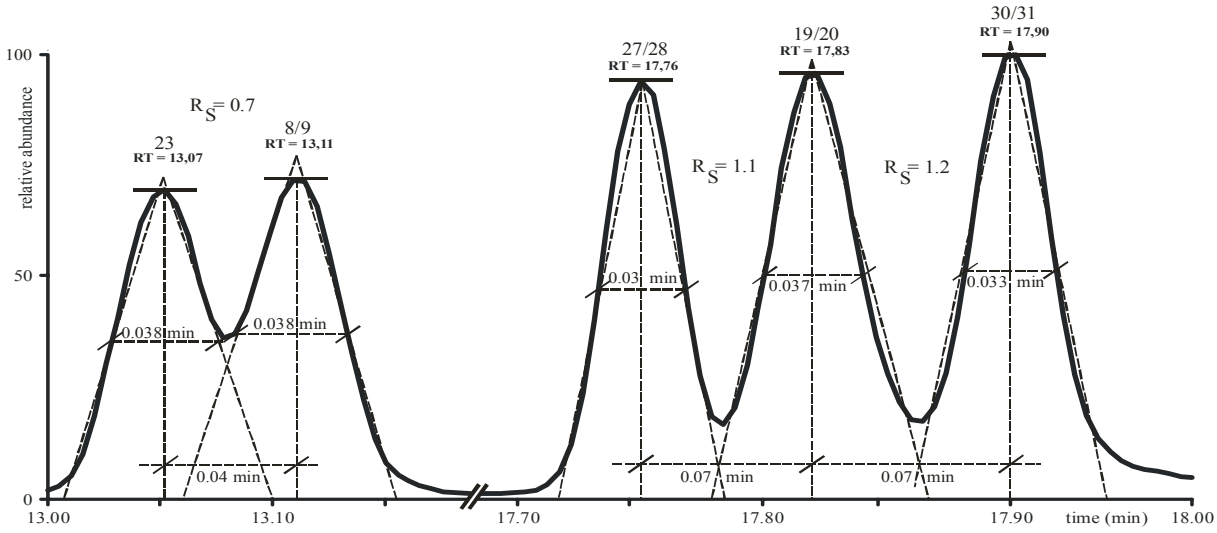



**Fig. 5.4: Not complete baseline resolved compounds on the DB-Wax column with the applied temperature program (Tab. 4.3): The resolution between nonan-2-one (23) and fenchone (8/9) was $R_S$ = 0.7, the resolution between α-terpineol (27/28) and borneol (19/20) was $R_S$ = 1.1, and between borneol (19/20) and verbenone (32/31) $R_S$ = 1.2.**

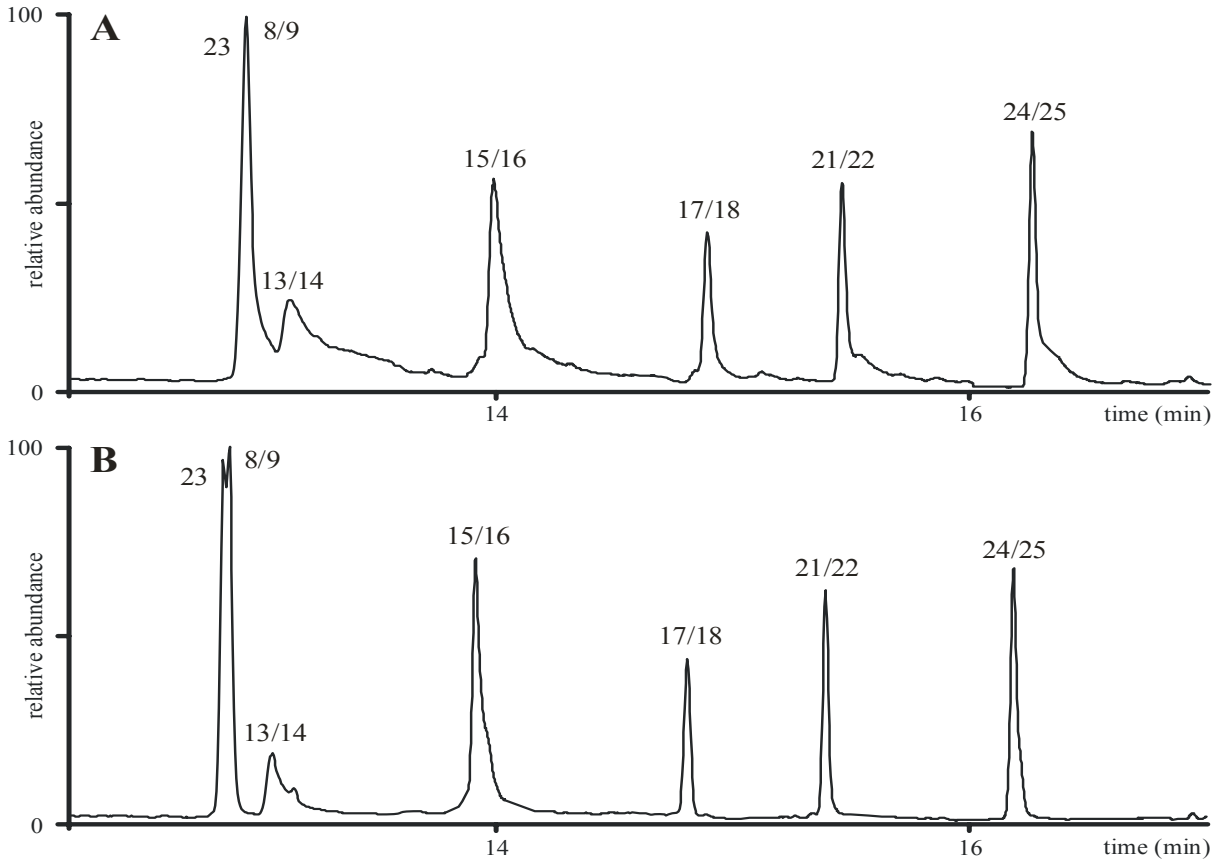



**Fig. 5.5: Example of an old and oxygen damaged DB-Wax capillary column (A) compared to a brand-new capillary column of the same type (B). The chromatogram range shows 23: nonan-2-one, 8/9: fenchone, 13/14: octan-3-ol, 15/16: 1-octen-3-ol, 17/18: camphor, 21/22: linalool, and 24/25: terpinen-4-ol.**

The tandem capillary system consisting of a HP-5 and HP-INNOWax did not separate the MVOC better than any single column. Because of increased length the separation time was almost twice as long (59 min vs. 30 min). Co-elution of several compounds occurred frequently as shown in Fig. 5.6. Thus, analysis were only performed on the single DB-Wax capillary column.

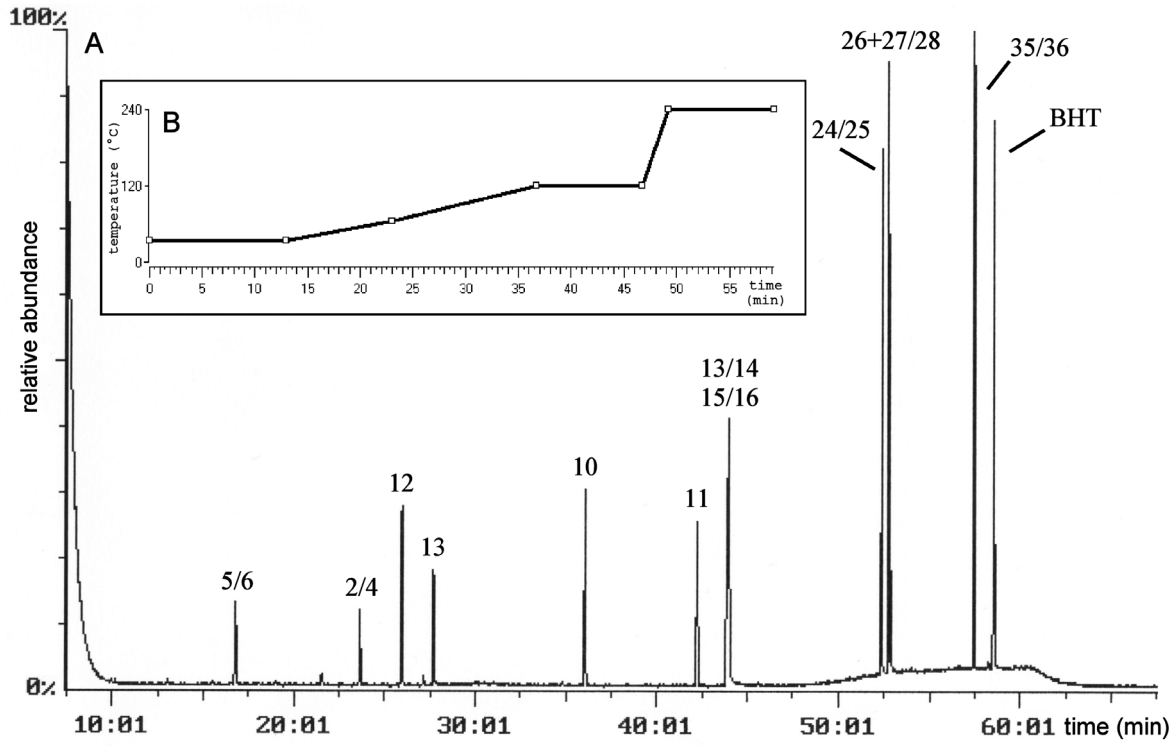



**Fig. 5.6: Chromatographic separation of 11 selected MVOCs on the tandem capillary system consisting of a HP-5 and a HP-Innowax capillary column (A): TIC chromatogram with** 5/6**: 2-methyl-butan-1-ol;** 2/4**: pentan-2-ol;** 12**: dimethyl disulfide,** 13**: 3-methyl-butan-1-ol;** 10**: heptan-2-one;** 11**: octan-3-one;** 13/14+15/16**: 1-octen-3-ol and 3-octanol;** 24/25**: terpinen-4-ol;** 26+27/28**:α-terpineol and γ-terpineol;** 35/36**: geosmin; BHT (The peak numbering follows the compound elution sequence on the BGB-174 with the chiral standard procedure.), (B): Temperature program.**

## MS detection

The scan mode of the MS detector was used for method development. The retention times of all selected MVOCs were determined. Additionally, first observations of background levels and contaminations were made. The achiral chromatographic separation of the MVOCs on the DB-Wax capillary column required a selected ion monitoring (SIM)-program consisting of 14

ion groups. One target ion, usually the base ion, together with one, or sometimes two qualifier ions were selected for each compound. The molecular ion was normally chosen as one of the qualifier ions. Ions of higher masses were favoured because of their higher significance and the lower background. Each mass was measured with a dwell time of 50 ms and a mass width of 0.1 amu.

Tab. 5.2 summarizes the chromatographic separation and detection on the DB-Wax capillary column including elution order and retention time (RT) of each compound as well as the monitored retention windows and the selected ions for each compound recorded in the SIM mode of the non-enantioselective method. Fig. 5.7 shows the corresponding chromatogram.

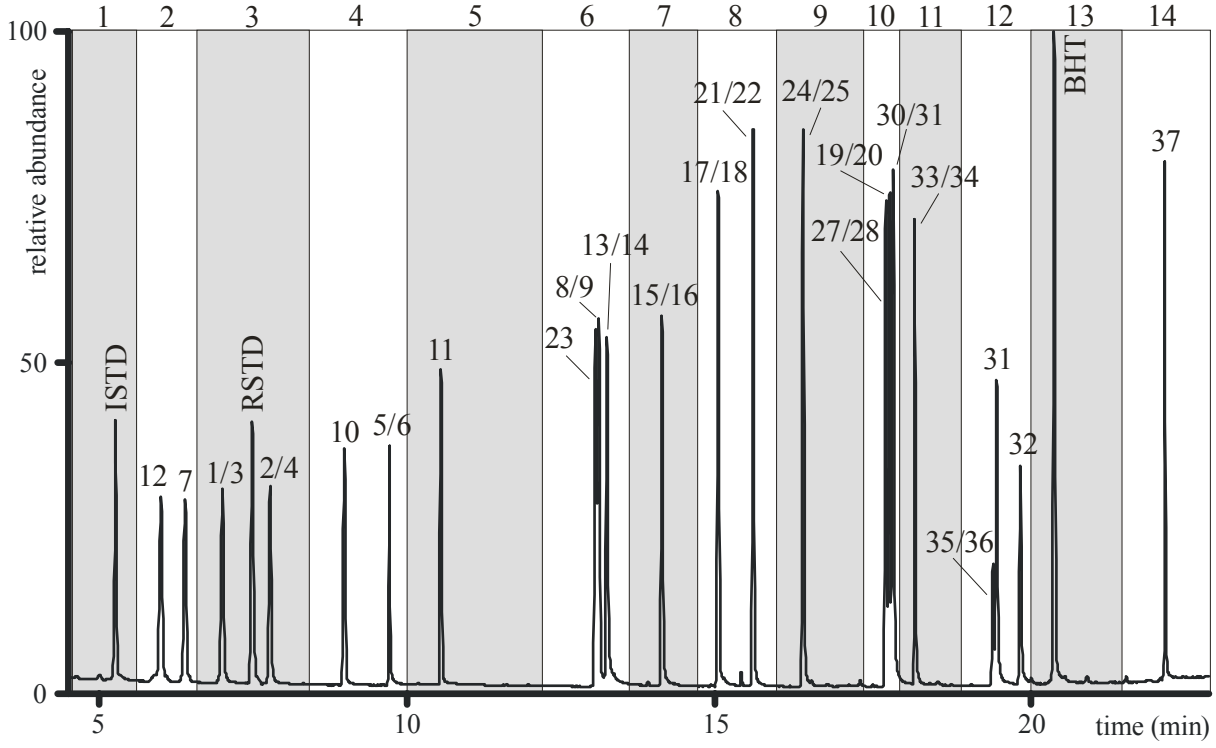



**Fig. 5.7: Overview of the non-enantioselective chromatographic separation with the 14 monitored retention windows of the selected 22 MVOCs on the DB-Wax capillary column. The compound numbering follows the elution sequence of the enantioselective chromatographic separation on the BGB-174 (refer to Tab. 5.2).**

**Tab. 5.2:** **Overview of the non-enantioselective chromatographic separation and detection method for the selected 22 MVOCs on the DB-Wax capillary column. (The compound numbering (#) follows the elution sequence of the enantioselective chromatographic separation on the BGB-174 (Tab. 5.8).**

| SIM-window | SIM-mass | AR ($s^{-1}$) | RT | compound (#) | bp. (°C) | $M_{W,\ isotopic}$ | BP | other characteristic masses |
|---|---|---|---|---|---|---|---|---|
| **4.50….5.50** | 69, 91, 93 | 6.67 | 5.13 | 1-chlorohexane (**ISTD**) | 134 | 120.07057 | **91** | **93**, **69**, 55, 56, 56, 41, 43, 39 |
| **5.50….6.60** | 58, 79, 94, 100 | 5.00 | 5.85 | methyl disulfide (12) | 109.7 | 93.99109 | **94** | **79**, 61, 64, 45 |
| | | | 6.28 | hexan-2-one (7) | 127 | 100.08881 | **43** | 100, 85, 71, **58**, 57 |
| **6.60….8.50** | 55, 67, 73, 82, 83 | 4.00 | 6.92 | 3-methyl-butan-2-ol* (1/3) | 112 | 88.08881 | 45 | **73**, **55**, 44, 43 |
| | | | 7.38 | chlorocyclohexane (**RSTD**) | 141 | 118.05492 | **67** | **83**, **82**, 55, 54, 41 |
| | | | 7.69 | pentan-2-ol* (2/4) | 120 | 88.08881 | 45 | **73**, **55**, 45, 44, 43 |
| **8.50…10.00** | 56, 57, 58, 71 | 5.00 | 8.86 | heptan-2-one (10) | 150 | 114.10446 | 43 | 114, **71**, 59, **58** |
| | | | 9.60 | 2-methyl-butan-1-ol* (5/6) | 129 | 88.08881 | **57** | **71**, 70, 59, **56**, 55, 41 |
| **10.00…12.10** | 72, 99 | 10.00 | 10.42 | octan-3-one (11) | 168 | 128.12011 | 43 | 128, **99**, 85, 86, **72**, 71, 57 |
| **12.10…13.60** | 58, 81, 83, 101, 142, 152 | 3.33 | 12.93 | nonan-2-one (23) | 192 | 142.13576 | **58** | **142**, 71, 59, 57, 43, 41 |
| | | | 12.96 | fenchone* (8/9) | 193 | 152.12011 | **81** | **152**, 109, 82, 80, 69, 41 (67, 79) |
| | | | 13.12 | octan-3-ol* (13/14) | 175 | 130.13576 | 59 | **101**, 84, **83**, 57, 55, 41 |
| **13.60…14.60** | 57, 72 | 10.00 | 13.99 | 1-octen-3-ol* (15/16) | 175 | 128.12011 | **57** | 99, 85, **72**, 43, 41, 39 |
| **14.60…15.90** | 71, 93, 95, 150 | 5.00 | 14.90 | camphor* (17/18) | 204 | 152.12011 | **95** | **150**, 110, 109, 108, 83, 81, 69, 67, 55, 41 |
| | | | 15.47 | linalool* (21/22) | 198 | 150.10446 | **71** | 136, 121, **93**, 83, 80, 69, 67 |
| **15.90…17.30** | 71, 111 | 10.00 | 16.27 | terpinen-4-ol* (24/25) | 212 | 154.13576 | **71** | 154, 136, **111**, 93, 86, 69, 67,55,43 |

*: Chiral compounds are marked with asterisk, **AR**: acquisition rate (ion masses scanned each second of the concerning retention window); **RT**: retention time; **bp**: boiling point; $M_{W,isotopic}$: isotopic molecular weight; **BP**: basis peak; **target ion of SIM-program**: underline mass; **qualifier ions**: bold typed masses

**Tab. 5.2: Continuation**

| SIM-window | SIM-mass | AR ($s^{-1}$) | RT | compound (#) | bp. (°C) | $M_{W,\ isotopic}$ | BP | other characteristic masses |
|---|---|---|---|---|---|---|---|---|
| **17.30…17.80** | 59, 95, 107, 110, 121, 135 | 3.33 | 17.61 | alpha-terpineol* (27/28) | 218 | 154.13576 | **59** | 136, **121**, 93, 92, 81, 67, 43 |
| | | | 17.69 | borneol* (19/20) | 216 | 154.13576 | **95** | 139, 136, **121**, **110**, 96, 93, 67, 55, 41 |
| | | | 17.75 | verbenone* (30/31) | 227.5 | 150.10446 | **107** | 150, **135**, 122, 108, 91, 80, 79 |
| **17.80…18.90** | 82, 93 | 10.00 | 18.09 | carvone* (33/34) | 279 | 150.10446 | **82** | 150, 108, 107, 106, **93**, 54 |
| **18.90…20.00** | 84, 109, 112, 125, 134 | 4.00 | 19.34 | geosmin* (35/36) | 270 | 182.16706 | **112** | 126, **125**, 97 |
| | | | 19.43 | *trans*-(-)-carveol* (31) | 226 | 152.12011 | **109** | 152, 137, 119, **84**, 83 |
| | | | 19.81 | *cis*-(-)-carveol* (32) | 226 | 152.12011 | **84** | 137, **134**, 119, 109, 83, 69, 55, 41 |
| **20.00…21.50** | 205, 220 | 10.00 | 20.32 | butylated hydroxyl-toluene (**BHT**; stabiliser) | 265 | 220.18271 | **205** | **220**, 206, 189, 177, 145 |
| **21.50…23.00** | 105, 150 | 10.00 | 22.19 | cuminol (37) | 246 | 150.10446 | 135 | **150**, 119, **105**, 107, 91, 79, 77 |

*: Chiral compounds are marked with asterisk, **AR**: acquisition rate (ion masses scanned each second of the concerning retention window); **RT**: retention time; **bp**: boiling point; $M_{W,isotopic}$: isotopic molecular weight; **BP**: basis peak; **target ion of SIM-program**: underline mass; **qualifier ions**: bold typed masses

Quantification and method validation of the non-enantioselective separation and detection

The results of the method validation were carried out with the internal standard method as described in chapter 4.6.2 (pg. 67). Correlation analysis was performed by the coefficient of determination (“Bestimmtheitsmaß”), $r^2$, that could be established for all compounds in the range of 0.97…0.99 with the exception of fenchone ($r^2 = 0.96$) (Tab. 5.3). It was considered as an acceptable linear fit for all 22 MVOCs.

Supplementary, a plot of the obtained data of each compound was visually inspected. The linear regression was calculated for the area ratio $\mathbf{P_A}$ versus the concentration of the compound **c** (pg $\mu L^{-1}$) as exemplary shown in Fig. 5.8 for octan-3-ol.

**Tab. 5.3: Coefficient of determination ($r^2$) for all 22 MVOCs obtained out of the linear regression (with an adjusted data weighting of 1/x; n = 27). (The compound numbering (#) follows the elution sequence on the BGB-174; compound listing follows the elution sequence on the DB-Wax.)**

| # | compound | $r^2$ | # | compound | $r^2$ |
|---|---|---|---|---|---|
| 12 | methyl disulfide | 0.9934 | 17/18 | camphore | 0.9958 |
| 1/3 | 3-methyl-butan-2-ol | 0.9952 | 21/22 | linalool | 0.9953 |
| 2/4 | pentan-2-ol | 0.9947 | 24/25 | terpinen-4-ol | 0.9822 |
| 10 | heptan-2-one | 0.9659 | 27/28 | alpha-terpineol | 0.9965 |
| 7 | hexan-2-one | 0.9935 | 19/20 | borneol | 0.9887 |
| 5/6 | 2-methyl-butan-1-ol | 0.9955 | 29/30 | verbenone | 0.9991 |
| 11 | octan-3-one | 0.9980 | 33/34 | carvone | 0.9935 |
| 23 | nonan-2-one | 0.9712 | 35/36 | geosmin | 0.9963 |
| 8/9 | fenchone | 0.9568 | 31 | *trans*-carveol | 0.9973 |
| 13/14 | octan-3-ol | 0.9994 | 32 | *cis*-carveol | 0.9944 |
| 15/16 | 1-octen-3-ol | 0.9657 | 37 | cuminol | 0.9953 |

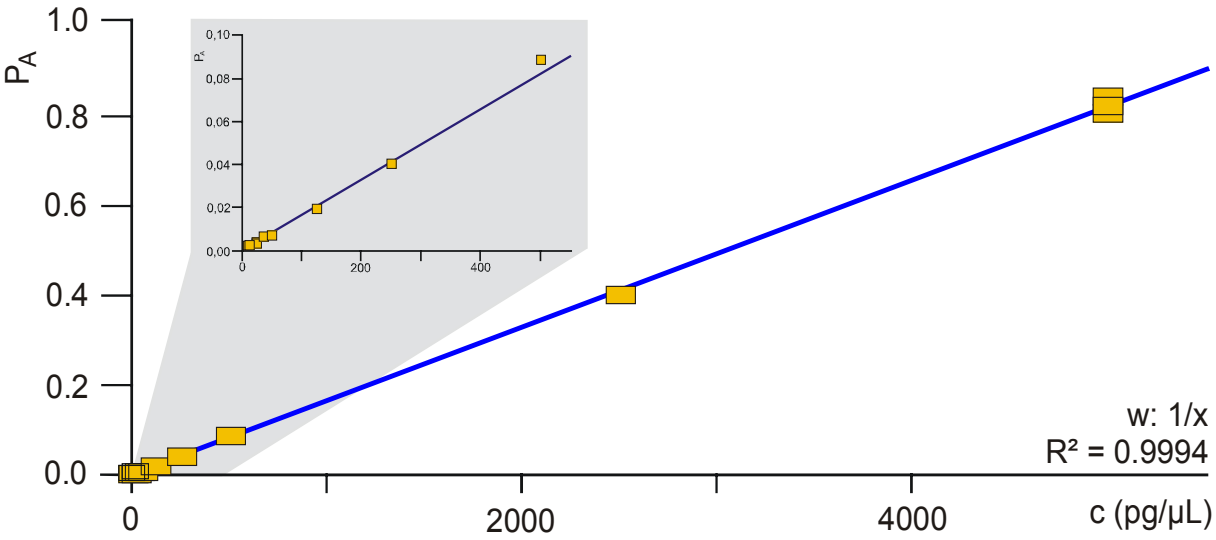



**Fig. 5.8: Visual linearity tests by linear regression for octan-3-ol in the concentration range of 0.01 to 5 ng $\mu L^{-1}$ within the non-enantioselective method ($P_A$: signal to area ratio; compare chapter 4.6.2 Eq. (6) on pg. 67)**

In summary, the linearity study verified that the response of the sample solutions in the concentration range of 0.01…5 ng $\mu L^{-1}$ (with nine calibration points, n = 27) was linear (Tab. 5.3).

The limit of detection (LOD) was defined as a signal-to-noise ratio (*S/N*) of 3:1. For quantification the compound signal had to exceed a minimum *S/N*-ratio of 10:1 (limit of quantification (LOQ)). Both limits were calculated for each compound (Tab. 5.4) by the *S/N*-values resulting from the Xcalibur™ module "signal-to-noise".

**Tab. 5.4: Limit of detection (LOD; S/N 3:1) and limit of quantification (LOQ; S/N 10:1) for all selected 22 MVOCs for the non-enantioselective GC-MS method using the SIM mode. (The compound numbering (#) follows the elution sequence on the BGB-174; compound listing follows the elution sequence on the DB-Wax.)**

| # | compound | LOD pg $\mu L^{-1}$ | LOQ pg $\mu L^{-1}$ | # | compound | LOD pg $\mu L^{-1}$ | LOQ pg $\mu L^{-1}$ |
|---|---|---|---|---|---|---|---|
| 12 | methyl disulfide | 0.8 | 2.7 | 17/18 | camphor | 3.2 | 10.6 |
| 1/3 | 3-methyl-butan-2-ol | 4.2 | 14.1 | 21/22 | linalool | 14.3 | 47.6 |
| 2/4 | pentan-2-ol | 9.4 | 31.3 | 24/25 | terpinen-4-ol | 6.8 | 22.7 |
| 10 | heptan-2-one | 4.9 | 16.4 | 27/28 | alpha-terpineol | 9.7 | 32.3 |
| 7 | hexan-2-one | 6.1 | 20.4 | 19/20 | borneol | 3.3 | 11.1 |
| 5/6 | 2-methyl-butan-1-ol | 16.7 | 55.6 | 30/31 | verbenone | 1.0 | 3.3 |
| 11 | octan-3-one | 7.0 | 23.3 | 33/34 | carvone | 5.5 | 18.2 |
| 23 | nonan-2-one | 4.2 | 14.1 | 35/36 | geosmin | 3.3 | 11.0 |
| 8/9 | fenchone | 1.1 | 3.6 | 31 | *trans*-carveol | 25.0 | 83.3 |
| 13/14 | octan-3-ol | 10.7 | 35.7 | 32 | *cis*-carveol | 85.7 | 285.7 |
| 15/16 | 1-octen-3-ol | 23.1 | 76.9 | 37 | cuminol | 9.5 | 31.7 |

The recovery (RC) for all selected MVOCs was calculated using the substance amount recovered from the sampler after solvent desorption ($m_{recovered}$) and the amount spiked onto the sampler ($m_{spiked}$):

$$RC[\%] = \frac{m_{recovered}}{m_{spiked}} * 100 \qquad (14)$$

The variability of the recovery rates of the volatile compounds was remarkably large and ranged from 5 % to 151 % (Tab. 5.5). The high RC values of methyl disulfide, heptan-2-one, and fenchone (RC: 148…151 %) must be viewed as outliers that were caused by dilution errors. During the validation and recovery experiments the reference solutions of these 3 compounds had to be remade with new reference compounds and new dilutions.

**Tab. 5.5: Relative recovery coefficient (RC), standard deviation (SD), and coefficient of variation (CV) of MVOCs with calibration solution at different concentration levels out of the passive sampler charcoal matrix including a between-runs precision of at least three analysis. Excluded are the outlying results of cuminol, carvone, *trans*- and *cis*-carveol. (The compound numbering (#) follows the elution sequence on the BGB-174; compound enumeration follows the elution sequence on the DB-Wax.)**

| # | compound | RC (%) | SD (%) | CV (%) |
|---|---|---|---|---|
| 12 | methyl disulfide | 151[a] | ± 1 | ± 1 |
| 1/3 | 3-methyl-butan-2-ol | 71 | ± 5 | ± 8 |
| 2/4 | pentan-2-ol | 95 | ± 5 | ± 5 |
| 10 | heptan-2-one | 149[a] | ± 4 | ± 3 |
| 7 | hexan-2-one | 122 | ± 1 | ± 1 |
| 5/6 | 2-methyl-butan-1-ol | 73 | ± 5 | ± 6 |
| 11 | octan-3-one | 85 | ± 3 | ± 4 |
| 23 | nonan-2-one | 47 | ± 5 | ± 11 |
| 8/9 | fenchone | 148[a] | ± 5 | ± 4 |
| 13/14 | octan-3-ol | 76 | ± 3 | ± 5 |
| 15/16 | 1-octen-3-ol | 117 | ± 4 | ± 4 |
| 17/18 | camphor | 105 | ± 4 | ± 4 |
| 21/22 | linalool | 111 | ± 5 | ± 4 |
| 24/25 | terpinen-4-ol | 54 | ± 4 | ± 8 |
| 27/28 | alpha-terpineol | 40 | ± 3 | ± 7 |
| 19/20 | borneol | 127 | ± 2 | ± 2 |
| 30/31 | verbenone | 40 | ± 4 | ± 9 |
| 33/34 | carvone | 12 | ± 3 | ± 29 |
| 35/36 | geosmin | 123 | ± 3 | ± 3 |
| 31 | *trans*-carveol | 8 | ± 8 | ± 109 |
| 32 | *cis*-carveol | 6 | ± 10 | ± 184 |
| 37 | cuminol | 5 | ± 11 | ± 226 |

[a] outlying observation caused by dilution errors

The low recovery rates of the less volatile compounds had large coefficients of variation e.g. for cuminol (RC: 5 %; SD: ± 11; CV: ± 226 %), carvone (RC: 12 %; SD: ± 3; CV: ± 29 %), *trans*-carveol (RC: 8 %; SD: ± 8; CV: ± 109 %), and *cis*-carveol (RC: 6 %; SD: ± 10; CV: ± 184 %). Gravimetric, volumetric, instrumental and calibration errors could be

excluded. Generally, no significant correlation between volatility and recovery was observed. Additionally, measurements according to Shields et al. (Shields and Weschler, 1987) demonstrated no significant evaporative loss of analytes during the desorption step even if the recovery of the desorption solvent varied (compare pg. 61). Nevertheless, desorption efficiency may not have been optimal for all 22 MVOCs with the selected solvent diethyl ether because of the wide spectrum of selected compound classes. However, pre-tests with dichloromethane, tetrachloromethane, and mixtures of dichloromethane and pentane (80 + 20 (v/v)) did not show any benefits compared to the selected solvent. Diethyl ether was the best available compromise with all its good solvent properties. The adsorption of cuminol, carvone, *trans*-, and *cis*-carveol onto the activated charcoal may have been comparatively strong and only partly reversible. Consequently, for these compounds the applied method could only be used as semiquantitative method.

**Tab. 5.6: The calculated between-runs precision of the non-enantioselective GC method described as relative standard deviation in % RSD of the single compound (number of parallels: n ≥ 6). (The compound numbering (#) follows the elution sequence on the BGB-174; compound listing follows the elution sequence on the DB-Wax.)**

| # | compound | % RSD | # | compound | % RSD |
|---|---|---|---|---|---|
| 12 | methyl disulfide | 3 | 17/18 | camphor | 4 |
| 7 | hexan-2-one | 3 | 21/22 | linalool | 4 |
| 1/3 | 3-methyl-butan-2-ol | 6 | 24/25 | terpinen-4-ol | 4 |
| 2/4 | pentan-2-ol | 4 | 27/28 | alpha-terpineol | 4 |
| 10 | heptan-2-one | 4 | 19/20 | borneol | 7 |
| 5/6 | 2-methyl-butan-1-ol | 5 | 30/31 | verbenone | 4 |
| 11 | octan-3-one | 4 | 33/34 | carvone | 4 |
| 23 | nonan-2-one | 5 | 35/36 | geosmin | 4 |
| 8/9 | fenchone | 13 | 31 | *trans*-carveol | 5 |
| 13/14 | octan-3-ol | 3 | 32 | *cis*-carveol | 6 |
| 15/16 | 1-octen-3-ol | 4 | 37 | cuminol | 6 |

Precision experiments were carried out as described in chapter 4.6.2 (pg. 67). The received values provide an estimate of between-runs precision including the within-run precision. The relative standard deviation (% RSD) varied between 3 and 7 % (exception fenchone 13 %).

The overall precision of the method summed up to 5 % (Tab. 5.6). The manual injection precision was calculated by the ISTD/RSTD-ratio to an interday deviation of ± 2 %. The BHT stabilizer concentration in the sampler matrix solution varied to ± 12 % for a series of 20 analyses measured in 12 days.

#### 5.1.6.2 Enantioselective determination

Variation of temperature, temperature rate and linear gas velocity greatly affected the resolution of enantiomers during method development. The best enantioselective separations were achieved with temperature rates not higher than 5 °C $min^{-1}$ in combination with low starting temperatures. As far as known today, selector-selectand interactions are strongly dependent on temperature (Schreier et al., 1995). At higher temperatures, the flexibility of the cyclodextrin (CD) derivatives increases, and the inclusion rate decreases.

In many instances, the resolution between enantiomers could be further improved with linear gas velocities that were higher than those considered optimum by the van Deemter curve. The typical optimum linear gas velocity for best enantiomeric separation was 80 cm $sec^{-1}$ with hydrogen as the carrier gas, but optimum linear gas velocities differed for chiral compounds and columns. The optimum linear gas velocity for helium as carrier gas was about 60 cm $sec^{-1}$. These results were in accordance with Grob et al. (Grob et al., 1990) who found that due to the dependence of selectivity on temperature, optimal resolution of enantiomers is often obtained under conditions not producing maximum separation efficiency. In this study, gas velocities above the minimum of the van Deemter curves resulted in optimal enantiomeric separations. However, several compounds did not show any increase in chiral resolution by different linear velocities, e.g. 1-octen-3-ol (Fig. 5.9).

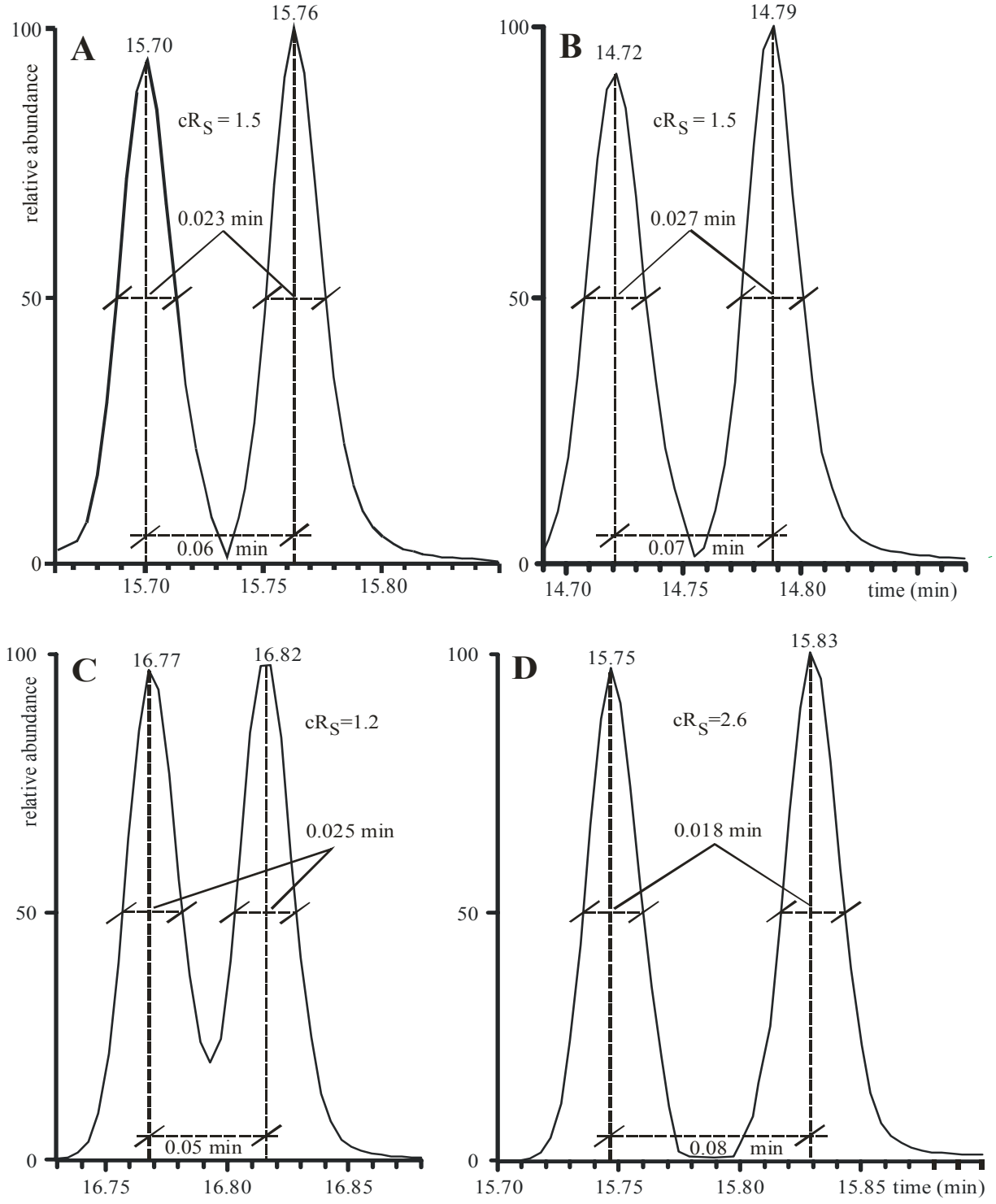



**Fig. 5.9: Different linear gas velocities ((A) and (C) 35 cm $s^{-1}$ / (B) and (D) 60 cm $s^{-1}$) within the same temperature program improved chiral resolution ($cR_S$) for most compounds, e.g. linalool ((C) $cR_S$ = 1.2 / (D) $cR_S$ = 2.6). An exception was e.g. 1-octen-3-ol ((A) and (B) $cR_S$ = 1.5).**

Changes in the enantiomer elution order of several compounds were observed when altering chromatographic parameters. Generally, chiral selectivity is controlled first by the number of

chiral centres available and second, by the strength of interacting forces which is determined by the nature of interacting atoms or groups (Beesley and Scott, 1998). Therefore, no prediction of the elution order of the (R)- and (S)-isomers was possible and elution order had to be empirically determined using enantiopure compounds.

Altogether 11 different enantioselective columns from 4 different manufacturers were tested with different stationary phases and/or different combinations of chiral selector and polysiloxane solvent. None of the selected chromatographic columns were available as immobilized and chemically bonded (cross-linked) phases. On the following pages the tested columns are discussed:

The FS-LIPODEX® E capillary manufactured by Macherey-Nagel GmbH & Co. KG (Düren, Germany) was coated with a pure and undiluted octakis-(2,6-di-O-pentyl-3-O-butyryl)-γ-cyclodextrin phase. It allowed the enantiomeric separation of only 7 of the 14 chiral MVOCs. Thereof 3 compounds were not sufficiently resolved ($cR_S$ « 1.0) (Fig. 5.10).

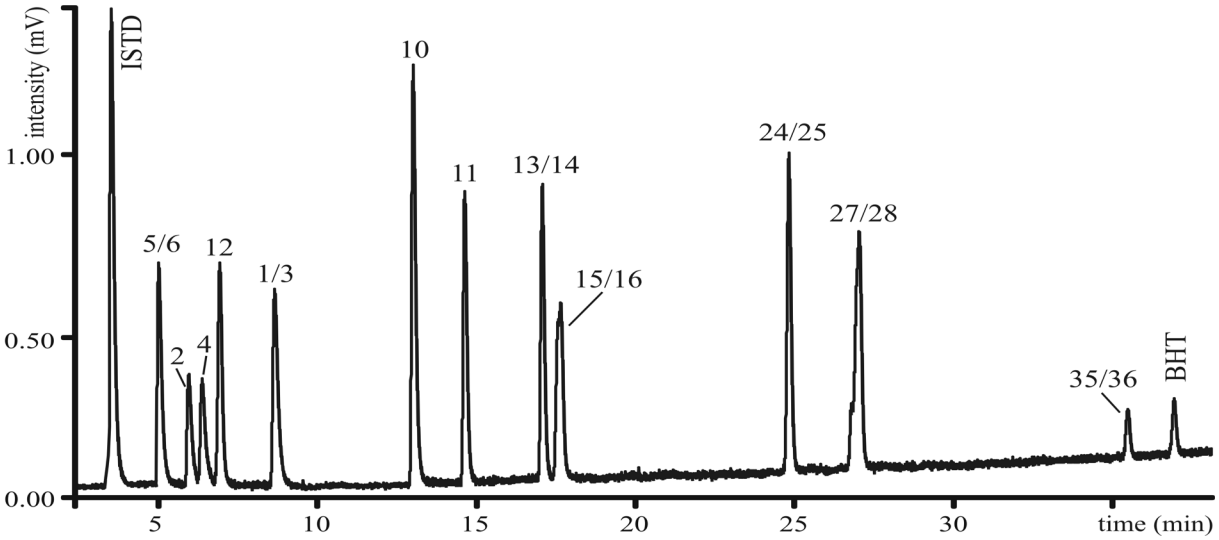



**Fig. 5.10: Performance test of the FS-LIPODEX® E with a selection of 8 chiral MVOCs on the Varian GC-FID system shows that out of the selected compounds only pentan-2-ol (2/4) was completely separated into enantiomers. (Compound numbering follows the elution sequence of Fig. 5.17 and is described in Tab. 4.10.)**

All other tested columns contained the derivatized CDs in dissolved polysiloxanes (compare chapter 1.4.2 (pg. 39)):

One of the most popular types of chiral stationary phase (CSP) presently used in GC represents the permethylated β-CD. Supelco (Buchs, Switzerland) offers this chiral selector as β-DEX 120 (20 % (w/w)) dissolved in 35 % diphenyl 65 % dimethyl polysiloxane (SPB$^{TM}$-35). It showed enantioselectivity only for 8 of the 14 chiral compounds (Fig. 5.11).

Changing to hexakis-(2,3,6-per-O-methyl)-α-cyclodextrin or octakis-(2,3,6-per-O-methyl)-γ-cyclodextrin did not improve the enantioselective separation of the tested MVOCs. In contrast to the heptakis-β-CD, the octakis-γ-CD separated only 5 chiral compounds and the hexakis-α-CD only 2 (1-octen-3-ol and carvone).

The *tert.*-butyldimethylated BGB-172 capillary column did not separate any of the MVOCs into enantiomers. Better results were obtained with the mono-*tert*-butyldimethylated cyclodextrins including the custom-made heptakis-(2,3-di-O-metyl-6-O-TBDMS)-β-cyclodextrin stationary phase by the Swiss Federal Research Station (Wädenswil, Switzerland), the FS-HYDRODEX® β-6TBDM capillary column by Macherey-Nagel GmbH & Co. KG (Tab. 4.5), and the β-DEX 325 by Supelco (Tab. 4.7). The first two cyclodextrins were both dissolved in OV-1701. The heptakis-(2,3-di-O-metyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin of the β-DEX 325 was dissolved in SPB$^{TM}$-20 (25 % (w/w)).

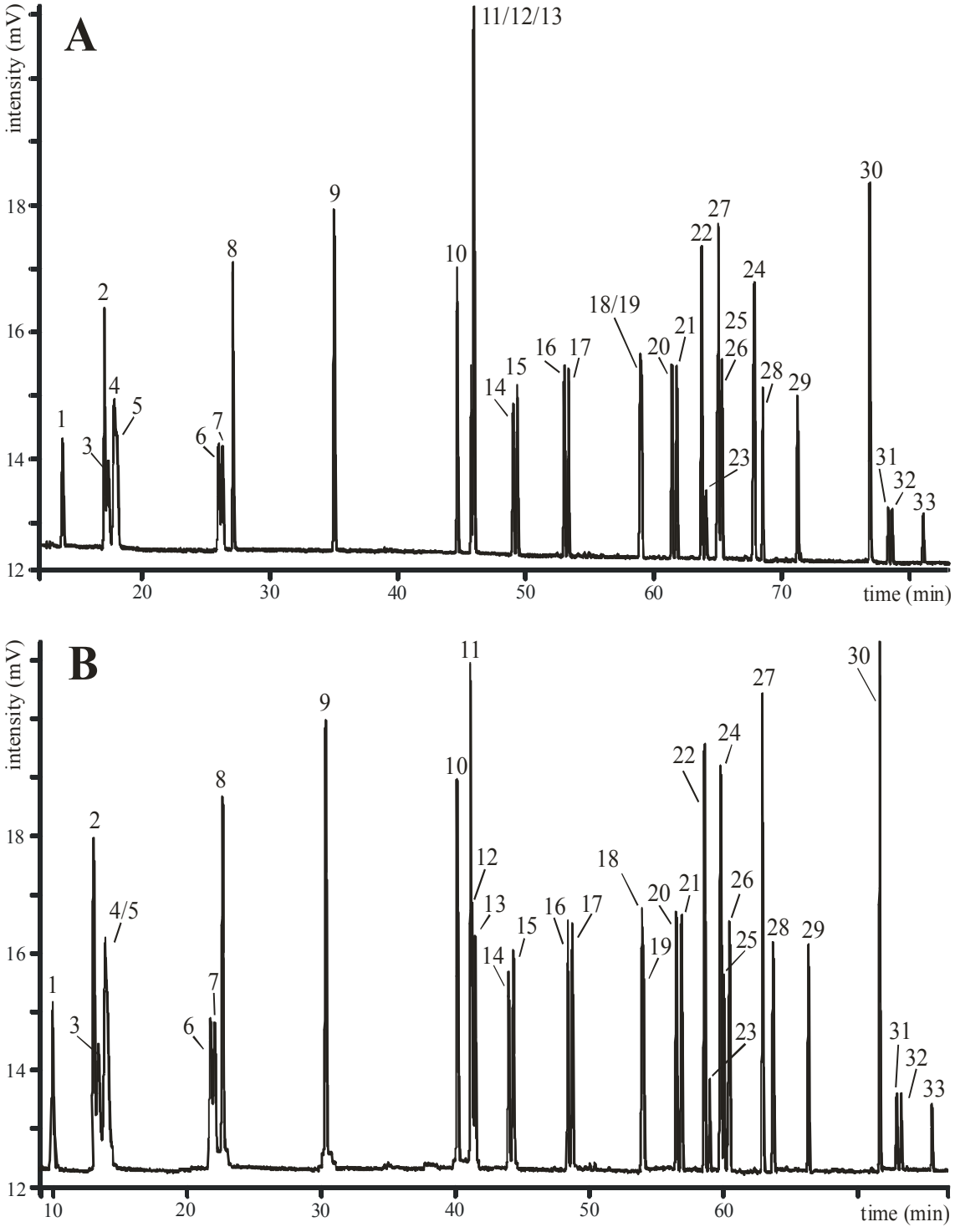



**Fig. 5.11: Separation of an MVOC standard solution on a Supelco β-DEX 120 capillary measured on the HP5890 II GC-FID system with $H_2$ as carrier gas. Both runs used the temperature program 2 (chapter 4.6.1, pg. 63) but different gas velocities were applied: (A) $v_{gas}$ = 50 cm $s^{-1}$, (B) $v_{gas}$ = 75 cm $s^{-1}$.**

The customer-made column only exhibited enantioselectivity for 2 chiral compounds which could partly be separated (octan-3-ol and geosmin). On the FS-HYDRODEX® β-TBDM

column 3 chiral MVOCs were resolved. Best results for the heptakis-(2,3-di-O-metyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin phases were obtained with the Supelco β-DEX 325. It was able to resolve 8 of the 14 chiral MVOCs. Additionally, the overall chromatographic performance was very good. The test indicated also that the enantioselective separation properties of the CSP were influenced by the age of the column. This phenomenon of CD degradation and reduced separation power by column storage and ageing is also described by Bichi et al. (Bicchi et al., 1991) and Schreier et al. (Schreier et al., 1995).

The best enantioselective separation of the MVOCs by the derivatized CDs was achieved with the 2,3-diacetylated and mono-6- *tert.*-butyldimethylated CSPs. Their polarity is higher due to the O-acyl groups compared to the O-alkyl groups. The BGB-174 column as well as the β-DEX 225 and γ-DEX 225 columns were tested. The latter had an extended size of the CD cavity by one D-glucopyranosyl unit (Fig. 1.11). The chiral selector of the other Supelco column and the BGB column was in both cases a heptakis-(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin. Both Supelco CSPs were dissolved in the slightly polar SPB$^{TM}$-20 (20 % diphenyl 80 % dimethyl polysiloxane) with a fraction of 25 % (w/w) of the chiral selector. The BGB cyclodextrin derivate was dissolved in BGB-1701 (14 % cyanopropylphenyl 86 % dimethyl polysiloxane) at a concentration of 50 % (w/w).

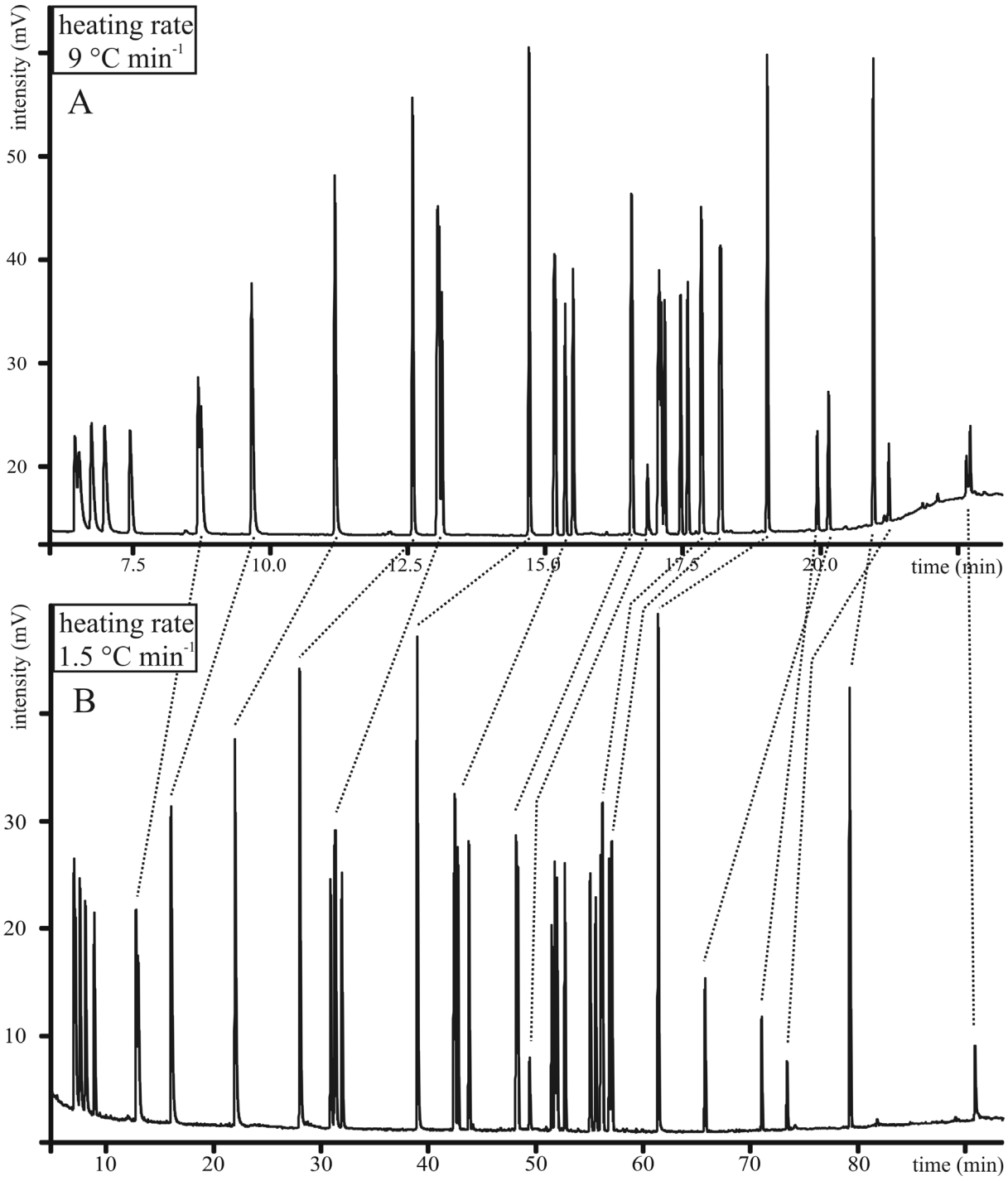



**Fig. 5.12: Different peak resolution and change of elution order of several MVOCs were obtained by temperature program 3 (A) and temperature program 2 (B) (chapter 4.6.1, pg. 63) on a Supelco γ-DEX 225 measured on the HP5890 II GC-FID system with a $H_2$ carrier gas velocity of 50 cm $s^{-1}$.**

The differences in selectivity were marginal between the β-CD and γ-CD derivates (Fig. 5.13) as well as between the two medium polar polysiloxane solvents. The γ-CD partly separated 11 chiral MVOCs and the β-CD partly separated 12 chiral MVOCs into enantiomers.

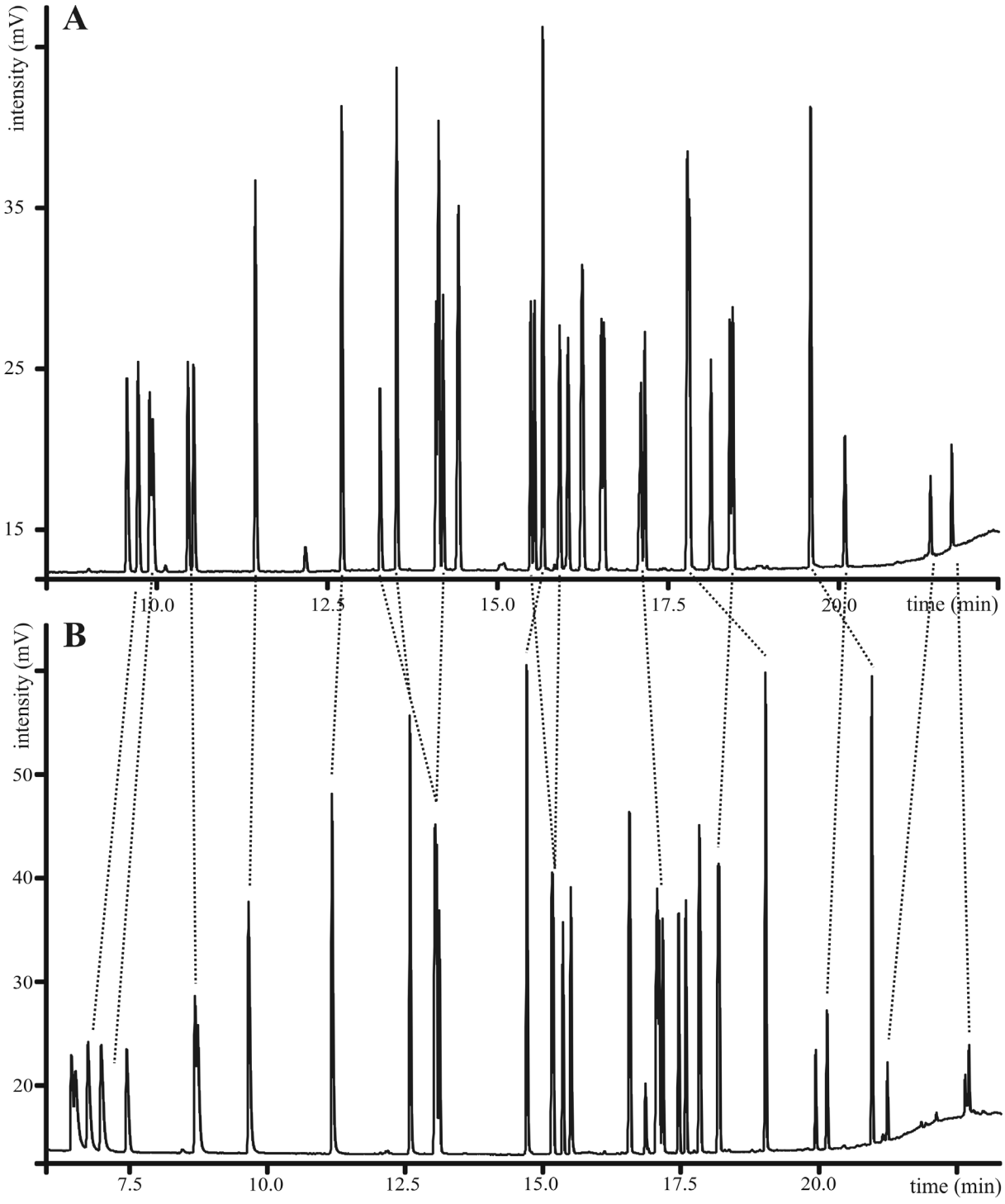



**Fig. 5.13: Selectivity differs between the β-DEX 225 (A) and γ-DEX 225 (B) using the same temperature program 3 (chapter 4.6.1, pg. 63).**

Best results with the heptakis-(2,3-di-O-acetyl-6-O- *tert.*-butyldimethylsilyl)-derivatized CDs were achieved using the BGB-174. After several optimization steps (carrier gas flow, heating rate) this column separated all chiral MVOCs except geosmin. Additionally, the 6 achiral

compounds, the 3 single enantiomers as well as the ISTD, RSTD and BHT were well separated within one GC run (Fig. 5.14, Fig. 5.17).

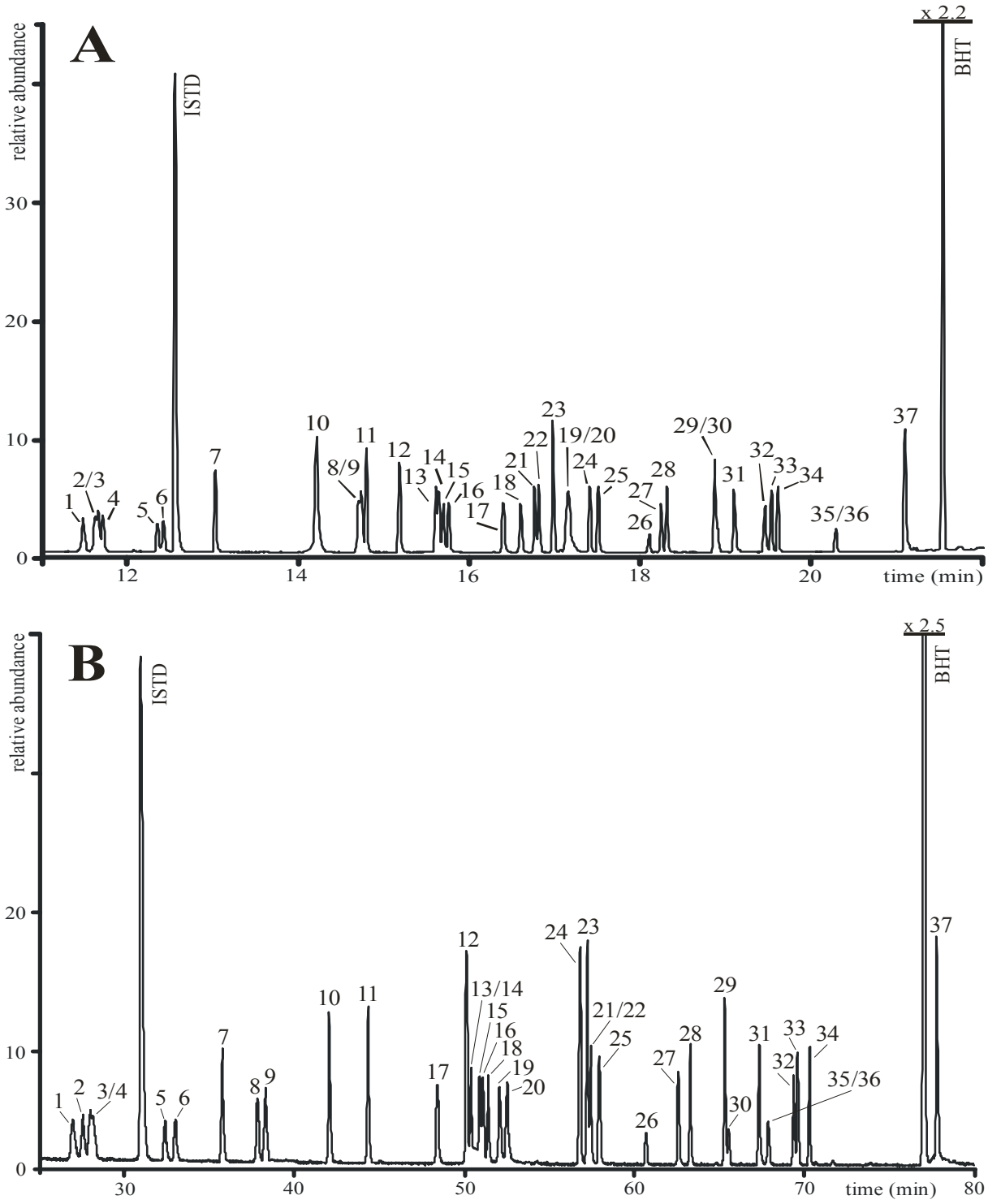



**Fig. 5.14: Separation optimization of MVOCs on BGB-174 analyzed with the Fisons GC-MS system in scan mode. Run (A): temperature program 3 with a heating rate of 9 °C min$^{-1}$ and a linear gas velocity of 35 cm s$^{-1}$. Run (B): temperature program 2 with a heating rate of 1.5°C min$^{-1}$ and a linear gas velocity of 60 cm s$^{-1}$. (Compound numbering follows the elution sequence of Fig. 5.17 and is described in Tab. 4.10.)**

The main difference between the BGB-174 and the Supelco β-DEX 225 column was the different dissolving polysiloxane (BGB-1701 vs SPB-20; Tab. 4.8) and therefore the changed polarity as well as the proportion of the CD (BGB-174: 50 % (w/w); Supelco: 25 % (w/w)).

The BGB-1701 is a medium polar polysiloxane of higher viscosity with a special selectivity due to the 7 % cyanopropyl content (Tab. 4.8). This "gum" phase forms a very stable film and has been proven as an ideal dissolving stationary phase for cyclodextrins and cyclodextrin derivates. It showed best properties for most chiral separation in the literature (König, 1993; Schreier et al., 1995; Schurig and Nowotny, 1990).

Additionally, the column bleed was tested for both heptakis-(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin phases, since they were neither immobilized nor chemically bonded (cross-linked). The chromatographic background that resulted from elution of stationary phase degradation or depolymerization products was comparably low for both columns and well acceptable for trace analysis. However, a significant rise of the baseline started about 40 °C below the upper temperature limit of the tested columns (about 180 °C for the BGB-174 and about 200 °C for the β-DEX 225) (Fig. 5.15). The bleed products consisted primarily of the well known low molecular thermally induced cyclic siloxanes.

BGB-174 with the 1:1 composition of heptakis-(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin and the medium polar BGB-1701 polysiloxane showed the best separation performance of all tested enantioselective phases. Consequently, the BGB column instead of the Supelco column was selected for the enantioselective MVOC analysis.

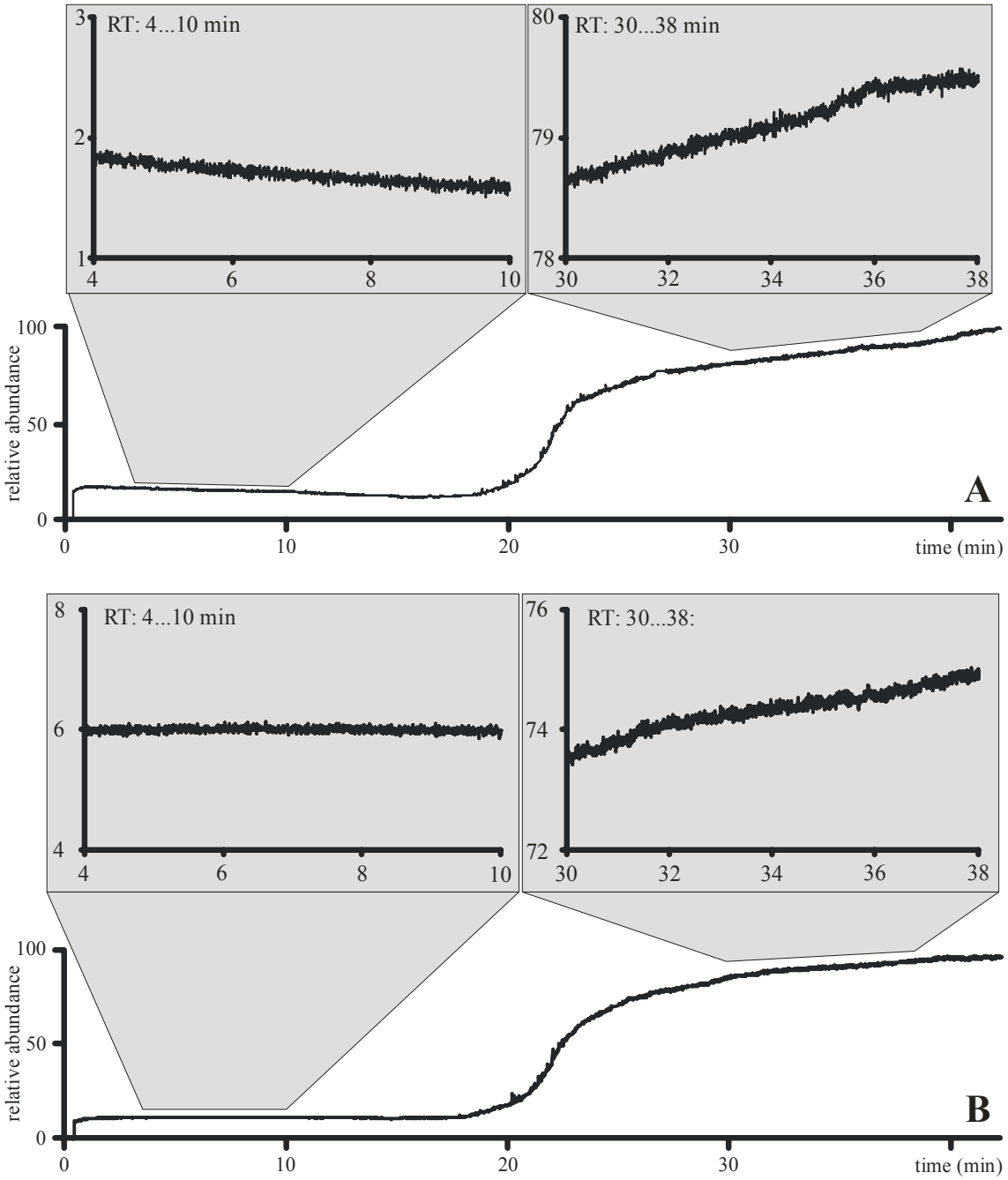



**Fig. 5.15: Bleed test of the BGB-174 (A) and the β-DEX 225 (B) determined by GC-MS using the scan mode and following the conditions in Tab. 4.9 applying bleed test 1.**

The achieved chiral resolution ($cR_S$) (see appendix 8.3. pg. 163) of all MVOCs is given in Tab. 5.7. 13 of 14 chiral MVOCs could be separated within one chromatographic run. However, the enantiomeric separation of geosmin (35/36) on this CSP was impossible. 7 enantiomer pairs were baseline separated ($cR_S \geq 1.5$) and, additionally, 3 compounds

showed a valley overlap of about 6 %. Also the diastereomers of (-)-carveol were baseline separated. Compromises covering the chiral resolution had to be made for octan-3-ol (13/14), borneol (19/20), and verbenone (30/31), which were only partly separated ($cR_S < 1.0$) (Tab. 5.7).

**Tab. 5.7: Chiral resolution ($cR_S$) of all selected 14 MVOCs on BGB-174 by GC-MS in the SIM mode (compare Fig. 5.17).**

| # | substance | resolution ($cR_S$) | separation |
|---|---|---|---|
| 1/3 | 3-methyl-butan-2-ol* | **2.71** | **baseline** |
| 2/4 | pentan-2-ol* | 1.08 | partially |
| 5/6 | 2-methyl-butan-1-ol* | **1.77** | **baseline** |
| 8/9 | fenchone* | 1.08 | partially |
| 13/14 | octan-3-ol* | 0.82 | partially |
| 15/16 | 1-octen-3-ol* | **1.52** | partially |
| 17/18 | camphor* | **5.81** | **baseline** |
| 19/20 | borneol* | 0.60 | partially |
| 21/22 | linalool* | 1.46 | partially |
| 24/25 | terpinen-4-ol* | **3.03** | **baseline** |
| 27/28 | alpha-terpineol* | **1.82** | **baseline** |
| 29/30 | verbenone* | 0.72 | partially |
| 33/34 | carvone* | **2.15** | **baseline** |
| 35/36 | geosmin* | 0.00 | n.s. |
| *31/32* | *trans-(-)-carveol* / cis-(-)-carveol** [3] | ***7.05*** | ***baseline*** |

* : chiral compounds; [3]: *cis-trans isomer separation* ; n.s.: not separated; baseline separation: $cR_S \geq 1.5$

In conclusion, the ring size of the CDs (Fig. 1.11 (pg. 40)) and, therefore, the cavity size did not influence the chiral separation significantly (Fig. 5.13). In fact, the polysiloxane used and the CD concentration had a larger influence on the chiral selectivity than the CD cavity. However, the substituents at C-2, C-3 and C-6 (compare Fig. 1.10) effected the enantiomeric selectivity dramatically.

Single compound pre-tests with GC-FID and high concentration MVOC solutions showed in general a reduced sample capacity of chiral stationary phases. Overloading of chiral compounds resulted in tailing and reduced enantiomer resolution (Fig. 5.16).

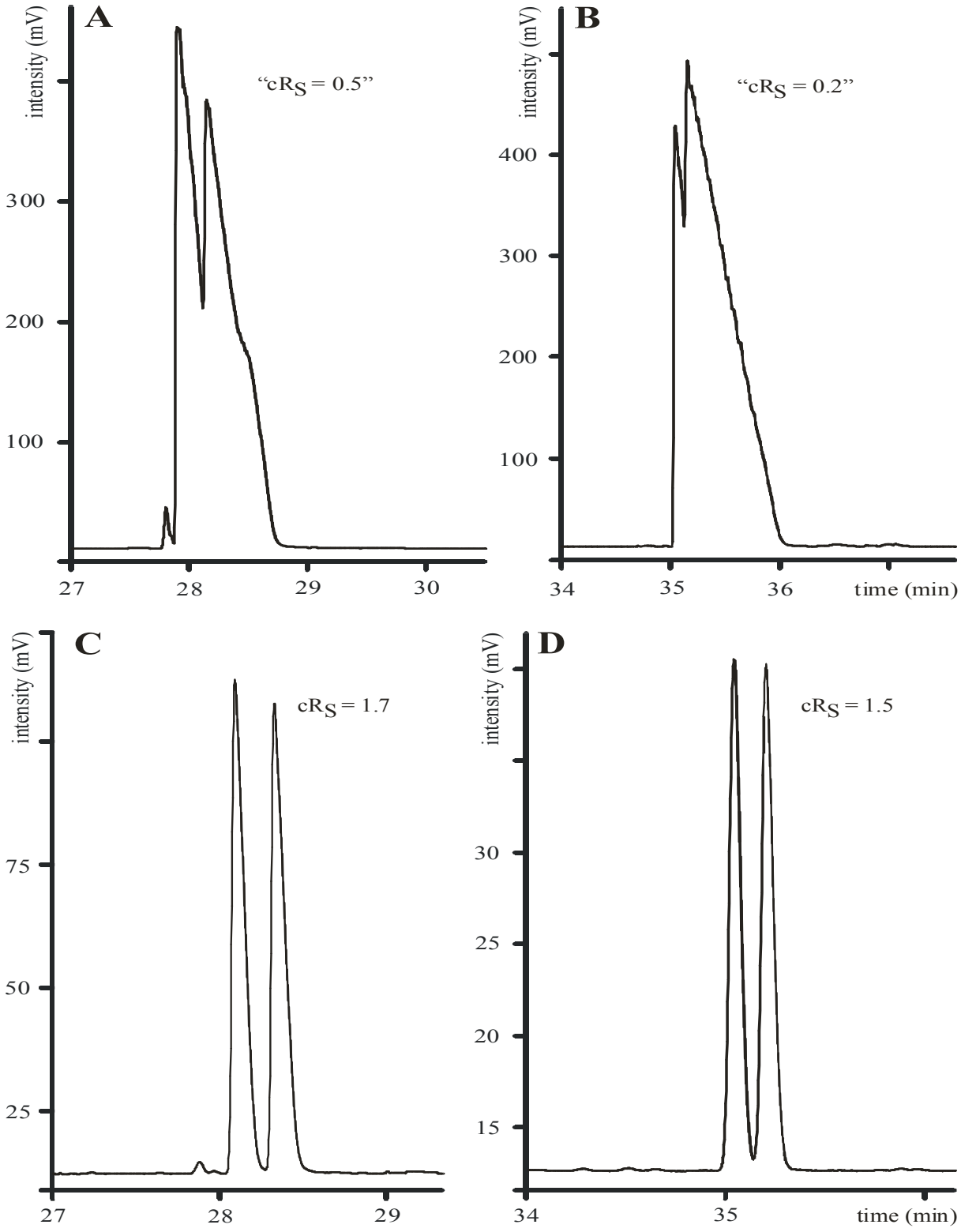



**Fig. 5.16: Reduced chiral resolution ($cR_S$) due to column overload during MVOC separation on a Supelco β-DEX 120. The same chromatographic conditions were used. Borneol (A/C) and terpinen-4-ol (B/D) were separated at a concentrations of 50 ng $\mu L^{-1}$ (A/B) and 5 ng $\mu L^{-1}$ (C/D).**

The retention gap routinely installed in front of all enantioselective columns had the main function to protect the analytical column and to increase column lifetime. However, it also improved separation and served as refocusing unit when used in combination with the cold on-column injector.

Special care was taken for column conditioning. Bicchi et al. (Bicchi et al., 1991) demonstrated the importance of the conditioning temperature and suggested that for the CD activation and best enantioselective performance a maximum temperature conditioning is needed. Therefore, all enantioselective columns were conditioned with hydrogen as carrier gas to their upper temperature limit.

A direct method-transfer from the GC-FID system to the GC-MS system was not possible and methods had to be adapted for the MS detection following the same procedures that were applied for the non-enantioselective chromatographic separation (pg. 94). The enantioselective chromatographic separation of the MVOCs on the BGB-174 capillary column required a SIM-program consisting of 15 retention windows. It started at 19 min and measured each of the selected masses with a fixed dwell time of 50 ms and a mass span of 0.1 amu. Tab. 5.8 and Tab. 4.10 summarizes the chromatographic separation and detection on the BGB-174 capillary column, the elution order and retention time (RT) of each compound as well as the monitored retention windows and the selected ions for each compound recorded in the SIM mode. Fig. 5.17 shows the resulting chromatogram.

**Tab. 5.8:** **Overview of the enantioselective chromatographic separation and detection method for the selected 22 MVOCs on the BGB-174 capillary column.**

| SIM-window | SIM-mass | AR ($s^{-1}$) | RT | # | compound | bp. (°C) | $M_{W.\ isotopic}$ | BP | other characteristic masses |
|---|---|---|---|---|---|---|---|---|---|
| **19.00…22.00** | 44, 55, 70, 73 | 5.00 | 20.32 | 1 | 3-methyl-butan-2-ol* | 112 | 88.08881 | 45 | **73**, **55**, 44, 43 |
| | | | 21.02 | 3 | | | | | |
| | | | 20.77 | 2 | pentan-2-ol* | 120 | 88.08881 | 45 | **73**, **55**, 45, 44, 43 |
| | | | 21.02 | 4 | | | | | |
| **22.00...23.70** | 56, 57, 69, 91, 93 | 4.00 | 22.67 | ISTD | 1-chlorohexane | 134 | 120.07057 | **91** | **93**, **69**, 55, 56, 56, 41, 43, 39 |
| | | | 22.97 | 5 | 2-methyl-butan-1-ol* | 129 | 88.08881 | **57** | **71**, 70, 59, **56**, 55, 41 |
| | | | 23.18 | 6 | | | | | |
| **23.70…25.00** | 58, 100 | 10.00 | 24.16 | 7 | hexan-2-one | 127 | 100.08881 | **43** | 100, 85, 71, **58**, 57 |
| **25.00…25.70** | 81, 152 | 10.00 | 25.51 | 8 | fenchone* | 193 | 152.12011 | **81** | **152**, 109, 82, 80, 69, 41 (67, 79) |
| | | | 25.62 | 9 | | | | | |
| **25.70…27.10** | 58, 67, 71, 72, 82, 99 | 3.33 | 25.88 | RSTD | chlorocyclohexane | 141 | 118.05492 | **67** | **83**, **82**, 55, 54, 41 |
| | | | 25.92 | 10 | heptan-2-one | 150 | 114.10446 | 43 | 114, **71**, 59, **58** |
| | | | 26.60 | 11 | octan-3-one | 168 | 128.12011 | 43 | 128, **99**, 85, 86, **72**, 71, 57 |
| **27.10…27.60** | 79, 94 | 10.00 | 27.48 | 12 | methyl disulfide | 109.7 | 93.99109 | **94** | **79**, 61, 64, 45 |
| **27.60…28.80** | 57, 72, 83, 95, 101, 152 | 3.33 | 27.80 | 13 | octan-3-ol* | 175 | 130.13576 | 59 | **101**, 84, **83**, 57, 55, 41 |
| | | | 27.85 | 14 | | | | | |
| | | | 27.93 | 15 | 1-octen-3-ol* | 175 | 128.12011 | **57** | 99, 85, **72**, 43, 41, 39 |
| | | | 28.02 | 16 | | | | | |
| | | | 28.16 | 17 | camphor* | 204 | 152.12011 | **95** | **150**, 110, 109, 108, 83, 81, 69, 67, 55, 41 |
| | | | 28.58 | 18 | | | | | |
| **28.80…29.50** | 58, 71, 93, 95, 110, 142 | 3.33 | 29.08 | 19 | borneol* | 216 | 154.13576 | **95** | 139, 136, **121**, **110**, 96, 93, 67, 55, 41 |
| | | | 29.15 | 20 | | | | | |
| | | | 29.20 | 21 | linalool* | 198 | 150.10446 | **71** | 136, 121, **93**, 83, 80, 69, 67 |
| | | | 29.27 | 22 | | | | | |
| | | | 29.39 | 23 | nonan-2-one | 192 | 142.13576 | **58** | **142**, 71, **59**, 57, 43, 41 |
| **29.50…30.30** | 71, 111 | 10.00 | 29.70 | 24 | terpinen-4-ol* | 212 | 154.13576 | **71** | 154, 136, **111**, 93, 86, 69, 67,55,43 |
| | | | 29.86 | 25 | | | | | |

**Tab. 5.8: continuation**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **30.30…31.10** | 93, 121 | 10.00 | 30.51 | 26 | gamma-terpineol* | n.n. | 154.13576 | **121** | 136, **93**, 154, 107, 81, 79 |
| | | | 30.75 | 27 | alpha-terpineol* | 218 | 154.13576 | **59** | 136, **121**, 93, 92, 81, 67, 43 |
| | | | 30.84 | 28 | | | | | |
| **31.10…31.90** | 84, 107, 109, 135 | 5.00 | 31.39 | 29 | verbenone* | 227.5 | 150.10446 | **107** | 150, **135**, 122, 108, 91, 80, 79 |
| | | | 31.44 | 30 | verbenone* | 227.5 | 150.10446 | **107** | 150, **135**, 122, 108, 91, 80, 79 |
| | | | 31.72 | 31 | *trans*-(-)-carveol* | 226 | 152.12011 | **109** | 152, 137, 119, **84**, 83 |
| **31.90…32.50** | 82, 84, 93, 109, 134 | 4.00 | 32.12 | 32 | *cis*-(-)-carveol* | 226 | 152.12011 | **84** | 137, **134**, 119, 109, 83, 69, 55, 41 |
| | | | 32.19 | 33 | carvone* | 279 | 150.10446 | **82** | 150, 108, 107, 106, **93**, 54 |
| | | | 32.29 | 34 | | | | | |
| **32.50…33.20** | 112, 125 | 10.00 | 32.72 | 35 | geosmin* | 270 | 182.16706 | **112** | 126, **125**, 97 |
| | | | n.s. | 36 | | | | | |
| **33.20…34.10** | 105, 150 | 10.00 | 33.94 | 37 | cuminol | 246 | 150.10446 | 135 | **150**, 119, **105**, 107, 91, 79, 77 |
| **34.10…35.00** | 205, 220 | 10.00 | 34.37 | BHT | butylated hydroxyl-toluene (stabiliser) | 265 | 220.18271 | **205** | **220**, 206, 189, 177, 145 |

*: Chiral compounds are marked with asterisk, **AR**: acquisition rate (ion masses scanned each second of the concerning retention window); **RT**: retention time; **bp**: boiling point; $\mathbf{M_{W,isotopic}}$: isotopic molecular weight; **BP**: basis peak; **target ion of SIM-program**: underline mass; **qualifier ions**: bold typed masses

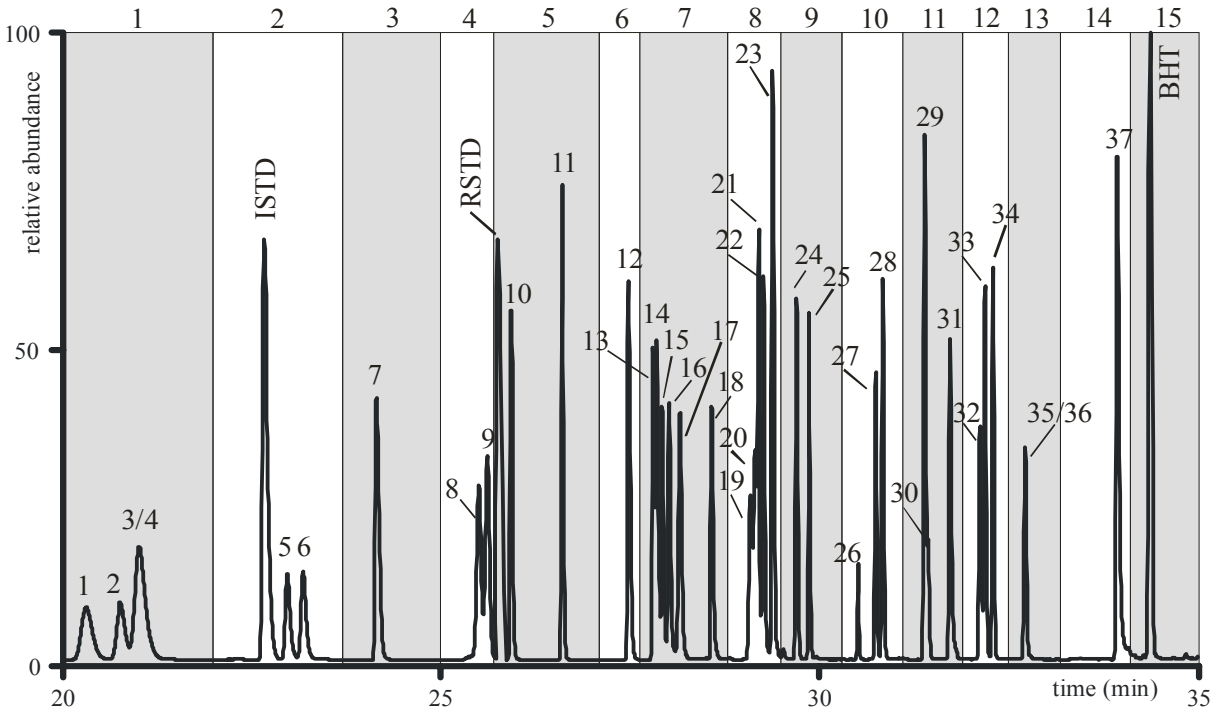



**Fig. 5.17: Overview of the enantioselective chromatographic separation with the 15 monitored retention windows of the selected 22 MVOCs on the BGB-174 capillary column (for details refer to Tab. 5.8).**

**Tab. 5.9:** **Summarizing overview of the retention time (RT) and relative retention time (RRT) of both applied methods (non-enantioselective methodology: Fig. 5.7 (pg. 95), enantioselective methodology: Fig. 5.17 (pg. 117)) (Compound assignment follows the elution order of the enantiomer chromatographic separation on the BGB-174.)**

| # | non-enantioselective method | | compound | enantioselective method | |
|---|---|---|---|---|---|
| | RT | RRT | | RT | RRT |
| ISTD | 5.13 | 1.00 | 1-chlorohexane (ISTD) | 22.67 | 1.00 |
| RSTD | 7.38 | 1.61 | chlorocyclohexane (RSTD) | 25.88 | 1.15 |
| 1 | 6.92 | 1.49 | 3-methyl-butan-2-ol* | 20.32 | 0.89 |
| 2 | | | | 21.02 | 0.92 |
| 3 | 7.69 | 1.70 | pentan-2-ol* | 20.77 | 0.91 |
| 4 | | | | 21.02 | 0.92 |
| 5 | 9.60 | 2.22 | 2-methyl-butan-1-ol* | 22.97 | 1.01 |
| 6 | | | | 23.18 | 1.02 |
| 7 | 6.28 | 1.31 | hexan-2-one | 24.16 | 1.07 |
| 8 | 12.96 | 3.13 | fenchone* | 25.51 | 1.13 |
| 9 | | | | 25.62 | 1.14 |
| 10 | 8.86 | 2.02 | heptan-2-one | 25.92 | 1.15 |
| 11 | 10.42 | 2.44 | octan-3-one | 26.60 | 1.18 |
| 12 | 5.85 | 1.20 | methyl disulfide | 27.48 | 1.22 |
| 13 | 13.12 | 3.18 | octan-3-ol* | 27.80 | 1.24 |
| 14 | | | | 27.85 | 1.24 |
| 15 | 13.99 | 3.41 | 1-octen-3-ol* | 27.93 | 1.24 |
| 16 | | | | 28.02 | 1.25 |
| 17 | 14.90 | 3.66 | camphor* | 28.16 | 1.25 |
| 18 | | | | 28.58 | 1.27 |
| 19 | 17.69 | 4.42 | borneol* | 29.08 | 1.30 |
| 20 | | | | 29.15 | 1.30 |
| 21 | 15.47 | 3.82 | linalool* | 29.20 | 1.30 |
| 22 | | | | 29.27 | 1.30 |
| 23 | 12.93 | 3.12 | nonan-2-one | 29.39 | 1.31 |
| 24 | 16.27 | 4.03 | terpinen-4-ol* | 29.70 | 1.32 |
| 25 | | | | 29.86 | 1.33 |
| 26 | 17.44 | 4.35 | gamma-terpineol* | 30.51 | 1.36 |
| 27 | 17.61 | 4.40 | alpha-terpineol* | 30.75 | 1.37 |
| 28 | | | | 30.84 | 1.38 |
| 29 | 17.75 | 4.44 | verbenone* | 31.39 | 1.40 |
| 30 | | | | 31.44 | 1.40 |
| 31 | 19.43 | 4.89 | *trans*-(-)-carveol* | 31.72 | 1.42 |
| 32 | 19.81 | 5.00 | *cis*-(-)-carveol* | 32.12 | 1.44 |
| 33 | 18.09 | 4.53 | carvone* | 32.19 | 1.44 |
| 34 | | | | 32.29 | 1.44 |
| 35 | 19.34 | 4.87 | geosmin* | 32.72 | 1.46 |
| 36 | | | | n.s. | n.d. |
| 37 | 22.19 | 5.65 | cuminol | 33.94 | 1.52 |
| BHT | 20.32 | 5.14 | BHT (stabiliser) | 34.37 | 1.54 |

* = chiral compounds

## 5.2 Real case sampling and method application

Real case sampling was performed and combined with the developed non-enantioselective and enantioselective GC-MS method (Tab. 4.3, Tab. 4.10, and Tab. 5.9) to detect the mould-characteristic MVOC pattern. The analytical procedure consisted of 3 individual steps: sampling, sample preparation, and GC-MS analysis.

### 5.2.1 Sampling time and sampler capacity

In the beginning of the project the passive samplers were exposed to indoor air over a period of 2 weeks as proposed by the manufacturer and done in other projects dealing with non-occupational measurements (e.g. (Pekar, 2000)). Experiments showed that the sampling interval of 28 days described the personal exposure better than any short-term measurement and resulted in a better enrichment of trace compounds.

The capacity of the sampler was never reached. The manufacturer defines the saturation capacity per compound in the range of 0.5 to 25 mg absolute (3M - Occupational Health and Environmental Safety Division, 1999). Shields et al. reported that during an 8-week sampling at environmental conditions no overload of the OVM 3500 occurred at any time (Shields and Weschler, 1987).

### 5.2.2 Total volatile organic compounds at real case sites

Several studies have been carried out to investigate the VOC load in air and showed that the indoor concentration of VOC generally exceeded the outdoor burden (Jones, 1999; Schleibinger et al., 2001). In 2003 Rehwagen et al. (Rehwagen et al., 2003) showed that the VOC concentration in indoor air is on average 10 times higher than outdoors.

The ISO standard 16000-6 (Kommission Reinhaltung der Luft (KRdL) im VDI und DIN, 2002) defines the total volatile organic compounds (TVOCs) as summation of all detected compounds eluting between hexane and hexadecane during analysis by GC-FID. In indoor areas an annual cycle of TVOCs has been observed with a peak concentration during the winter months. VOC measurements found complex mixtures comprising over 200 individual compounds. Several environmental surveys since 1985 showed that the average annual mean TVOC concentration indoors was in the range of 400…1000 µg $m^{-3}$ strongly dependent on the sampling site and emission sources. Nevertheless, extreme values up to 20'000 µg $m^{-3}$ were reported (Crump, 2001; Gabrio, 2001; Oppl and Braun, 1999; Waeber and Wanner, 1997).

### 5.2.3 Background sampling

Background sampling in several non mould contaminated apartments was performed to gain additional information about possible interacting compounds. These GC-MS measurements showed rather high VOC values in the indoor air compared to the MVOC target compounds (Fig. 5.18, Fig. 5.21, Fig. 5.19). The random sampling confirmed the great variety of indoor VOC burden at different locations. Higher concentrations of terpenes (α-pinene, β-pinene, δ-3-caren, δ-2-carene, limonene) were found almost everywhere, but could often be correlated with wood furniture, panelling, or bio paints. However, none of these sampling sites showed the analytical pattern of the MVOCs expected out of the selection considerations in chapter 3.

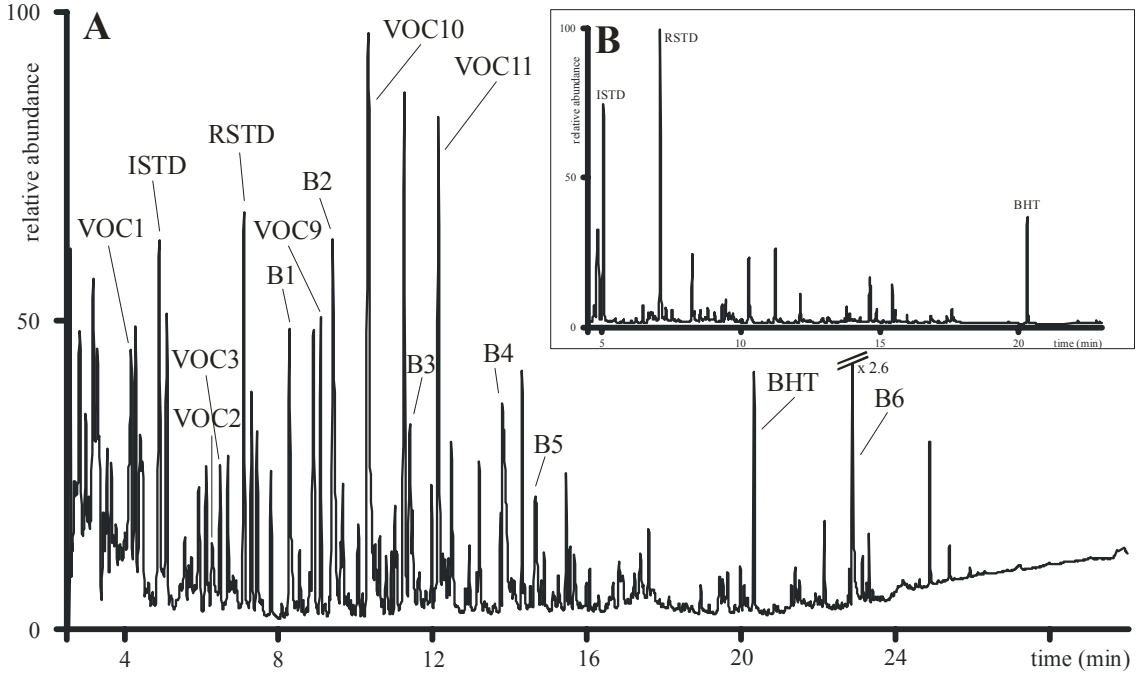



**Fig. 5.18: Typical indoor air burden of a non mould contaminated apartment analysed in scan (A) and SIM (B) mode with the non-enantioselective methodology (see Fig. 5.7): VOC1: α-pinene, VOC2: n-hexanal, VOC3: β-pinene, VOC9: limonene, VOC10: 2-(2-ethoxyethoxy)ethanol, VOC11: ethoxy-1-propanal, B1: butan-1-ol, B2: dimethylsulfone, B3: but-2-oxy-ethan-1-ol, B4: acetic acid (out of the solvent), B5: ethyl-2-hexan-1-ol, B6: caprolactam, ISTD: 1-chlorhexane, RSTD: chlorocyclohexane, BHT: butylated hydroxytoluene (solvent stabilizer).**

## 5.2.4 Additional reference points for the indoor air analysis

Supplementary references for real case sampling were given by the nature of the sampled environment. Butan-1-ol (CAS [71-36-3]) and 2-methylpropan-1-ol (CAS [78-83-1]) are typical present in the indoor environment and could be adapted to identify faulty sampling. Both compounds are typical indoor contaminants with ubiquitous concentration of at least 1 $\mu g\ m^{-3}$ (Lorenz, 2001). However, only 2-methylpropan-1-ol could serve as control compound of flawless sampling as butan-1-ol was part of the passive sampler blanks (see B1 and VOC4 in Fig. 5.2, Fig. 5.21, Fig. 5.19).

### 5.2.5 Detection of MVOCs in a real case sample

The analysis of several passive sampler of the mould suspected apartment building in southern Germany (chapter 4.5.2.2, pg. 59) showed, first of all, the typical high indoor VOC burden (chapter 5.2.3). Co-elution from non-target compounds were very common, creating very complex chromatograms that were difficult to interpret, especially, if detection was performed in the SIM mode. Nevertheless, most of the selected mould indicator substances were found in trace amounts in the SIM analysis. Only hexan-2-one, 3-methyl-butan-2-ol, and linalool showed elevated concentrations. The last 5 eluting target compounds carvone, geosmin, *trans*-, and *cis*-carveol, as well as cuminol were not detected (Fig. 5.19). Additionally, 2-methyl-protan-1-ol could be detected in the GC-MS scan mode and confirmed the good sampling quality.

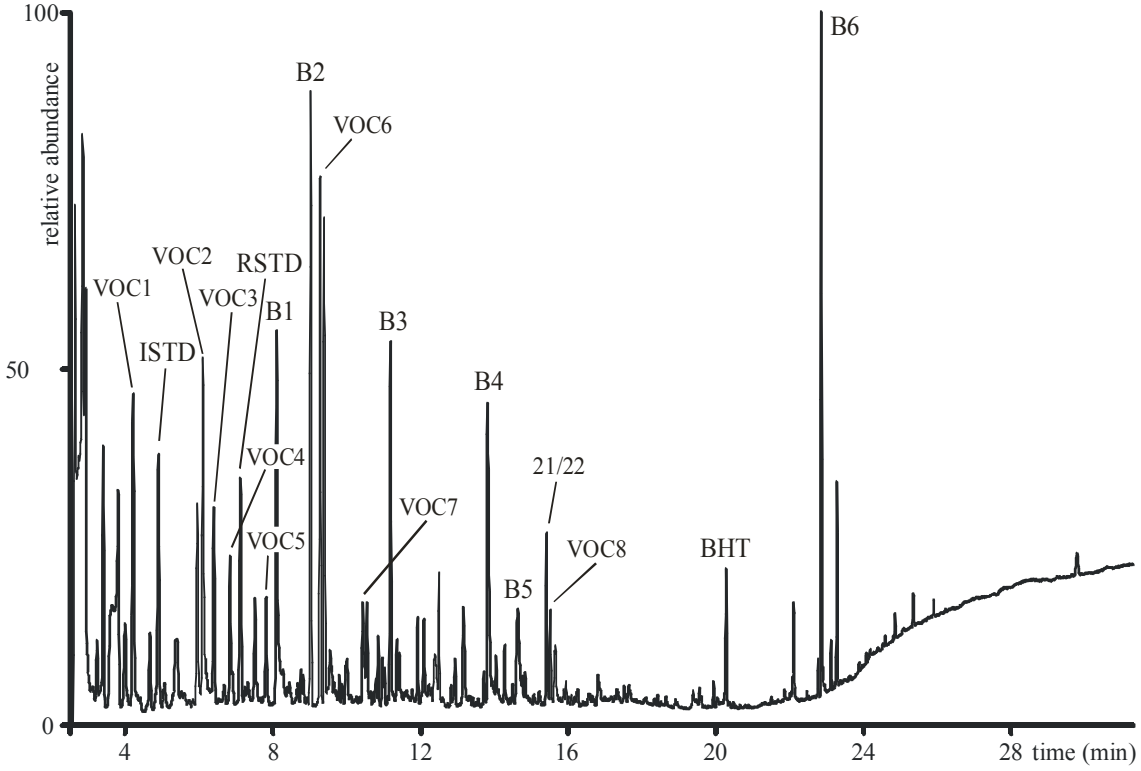



**Fig. 5.19: Real case sample analyzed with the non-enantioselective methodology (see Tab. 5.2) using the scan mode: VOC1: α-pinene, VOC2: n-hexanal, VOC3: β-pinene, VOC4: 2-methyl-propan-1-ol, VOC5: δ-3-carene, VOC6: eucalyptol, VOC7: pentan-1-ol, VOC8: linalyl butyrate, B1: butan-1-ol, B2: dimethylsulfone, B3: but-2-oxy-ethan-1-ol, B4: acetic acid (out of the solvent), B5: ethyl-2-hexan-1-ol, B6: caprolactam, ISTD: 1-chlorhexane, RSTD: chlorocyclohexane, BHT: butylated hydroxytoluene (solvent stabilizer), 21/22 linalool.**

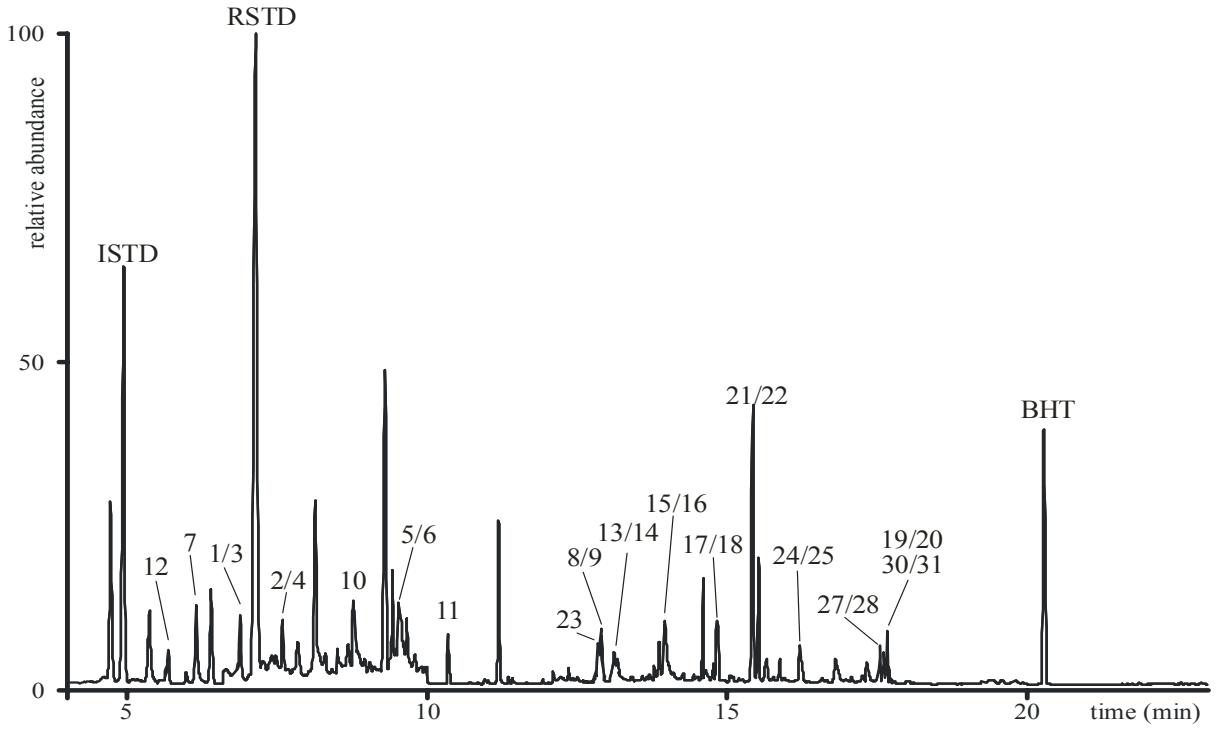



**Fig. 5.20: Real case sample analyzed with the non-enantioselective methodology (see Tab. 5.2) in SIM mode. (The peak declaration follows the elution sequence of the non-enantioselective chromatographic separation on the BGB-174)**

The detected concentrations and calculated amounts (compare chapter 4.6.4 (pg. 76)) of the selected MVOC in the air of the apartment are summarized in Tab. 5.10 (Fig. 5.20). The concentration of octan-3-ol was below the LOQ and was thus not taken into account for quantification.

**Tab. 5.10:** **Detected MVOCs at a real case site and their quantification (concentration in air) under the consideration of the method validation (pg. 97 et seqq., RC: recovery coefficient). (The compound numbering (#) follows the elution sequence of the enantioselective chromatographic separation on the BGB-174)**

| # | substance | amount detected in injected volume of 2 µL (pg) | LOD --- LOQ (pg µL$^{-1}$) | total amount desorbed from passive sampler in 1 mL solvent (pg) | total amount trapped on passive sampler incl. RC (ng) | trapped volume of compound (m³) | concentration in air (ng m$^{-3}$) |
|---|---|---|---|---|---|---|---|
| 12 | methyl disulfide | 47 | 1.0<br>2.8 | 522 | 0.345 | 1.32 | 0.253 |
| 7 | hexan-2-one | 510 | 4.2<br>14.1 | 5667 | 7.981 | 1.15 | 6.665 |
| 1/3 | 3-methyl-butan-2-ol* | 355 | 9.4<br>31.3 | 3946 | 4.153 | 1.27 | 3.150 |
| 2/4 | pentan-2-ol* | 173 | 4.9<br>16.4 | 1922 | 1.290 | 1.21 | 1.025 |
| 10 | heptan-2-one | 245 | 6.1<br>20.4 | 2723 | 2.232 | 1.08 | 1.998 |
| 5/6 | 2-methyl-butan-1-ol* | 337 | 16.7<br>55.6 | 3750 | 5.137 | 1.21 | 4.097 |
| 11 | octan-3-one | 195 | 7.0<br>23.3 | 2163 | 2.544 | 1.21 | 2.427 |
| 23 | nonan-2-one | 20 | 4.2<br>14.1 | 222 | 0.472 | 1.01 | 0.480 |
| 8/9 | fenchone* | 53 | 1.1<br>3.6 | 584 | 0.395 | 0.95 | 0.453 |
| 13/14 | octan-3-ol* | 22 | 10.7<br>35.7 | < LOQ? | < LOQ? | 0.84 | < LOQ? |
| 15/16 | 1-octen-3-ol* | 92 | 23.1<br>76.9 | 1026 | 0.877 | 0.98 | 0.849 |
| 17/18 | camphor* | 177 | 3.2<br>10.6 | 1963 | 1.869 | 1.00 | 2.166 |
| 21/22 | linalool* | *677* | 14.3 | *7521* | *6.776* | 1.00 | *7.151* |
| | *linalool-background* | *-246* | | *-2733* | *-2462* | | *-2.695* |
| | linalool* corrected | 431 | 47.6 | 4788 | 4314 | | 4.721 |
| 24/25 | terpinen-4-ol* | 60 | 6.8<br>22.7 | 672 | 1.244 | 0.83 | 1.535 |
| 27/28 | alpha-terpineol* | 26 | 9.7<br>32.3 | < LOQ? | < LOQ? | 0.83 | < LOQ? |
| 19/20 | borneol* | 121 | 3.3<br>11.1 | 1340 | 1.055 | 0.91 | 1.264 |
| 29/30 | verbenone* | 31 | 1.0<br>3.3 | 343 | 0.856 | 0.78 | 0.979 |
| 33/34 | carvone* | < LOD? | 5.5<br>18.2 | < LOD? | < LOD? | 0.78 | < LOD? |
| 35/36 | geosmin* | < LOD? | 3.3<br>11.0 | < LOD? | < LOD? | 0.80 | < LOD? |
| 31 | *trans*-(-)-carveol* | < LOD? | 25.0<br>83.3 | < LOD? | < LOD? | 0.84 | < LOD? |
| 32 | *cis*-(-)-carveol* | < LOD? | 85.7<br>285.7 | < LOD? | < LOD? | 0.80 | < LOD? |
| 37 | cuminol | < LOD? | 9.5<br>31.7 | < LOD? | < LOD? | 0.80 | < LOD? |

The background sample collected in the staircase at the main entrance of the apartment building showed increased linalool level (Fig. 5.21).

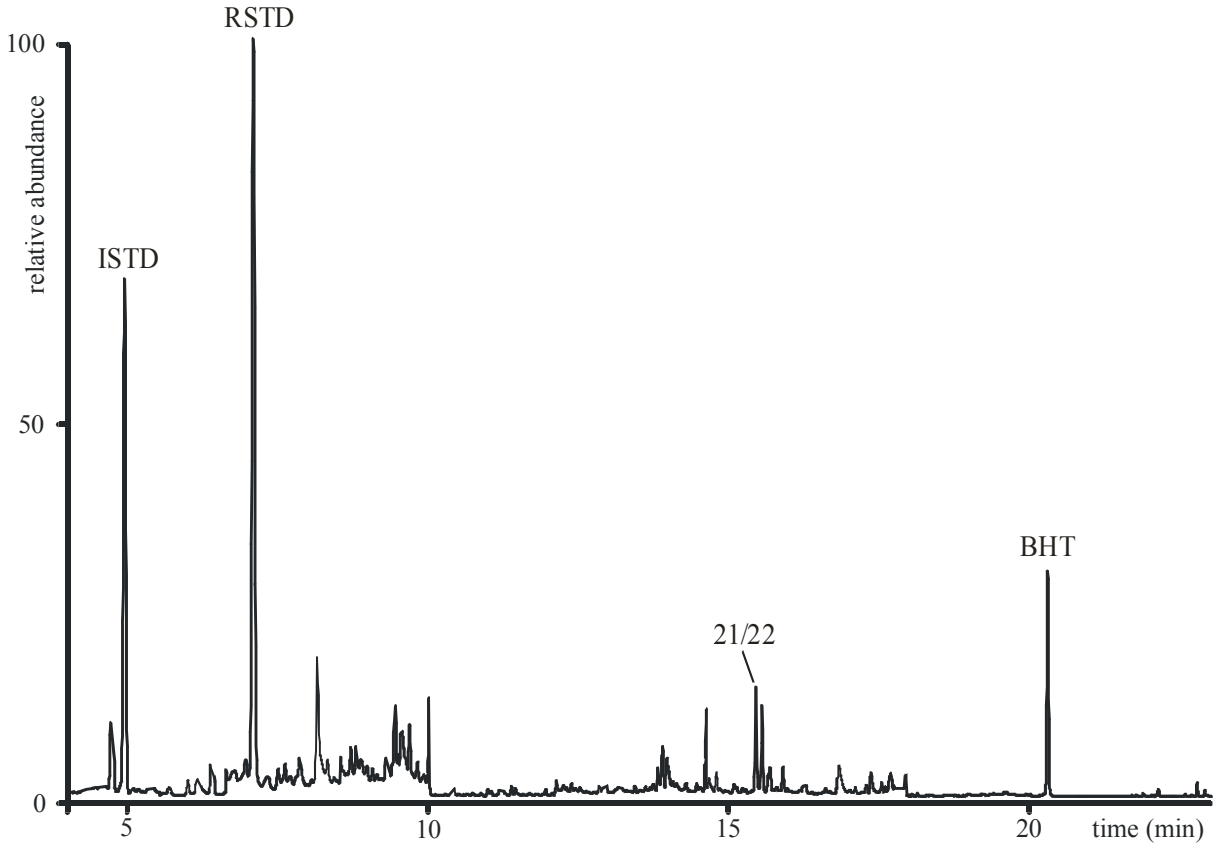



**Fig. 5.21: Background of the sampled side analyzed with the non-enantioselective methodology (see Tab. 5.2) in SIM mode: ISTD: 1-chlorhexane, RSTD: chlorocyclohexane, 21/22: linalool, BHT: butylated hydroxytoluene (solvent stabilizer).**

Therefore, the linalool concentration was corrected for quantification by the detected background concentration. The total amount of the detected MVOCs in the apartment summed up to 33 ng $m^{-3}$. These measured values were low compared to the TVOC concentrations by Seifert, who suggested normal values ranging between 20 to 100 µg $m^{-3}$ for different chemical classes (Berglund et al., 1997; Seifert, 1992; Seifert, 1999) (see chapter 1.1.3, pg. 9), but were still increased compared to the background concentration.

Overall, 16 of the 22 individual compounds were detected and contributed to the MVOC pattern. Taking this analytical pattern as a fingerprint the detected MVOC indicated a possible fungal contamination to some extend. The enantioselective GC-MS analysis for compound conformation could not contribute to this result. The concentrations of the chiral compounds were far too low and the sensitivity of the enantioselective method was not high enough. However, further on-site investigation showed a major hidden mould contamination between the fitted kitchen furniture and the inadequately isolated outside wall resulting from a severe water-damage of the flat-roof house. The soaked construction materials (wall paper, plaster and brick) in combination with tight sealing and heated room created an ideal environment for fungal colonisation.

# 6 Conclusions

Analysis of VOCs produced by mould was used as indicator of microbial growth. However, the use of these so-called microbial volatile organic compounds (MVOCs) to detect fungal growth in buildings is limited. The diversity of the MVOCs is extensive and far over 500 compounds are known today (Tab. 1.5). The most commonly reported compounds are hydrocarbons (e.g. octane), alcohols (e.g. 2-methyl-1-butanol), aldehydes and ketones (e.g. octan-3-one), esters (e.g. ethyl acetate), ethers and furans (e.g. 2-methylfuran), terpenes and terpene derivatives (e.g. geosmin), nitrogen and sulphur compounds (e.g. pyridine and dimethyl disulfide). Only a few MVOCs act in combination as universal fungal signature compounds and as possible general marker pattern. 22 most characteristic compounds were chosen for this study as reference compounds (Tab. 3.1).

This work showed that the chromatographic separation and an unequivocal mass spectrometric detection is possible of the selected MVOCs. The chromatographic separation could be performed either non-enantioselective or enantioselective within one single run.

Cold on-column injection was most suitable for the MVOC ether solution. The non-vaporizing technique reduced the thermal stress of the analytes and ensured a qualitative transfer of the solutes into the column. A routinely installed retention gap and guard column was used to achieve optimal focussing of the compounds.

Best non-enantioselective chromatographic separation of the 22 MVOCs was obtained by the DB-Wax capillary. With this 30 m long polyethylene glycol (PEG) column separation

problems were minimized when using the apolar HP-1 and HP-5 MS as well as the polar HP-INNOWax capillary column.

The standard procedure including reference solution preparation, sampling simulation and desorption as well as non-enantioselective GC separation and MS detection was validated as a reliable semiquantitative method for trace analysis of MVOCs. Quantification of the target compounds accumulated on the passive sampler was performed using the internal standard method. Of the evaluated standards 1-chlorohexane was best suitable as internal standard (ISTD) and chlorocyclohexane as recovery standard (RSTD). The non-enantioselective GC-MS method was linear over a concentration range of 0.01 to 5 ng $\mu L^{-1}$ with a coefficient of determination ($r^2$) between 0.96 and 0.99. The recovery rate ranged from 40 to 127 % for the majority of compounds. The between-runs precision was 2 to 7 %. The limit of detection was 1 to 86 pg $\mu L^{-1}$ (S/N-ratio 3:1) and the limit of quantification was 2 to 286 pg $\mu L^{-1}$ (S/N-ratio 10:1). During the validation process two groups of outliers set off while recovery tests were made. One group (methyl disulfide, heptan-2-one, fenchone) was caused by a faulty dilution; the other group with higher boiling MVOC was associated with a problematic compound-adsorbent-solvent-interaction.

The heptakis-(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin dissolved in the medium polar BGB-1701 polysiloxane (50 % (w/w)) of BGB Analytik AG (Zurich, Switzerland) (BGB-174) demonstrated excellent enantioselectivity for 13 of the 14 chiral MVOCs and showed overall the best enantioselective chromatographic capabilities.

The detection of the majority of the 22 MVOCs on a real case site was possible by passive sampling and solvent desorption. 16 of the 22 compounds contributed to the analytical

fingerprint pattern and indicated possible fungal contamination. These analytical results were confirmed by on-site investigations that followed. However, real case studies showed that the concentration of the MVOCs in indoor air was very low. The general VOC burden of the indoor air was up to several orders of magnitude higher. The variety of compounds and the high background concentration made an unequivocal identification of the trace amounts very difficult and led easily to misinterpretations even with the high sensitive and selective SIM-MS detection. The quantification of all compounds was not possible because of the low MVOC concentration sometimes far below the LOQ.

The enantioselective GC-MS analysis of the MVOCs in indoor air was not suitable for the observed concentration range. The sensitivity of the enantioselective method was too low to unequivocal differentiate the source of the detected compounds. Any differentiation between naturally occurring mould compounds and synthetic/anthropogenic racemates present in indoor air was not possible. These problems need to be investigated further, before enantioselective GC-MS analysis in combination with passive sampling of indoor air can become a way of detecting indoor mould.

Furthermore, passive sampling experiments showed the difficulties and the error share to different (unknown) environmental conditions to obtaining a representative sample. The detectable amount of compounds in air with a passive sampler is a direct function of the sampling rate (SR), the sampling time (t), the blank values of the unexposed samplers, as well as temperature, humidity and air velocity (see chapter 1.3.2, pg. 26) in addition to the HRGC-MS method parameters as discussed in chapter 4 (e.g. the reproducibility, the sensitivity of the GC detector, the selectivity of the GC column).

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# 8 Appendix

## 8.1 Characterization of the selected chemical compounds

The following tables contain information about the 22 selected MVOCs. The compounds are arranged by the elution sequence of the enantioselective chromatographic separation on the BGB-174 column that defined the compound numbering in this work. Supplementary, a set of data on the reference compounds (8.1.2 Standards), the solvent and the solvent stabilizer (8.1.3 Solvent) are listed. The data are assembled by literature research ((Budavari, 1996; Lide, 1993; Stecher, 1968; Weast, 1988; Windholz, 1983) and electronic data sources (http://chemfinder.cambridgesoft.com/: Chemosoft (CambridgeSoft Corporation, 2004), http://webbook.nist.gov/chemistry/: NIST (NIST Standard Reference Data, 2004), http://www.chemdat.info/: (Merck KGaA, 2004), http://www.sigmaaldrich.com/: Fluka Chemie GmbH (Buchs, Switzerland)), but does not lay any claim on completeness.

All data refer to a temperature of 20 °C and standard pressure of 101.325 kPa when not stated otherwise. Chiral compounds are labelled with superscript asterisk at their name. Vacant positions in the data sets were not found in the mentioned references.

A small selection of literature references of each compound is provided. These references are cited as follows:

- **FT-IR**: Reference to the page location of the spectrum in “The Aldrich Library of FT-IR Spectra”. The number in boldface type is the edition, Roman volume number

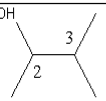
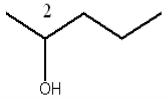
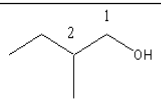
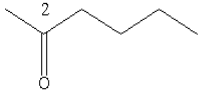
is in parentheses followed by the page number. The letter description refers to the position of the spectrum on a page (top, middle, bottom) (Pouchert, 1997).

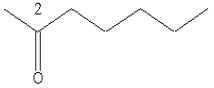
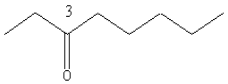
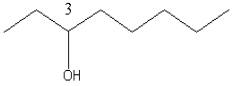
- **FT-NMR**: Reference to the page location of both the carbon and proton spectra in "The Aldrich Library of $^{13}C$ and $^{1}H$ FT NMR Spectra". The number in boldface type is the edition, volume number is in parentheses followed by the page number. The letter description refers to the position of the spectrum on a page (top, middle, bottom) and to the chemical structure diagram of the product. The carbon-13 spectrum is displayed immediately above the proton spectrum.(Pouchert and Behnke, 1993)

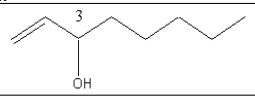
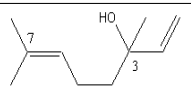
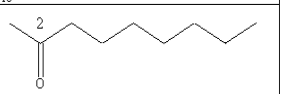
- **Beilstein**: Reference to "Beilstein Handbuch der Organischen Chemie". The number in boldface type is the volume number, followed by the Roman supplement number and the page number. Beilstein **6**, I, 556 refers to volume 6, 1st supplement, page 556 (Luckenbach, 1984; Prager et al., 1918).

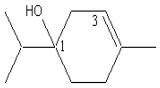
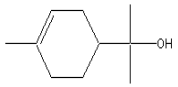
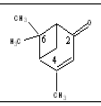
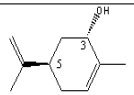
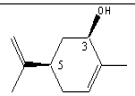
- **Merck**: Reference to "The Merck Index". The number in boldface type is the edition, followed by the monograph number of the Merck Index (Budavari, 1996).

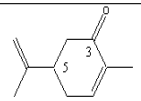
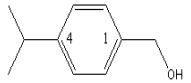
The mass spectra of the listed compounds were recorded by the Fisons MD800 (Fisons Instruments, Manchester, United Kingdom) mass spectrometer along with the practical work. The obtained spectra are based on the reference compounds listed in chapter 4.2 (pg. 50) and were included as user database in the "Library Browser" of the Xcalibur™ data system software parallel to the NIST/EPA/NIH Mass Spectral Database (version 1.7 ©1999, National Institute of Standard an Technology, Gaithersburg, MD, USA) and the Wiley Registry of Mass Spectral Data (7th edition ©2000, Wiley Interscience, New York, NY, USA).

| Trivial name | 3-methyl-butan-2-ol* | pentan-2-ol* | 2-methyl-butan-1-ol* | hexan-2-one |
|---|---|---|---|---|
| # | **1 / 3** | **2 / 4** | **5 / 6** | 7 |
| **Synonymous/ commercial name** | 1,2-dimethyl-1-propanol; methylisopropylcarbinol; NSC 71162; sec-Isoamyl alcohol; Isopropyl methyl carbinol | sec-amyl alcohol; sec-pentanol; methylpropylcarbinol; 1-methylbutanol; 2-pentyl alcohol; pentanol-2; pentan-2-ol; sec-n-amyl alcohol; isoamyl alcohol, secondary; sec-pentyl alcohol; 2-hydroxypentane | 2-methyl-n-butanol; 2-methylbutyl alcohol;active amyl alcohol; active primary amyl alcohol; NSC 8431; primary active amyl alcohol; sec-butylcarbinol | MBK; methyl n-butyl ketone; propyl acetone; hexan-2-one; MNBK; 2-oxohexane |
| **Systematic name** | DL-3-methyl-2-butanol; (±)-3-methyl-2-butanol | 1-methyl-butan-1-ol | DL-2-methyl-1-butanol; (±)-2-methyl-1-butanol | methyl-tert-butyl ketone |
| **CAS** | 598-75-4 (±); 1572-93-6 (-); 1517-66-4 (+) | 6032-29-7 (±); 31087-44-2 (-) (R); 26184-62-3 (+) (S) | 137-32-6 (±); 1565-80-6 (-) | 591-78-6 |
| **BRN** | 1718800 (±) | 1718819 (±); 4652311 (-); 1718820 (+) | 1361351 (±); 1718809 (-) | 1737676 |
| **Molecular formula** | $C_5H_{12}O$ | $C_5H_{12}O$ | $C_5H_{12}O$ | $C_6H_{12}O$ |
| **Structure / chemical constitution** |   |   |   |   |
| $M_W$ (amu) | 88.1492 | 88.1482 | 88.1492 | 100.1602 |
| **SR** ($cm^3\ min^{-1}$) | 32.7 | 31.2 | 31.1 | 29.7 |
| **Appearance, colour, odour etc. (physical state at 20 °C)** | | colourless, combustible liquid, characteristic hot spicy odour or mild green fusel oil | combustible liquid | colourless liquid; characteristic, medical, hot odour |
| **t.-o. conc.** ($\mu g\ m^{-3}$) | | | 30 (Böck, 2001) | 708 |
| **mp** (°C) | | -50 | -70 | -57 |
| **bp** (°C) | 112 | 119…121 | 129 | 127 |
| **Solubility** ($g\ L^{-1}$) | | 0.135 (in ethanol) | | |
| **Water solubility** ($g\ L^{-1}$) | 28 | 0.135 | 36 | 0.014 |
| **Vapour pressure (Pa)** | 300 | 300 | 450 | 11 |
| **ρ** ($g\ cm^3$) | 0.818 | 0.809 | 0.82 | 0.811 |
| $n^{20}_D$ | 1.4097 | 1.4055 | 1.4107 | 1.4007 |
| $[\alpha]^{20}_D$ | + 5° (undiluted) at 18 °C (+) | +13° (undiluted) at 25 °C (+); -13° (undiluted) at 25 °C (-) | - 5.5 …-6.5° (undiluted); - 6.3 ± 0.5°, c = 10 % in EtOH | |
| **Hazard indications**<br>**R-phrases**<br>**S-phrases**<br>**Swiss poison class** | irritant; flammable harmful liquid<br>10-20<br>24/25<br>4 | irritant; harmful liquid<br>10-20-37-66<br>46<br>4 | irritant; harmful liquid<br>10-20<br>24/25<br>4 | toxic liquid<br>10-48/23-62-67<br>36/37-45<br>4 |
| **selected supplier**<br>supplier remark: | Fluka Chemie GmbH (Buchs, CH); product #: 66020; purity: ≥ 98.0 % (GC) | Fluka Chemie GmbH (Buchs, CH); product #: 76943; purity: ≥ 99.5 % (GC) | Fluka Chemie GmbH (Buchs, CH); product #: 65980 (S)-(-)-2-Methylbutanol; purity: 95% (GC)<br>main impurity: 3-methyl-1-butanol | Fluka Chemie GmbH (Buchs, CH); product #: 02473; purity: ≥ 99.5 % (standard for GC, GC)<br>limited shelf life, expiry date! |
| **Literature** FT-IR : | 2(I), 176D; 1(I), 122C | 2(I), 171B; 1(I), 118D | 2(I), 166D; 3(I) 161C | 2(I), 643B; 1(I), 408A |
| FT-NMR : | 1(I),183C | 1(I), 176C; (-): 1(I), 177A; (+): 1(I), 177B | 1(I), 170C; 2(I), 107A | 1(I), 635B |
| Beilstein : | 1, 391, I 196, II 425, III 1630, IV 1675 | 1, 384, I 193, II 418, III 1609, IV 1656 | 1, 388, IV 1666 | 1, 690, IV 3298 |
| Merck : | 13, 6058; 12, 6110; 11, 5953 | 13, 7196; 12, 7258; 11, 7075 | 13, 6057; 12, 6109; 11, 5952 | 13, 6060; 12, 6112; 11, 5955 |
| **Remarks** | | | | |
| **Assignment of mould species** | Aspergillus spp (A.niger, A. versicolor) Alternaria spp alternata, Cladosporium herbarum, Penicillium spp (P.expansum, P.coprohilum) (Böck et al., 1998; Larsen and Frisvad, 1995) | Penicillium sp. (P.palitans) (Wilkins et al., 2003) | Penicillium spp (P. expansum), Aspergillus spp (A. versicolor, A. niger, A. alternata), Cladosporium herbarum (Böck et al., 1998; Fischer et al., 2000; Keller et al., 1998; Larsen and Frisvad, 1995; Larsen and Frisvad, 1995; Lorenz, 2001; Ström et al., 19 | Penicillium sp. (Larsen and Frisvad, 1995) |

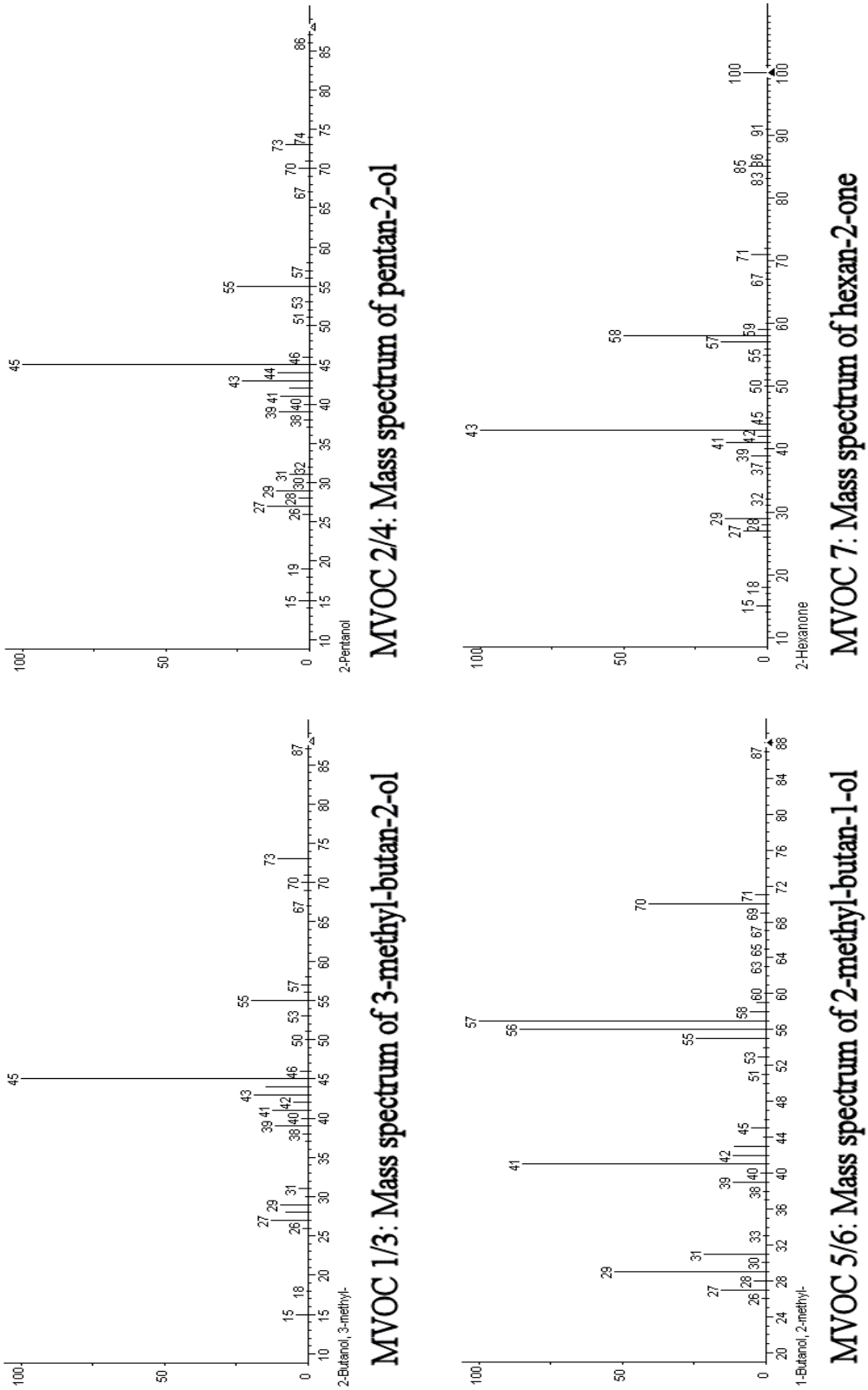
| Trivial name | fenchone* | heptan-2-one | octan-3-one | methyl disulfide | octan-3-ol* |
|---|---|---|---|---|---|
| # | 8 / 9 | 10 | 11 | 12 | 13 / 14 |
| **Synonymous/ commercial name** | 1,3,3 trimethyl-2-norbomanone; 1,3,3 trimethylnorcamphor; 1,3,3 trimethyl-bicyclo[2.2.1]heptane-2-one; 1,3,3 trimethyl-2-norcamphanone | MAK; n-amyl methyl ketone; butyl acetone; heptan-2-one; ketonec-7; amyl methyl ketone; butyl acetone; 2-ketoheptane; amyl-methyl-cetone; 1-methylhexanal; butylacetone; 2-oxoheptane; NSC 7313; 1-methylhexanal | n-amyl ethyl ketone; n-octanone-3; amyl ethyl ketone; ethyl amyl ketone; 3 oxooctane; ethyl n-amyl ketone; EAK; octan-3-one; ethyl n-pentylketone; NSC 60161 | (methyldithio)methane; 2,3-dithiabutane; dimethyl disulphide; DMDS | amylethylcarbinol; ethyl-n-amyl carbinol; Ethyl pentyl carbinol; 1-Ethyl-1-hexanol; DL-3-octanol |
| **Systematic name** | 1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-one | n-pentyl methyl ketone | Ethyl pentyl ketone | dimethyl disulfide | (±)-octan-3-ol |
| **CAS** | 1195-79-5 (±); 4695-62-9 (+); 7787-20-4 (-) | 110-43-0 | 106-68-3 | 624-92-0 | 589-98-0 (±) |
| **BRN** | 2206555 (+); 2042710 (-) | 1699063 | 1700021 | 1730824 | 1719310 (±) |
| **Molecular formula** | $C_{10}H_{16}O$ | $C_7H_{14}O$ | $C_8H_{16}O$ | $C_2H_6S_2$ | $C_8H_{18}O$ |
| **Structure / chemical constitution** |  |  |  |  |  |
| $M_W$ (amu) | 152.2358 | 114.1045 | 128.2138 | 94.2016 | 130.2296 |
| **SR** ($cm^3\ min^{-1}$) | 21.6 | 27.7 | 26.0 | 33.9 | 25.3 |
| **Appearance, colour, odour etc. (physical state at 20 °C)** | bitter tasting oily liquid, camphor-like odour | clear, colourless combustible liquid; mild banana oil, spicy, piquant | colourless liquid; fruity, spicy | pale yellow liquid, stinky and unpleasant odour | colourless, oily combustible liquid; nutty, herbaceous, with a odour of melon, citrus, or cheese, mushroom |
| **t.-o. conc.** ($\mu g\ m^{-3}$) | | 94 | 324 | 0.1 | |
| **mp** (°C) | 5 | -31 | -23 | -84.7 | -45 |
| **bp** (°C) | 193 | 151 | 168 | 109.7 | 174…176 |
| **Solubility** ($g\ L^{-1}$) | soluble in ethanol | | | | |
| **Water solubility** ($g\ L^{-1}$) | insoluble in water | 0.0043 | insoluble | < 0.001 | |
| **Vapour pressure (Pa)** | 61.7 | 630.62 | 200 | 3826 | 68.3 |
| **ρ** ($g\ cm^3$) | 0.947 | 0.815 | 0.822 | 1.046 | 0.819 |
| $n^{20}_D$ | 1.4620 | 1.4087 | 1.415 | 1.5260 | 1.426 |
| $[\alpha]^{20}_D$ | +61 ± 1 °(undiluted); -52 ± 2 ° (undiluted) | | | | |
| **Hazard indications** | | irritant; harmful liquid | irritant; harmful liquid | highly flammable, irritant liquid, dangerous for the environment | irritant; harmful liquid |
| **R-phrases** | 10 | 10-20/22 | 10-36/37/38 | 11-20/22-36-51/53 | 36/37/38 |
| **S-phrases** | 23-24/25 | 24/25 | 26-36 | 16-26-60 | 26-36 |
| **Swiss poison class** | | 4 | 4 | 2 | 4 |
| **selected supplier** | **(+)-fenchone**: Fluka Chemie GmbH (Buchs, CH); product #: 46208; purity: ≥ 99.5 % (terpene standard for GC, GC). **(–)-fenchone**: Aldrich Chemical Corp. (Milwukee, USA); product #: 196436; purity: ≥ 98.0 % (GC). | Fluka Chemie GmbH (Buchs, CH); product #: 68592; purity: ≥ 98.0 % (GC) | Fluka Chemie GmbH (Buchs, CH); product #: 04659; purity: ≥ 97.0 % (GC) | Fluka Chemie GmbH (Buchs, CH); product #: 40221; purity: ≥ 98.0 % (GC) | Acros Organics (Geel, Belgium); product #: 203770500; purity: ≥ 99 % (GC) |
| supplier remark: | **(+)**: limited shelf life, expiry date!; **(-)**: % | | | | |
| **Literature** FT-IR : | 2(I), 684A; 1(I), 442B | 2 (1), 644C, 1(1), 408D | 2(I), 646C; 1(1), 410C | 1(I), 264C | 2 (I), 175A; 1 (I), 121A |
| FT-NMR : | 1(1), 678B | 1(1), 636C | 1(1), 639A | 1(1), 425B | (1), 181C |
| Beilstein : | 7, IV 212 | 1, 699, I 358, II 753, III 2851, IV 3318 | 1, 706, I 362; III 2876; IV 3341 | 1, 291 I 145, II 278, III 1219, IV 1281 | 1, I 208, II 452, III 1723, IV 1779 |
| Merck : | 13, 3995; 12, 4008 | 13, 4683; 12, 4698; 11, 4584 | 13, 3799; 12, 3810; 11, 3721 | | 12, 6110; 11, 5953 |
| **Remarks** | Further readings: ApSimon 4, 529, 546; Gildemeister 3 c, 330 ff.; Pharm. Unserer Zeit 14, 11 (1987); Review: Food Cosmet. Toxicol. 14, Suppl., 769 ff. (1976); Ullmann (5.) A 11, 173; Synth.: Justus Liebigs Ann. Chem. 1981, 2093 | | | | |
| **Assignment of mould species** | | Penicillium sp. (P.decumbens, P.olsonii) (Larsen and Frisvad, 1995) | Penicillium sp. (P.camemberti, P.chrysogenum, P.commune, P.discolor, P.galbrun); Aspergillus sp. (A.niger); Fusarium sp. (F.sporotrichoides) (Kaminski et al., 1974; Larsen and Frisvad, 1995; Schnürer et al., 1999; Wilkins et al., 2003) | Penicillium sp. (P.chrysogenum, P.crustosum) Aspergillus sp. (A.versicolor) (Larsen and Frisvad, 1995; Wilkins et al., 2003) | Penicillium sp (P.camemberti, P.chrysogenum, P.commune, P.tricolor, P.verrucosum), Aspergillus sp. (A.niger, A.parasiticus) (Kaminski et al., 1974; Larsen and Frisvad, 1995) |

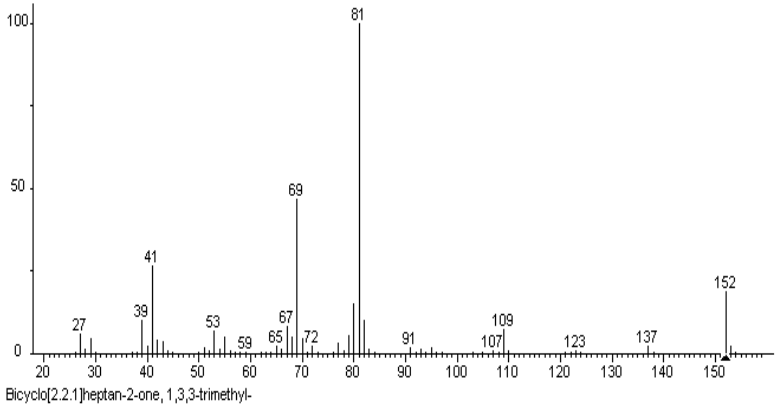
| Trivial name | 1-octen-3-ol* | camphor* | borneol* | linalool* | nonan-2-one |
|---|---|---|---|---|---|
| # | **15 / 16** | **17 / 18** | **19 / 20** | **21 / 22** | **23** |
| **Synonymous/ commercial name** | vinyl pentyl carbinol; flowtron mosquito attractant; morillol; pentyl vinyl carbinol; vinyl hexanol; mushroom alcohol; pentyl vintyl carbinol; amyl vinyl carbinol; 3-hydroxy-1-octene; matsutake alcohol; matsutakeol | camphora, 2-bornanon, alcanfor, 1,7,7 trimethylnorcamphor; 2-bornanone; 2-camphanone; alphanon; borneo camphor; root bark oil | endo-borneol; borneocamphor; endo-2-bornanol; sumatra camphor; 1-bornyl alcohol; ngai camphor; 2-camphanol; NSC 60223; endo-2-hydroxy-1,7,7-trimethylnorbornane | licareol; linalyl alcohol; allo-ocimenol; 3,7-dimethylocta-1,6-dien-3-ol; linanool; NSC 378 | methyl n-heptyl ketone; ß-nonanone; methyl heptyl ketone; NSC14760 |
| **Systematic name** | n-oct-1-en-3-ol | 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one | 1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-ol | 2,6-dienthyl octa-2,7-dien-6-ol | heptyl methyl ketone |
| **CAS** | 3391-86-4 (±); 24587-53-9 (+) (S); 3687-48-7 (-) (R) | 76-22-2 (±); 464-48-2 (-); 464-49-3 (+) | 507-70-0 (±); 464-43-9 (-) (1S); 464-43-7 (+) (1R) | 78-70-6 (±); 126-90-9 (+) (S); 126-91-0 (-) (R) | 821-55-6 |
| **BRN** | 1744110 | 1907611 (±); 2042745 (-); 1907612 (+) | 2038056 (+); 2038053 (-) | 1721488 (±); n.n. (+); 1721487 (-) | 1743645 |
| **Molecular formula** | $C_8H_{16}O$ | $C_{10}H_{16}O$ | $C_{10}H_{18}O$ | $C_{10}H_{14}O$ | $C_9H_{18}O$ |
| **Structure / chemical constitution** |  |  |  |  |  |
| $M_W$ (amu) | 128.2138 | 152.2358 | 154.2516 | 154.2516 | 142.2406 |
| **SR** ($cm^3\ min^{-1}$) | 25.6 | 21.4 | 20.7 | 23.5 | 24.4 |
| **Appearance, colour, odour etc. (physical state at 20 °C)** | colourless, combustible liquid; (-): harsh, mushroom, earthy; (+): fusty, herbaceous | colourless or white crystals | colourless solid crystals; (+): pungent, camphor-like with a slightly sharp, earthy-peppery tang | colourless liquid; (+): sweet, petigrain (Brenna et al., 2003); (-): woody, lavender (Brenna et al., 2003) | colourless to pale yellow oily combustible liquid |
| **t.-o. conc.** ($\mu g\ m^{-3}$) | 16 | | | 0.006 mg L 1 in H2O; 0.025 mg L 1in 12 % C2H5OH | |
| **mp** (°C) | | 178.6 | | | -21 |
| **bp** (°C) | 174 | 204 | 206…209 | 199 | 193 |
| **Solubility** ($g\ L^{-1}$) | | | | soluble in ethanol | |
| **Water solubility** ($g\ L^{-1}$) | no soluble | insoluble in water | insoluble | insoluble | insoluble |
| **Vapour pressure (Pa)** | | 30 | 9 | 12 | |
| **ρ** ($g\ cm^3$) | 0.833 | 0.9853 | 1.011 | 0.863 | 0.821 |
| $n^{20}_D$ | 1.437 | | | 1.462 | 1.4207 |
| $[\alpha]^{20}_D$ | | 43 ° (c = 10, C2H5OH) | -37.0 ± 1° (c = 5 in EtOH) | -18 ± 3° (undiluted) | |
| **Hazard indications**<br>**R-phrases**<br>**S-phrases**<br>**Swiss poison class** | irritant; harmful liquid<br>22-36/38<br>26-36<br>4 | highly flammable, harmful<br>11-22-36/37/38<br>16-26<br>3 | highly flammable; harmful solid<br>11-43<br>6-36/37 | corrosive<br>34<br>26-36/37/39-45<br>4 | irritant; harmful liquid<br>36/37/38<br>26-36 |
| **selected supplier**<br>supplier remark: | Acros Organics (Geel, Belgium); product #: 129470050; purity: ≥ 98 % (GC) | | **(+)-borneol:** Fluka Chemie GmbH (Buchs, CH); product #: 15597; purity: ≥ 98.0 % (GC: enantiomeric ratio ≥ 98:1).<br>**(-)-borneol:** Fluka Chemie GmbH (Buchs, CH); product #: 63969; purity: ≥ 99.5 % (standard for GC, GC: enantiomeric ratio ≥ 99.5:0.5).<br>limited shelf life, expiry date! | Fluka Chemie GmbH (Buchs, CH); product #: 62139 (−)-Linalool; purity: ≥ 95.0 % (GC) | Fluka Chemie GmbH (Buchs, CH); product #: 63969; purity: ≥ 99.5 % (standard for GC, GC)<br>limited shelf life, expiry date! |
| **Literature** FT-IR : | 2(I), 205A; 1(I), 143C | 2(I), 684B; 1(I), 441D | 2(I), 236B | 2 (I), 213B; 1(I), 148C | 2(I), 646D; 1(I), 410D |
| FT-NMR : | 1(1), 215C | 1(1), 678C | 2(1), 154C | 1(1), 225B | 2(1), 374C |
| Beilstein : | 1, III 1941, IV 21631 | 7, 135; I 84; II 103; III 40, IV 214 | 6, 72, I 47; II 81; III 295 | 1, 460; I 238; II 510; III 2009; IV 2278 | 1, 709, I 365; II 761; III 2887; IV 3353 |
| Merck : | 11, 1764 | 13, 1739; 12, 8907; 11, 1738 | 13, 1366; 12, 1366; 11, 1338 | 13, 5517; 12, 5520; 11, 5373 | 11, 2219 |
| **Remarks** | | biosynthetic oxidation product from borneol | > camphor is the biosynthetic oxidation product of borneol > (1R)(+)-borneol has been found in the oils of rosemary, lavender, spike lavandin, oilbanum, nutmeg, Dryobalanops spp., Dipterocarpaceae, Cupressaceae and Zingiberaceae sp. (Ravid et al., 1996) | Penicillium sp. (P.decumbens, P.italicum, P.roqueforti) (Larsen and Frisvad, 1995) | |
| **Assignment of mould species** | Penicillium sp. (P.glabrum, P.verrucosum, P.chrysogenum, P.camemberti, P.cummune, P.tricolor) Aspergillus sp. (A.versicolor, A.niger), Cladosporium sp., Alternaria sp., Fusarium sp. (Böck et al., 1998; Kaminski et al., 1974; Larsen and Frisvad, 1995) | | | | Penicillium sp. (P.olsonii) (Larsen and Frisvad, 1995) |

| Trivial name | terpinen-4-ol* | alpha-terpineol* | verbenone* | trans-(-)-carveol* | cis-(-)-carveol* |
|---|---|---|---|---|---|
| # | 24 / 25 | 27 / 28 | 29 / 30 | 31 | 32 |
| Synonymous/ commercial name | p-menth-1-en-4-ol; 4-carvomenthenol; terpene-4-ol; terpinene-4-ol; origanol; melaleucol; 1-(1-methylethyl)-4-methyl-3-cyclohexen-1-ol; 1-terpinen-4-ol; NSC 147749; | α,α-4-trimethyl-3-cyclohexene-1-methanol; p-menth-1-en-8-ol; 1-methyl-4-isopropyl-1-cyclohexen-8-ol | 2-pinen-4-one; berbenone | *trans*-(-)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol; p-mentha-6,8-dien-2-ol, (E)-carveol; NSC 319644; *trans*-carveol; *trans*-mentha-1,8-dien-6-ol | *cis*-(+)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol; p-Mentha-6,8-dien-2-ol, (Z)-carveol; NSC 319644; *cis*-carveol; *cis*-mentha-1,8-dien-6-ol |
| Systematic name | 1-methyl-4-isopropyl-1-cyclohexen-4-ol | 2-(4-methyl-cyclohex-3-enyl)-propan-2-ol | 4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-one | (1S,5R)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol | (1R,5R)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol |
| CAS | 562-74-3 (±); 2438-10-0 (+); 20126-76-5 (-) | 98-55-5 (±); 10482-56-1 (-); 7785-53-7 (+) | 80-57-9 (±); 1196-01-6 (-) (1S,5S-form resp. L-form); 18309-32-5 (+) (1R,5R-form resp. D-form) | 1197-07-5 (-) trans; 99-48-9 (mixture of isomers) | 1197-06-4 (-) cis; 99-48-9 (mixture of isomers) (-) |
| BRN | 2411938 (+) | 2325137 (-); 2041428 (+) | 1907623 (-) | | 6269416 (mixture of isomers) (±) |
| Molecular formula | $C_{10}H_{18}O$ | $C_{10}H_{18}O$ | $C_{10}H_{14}O$ | $C_{10}H_{16}O$ | $C_{10}H_{16}O$ |
| Structure / chemical constitution |  |  |  |  |  |
| $M_W$ (amu) | 154.2516 | 154.2516 | 150.1045 | 152.1201 | 152.1201 |
| SR ($cm^3 min^{-1}$) | 20.1 | 20.0 | 21.6 | 20.3 | 20.3 |
| Appearance, colour, odour etc. (physical state at 20 °C) | colourless oily liquid with spicy, earthy, and woody terpenoid and nutmeg-like odour | colourless to slightly yellow, slightly viscous liquid, fresh sweet lilac floral | yellow viscous liquid with an odour like camphor, menthol, and celery | | |
| t.-o. conc. ($\mu g\ m^{-3}$) | | | | | |
| mp (°C) | | 34.5 | 6.5 | | |
| bp (°C) | 212 | 218 | 227…228 | 226 | 226 |
| Solubility ($g\ L^{-1}$) | | | soluble in ethanol and other organic solvents | | |
| Water solubility ($g\ L^{-1}$) | | insoluble | insoluble | | |
| Vapour pressure (Pa) | 6.4 | 3.8 | 10.3 | 1.56 | 1.56 |
| ρ ($g\ cm^3$) | 0.933 | 0.933 | 0.978 | 0.968 | 0.968 |
| $n^{20}_D$ | 1.4799 | 1.474 | 1.4993 | | 1.496 |
| $[\alpha]^{20}_D$ | + 30 ° (undiluted); - 32 ° (undiluted) | +25 ± 10 °, neat (with a share of 30…45 % (-)-enantiomer!) | -177 ± 5 ° (undiluted) | | -112° (c = 1, $CHCl_3$) |
| Hazard indications | irritant; harmful liquid | irritant; harmful liquid | | irritant; harmful liquid | irritant; harmful liquid |
| R-phrases | 22-36/37/38 | 36/37/38 | | 36/37/38 | 36/37/38 |
| S-phrases | 26-36 | 26-36 | 23-24/25 | 26-37/39 | 26-37/39 |
| Swiss poison class | | 4 | 2 | | |
| selected supplier | (+)-terpinen-4-ol: Fluka Chemie GmbH (Buchs, CH); product #: 86477; purity: ≥ 99.0 % (terpene standard for GC, enantiomeric ratio ~2:1, GC)<br>(-)-terpinen-4-ol: Acros Organics (Geel, Belgium); product #: 270430250; purity: ≥ 97 % (GC). | Acros Organics (Geel, Belgium); product #: 203740500; purity: ≥ 97 % (mixture of alpha- and gamma-terpineol, GC)<br>Acros Organics (Geel, Belgium); product #: 203740500; purity: ≥ 98 % (mixture of mixture of isomers, GC). | Fluka Chemie GmbH (Buchs, CH); product #: 94882 (1S)-(−)-Verbenone; purity: ≥ 99.5 % (terpene standard for GC, GC) | refer to cis-(-)-carveol | Aldrich Chemical Corp. (Milwukee, USA); product #: 192384; purity: ≥ 97.0 % (sum of isomers, GC) |
| supplier remark: | **(+)**: limited shelf life, expiry date! Sensitive to air; keep under argon; +4 °C. Highly purified monoterpene | | limited shelf life, expiry date! Isomerization to 2(10)-pinen-4-one | | mixture of isomers ~60:40; storage temp. 2-8°C |
| Literature FT-IR: | | 1(I), 154B | 2(I), 701B; 1(I), 444A | refer to cis-(-)-carveol | 2(1), 245A; 1(1), 165C |
| FT-NMR: | | 1(1), 259A | 1(1), 698B | | 1(1), 261A |
| Beilstein: | 6, *55*, III 240 | 6, 58; I 41; II 67; III 247; IV 252 | 7, 161; IV 327 | | 6, I 6 |
| Merck: | | 13, 9248; 12, 9316; 11, 9103 | 13, 10094; 12, 10094 | | 12, 6110; 11, *5953* |
| Remarks | Component of Melaleuca alternifolia (tea tree) oil and lavender oil (from Lavandula specc.); misaroma of orange oil | > component of Pinus sylvestris (Pine) oil<br>> leaf essential oils of the genus Leptospermum (Myrtaceae) in eastern Australia. Part 1. Leptospermum brachyandrum and Leptospermum pallidum groups. Brophy, Joseph J. et al.; Flavour Fragrance J., 13(1), 19-25, | > Chemie in unserer Zeit 19, 11-21 (1985)<br>> component of Ledum palustre (Rosemary) oil | | |
| Assignment of mould species | | Penicillium sp. (P. unlpinum) (Larsen and Frisvad, 1995) | | | |

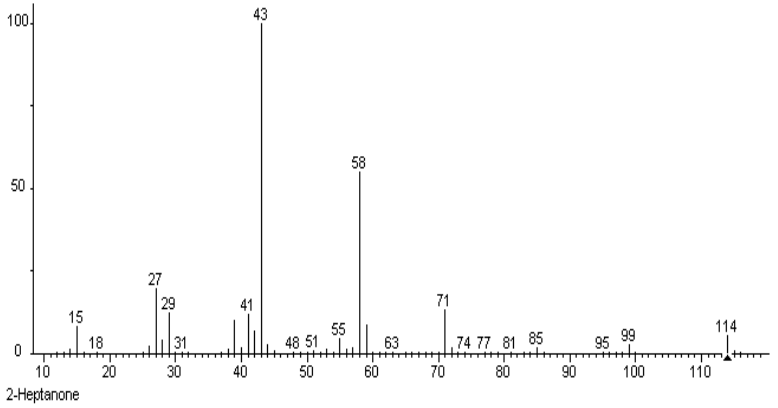
| **Trivial name** | **carvone*** | **geosmin*** | **cuminol** |
|---|---|---|---|
| # | **33 / 34** | **35 / 36** | **37** |
| **Synonymous/ commercial name** | 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one; p-mentha-6,8-dien-2-one; carvol; karvon; (sup 6,8)-(9)-terpadienone-2; 1-methyl-4-isopropenyl-6-cyclohexen-2-one; 2-methyl-5-isopropenyl-2-cyclohexen-1-one; 6,8-p-menthadien-2-one | octahydro-4,8a-dimethyl-4a(2H)-naphthalenol; 2,6 dimethyl bicyclo[64.4.06]decan-1-ol; (4a,4aa,8ab)-octahydro-4,8a-dimethyl-4a(2H)-naphthalenol; octahydro-4a,8ab-dimethyl-4aa(2H)-naphthol | cuminyl alcohol; cuminic alcohol; p-cumic alcohol; p-cymen-7-ol; p-Isopropyl benzyl alcohol; cumyl alcohol; 4-Isopropyl benzyl alcohol; cumin alcohol; NSC 15672 |
| **Systematic name** | 5-isopropenyl-2-methyl-cyclohex-2-enone | 2,6-dimethyl bicyclo[4.4.0]decan-1-ol | 4-(1-methylethyl)-benzenemethanol |
| **CAS** | 99-49-0 (±); 2244-16-8 (+); 6485-40-1 (-) | 16423-19-1 (±); 104873-46-3 (+) (4R, 4aS, 8aR); 19700-21-1 (-) (4S, 4aS, 8aR) | 536-60-7 |
| **BRN** | 2042970 (+); 2206714 (-) | | |
| **Molecular formula** | $C_{10}H_{14}O$ | $C_{12}H_{22}O$ | $C_{10}H_{14}O$ |
| **Structure / chemical constitution** |  |  |  |
| $M_W$ (amu) | 150.1045 | 182.1671 | 150.1045 |
| **SR** ($cm^3$ $min^{-1}$) | 20.5 | 17.8 | 20.6 |
| **Appearance, colour, odour etc. (physical state at 20 °C)** | (+): caraway (Brenna et al., 2003); (-): spearmint (Brenna et al., 2003) | oily liquid, odour of freshly ploughed soil earth | |
| **t.-o. conc.** ($\mu g\ m^{-3}$) | | 7.6 | |
| **mp** (°C) | 230 | | 28 |
| **bp** (°C) | 279 | 270 | 249 |
| **Solubility** ($g\ L^{-1}$) | | | |
| **Water solubility** ($g\ L^{-1}$) | insoluble in water | | |
| **Vapour pressure (Pa)** | 8.75 | 0.124 | 1.65 |
| **ρ** ($g\ cm^3$) | 0.959 | 1.018…1.023 at 25 °C | 0.982 |
| $n^{20}_D$ | 1.4985 | | 1.5186 |
| $[\alpha]^{20}_D$ | +55…57 ° (undiluted) ; -60…62 ° (undiluted) | -16.5° (undiluted) | |
| **Hazard indications** | | toxic | irritant; harmful liquid |
| **R-phrases** | 22 | 11, 23/25, 36/38 | 22-38 |
| **S-phrases** | 36 | 45, 16, 23.3, 7, 24 | 26-36 |
| **Swiss poison class** | 4 | | |
| **selected supplier** | **(+)-carvone**: Fluka Chemie GmbH (Buchs, CH); product #: 22070; purity: ≥ 98.5 % (terpene standard for GC, GC). **(-)-carvone**: Fluka Chemie GmbH (Buchs, CH); product #: 22060; purity: ≥ 99.0 % (terpene standard for GC, GC). | Sigma Chemical Corp. (St. Louis, USA); product #: G5908; purity: ≥ 98.0 % (GC) purum | Across Organics (Geel, Belgium); product #: 187800250; purity: ≥ 97 % (GC) |
| supplier remark: | limited shelf life, expiry date! | approx. 2 mg mL-1 in methanol, storage temp. -20 °C | |
| **Literature** FT-IR : | 1(I), 449C | | 2 (II), 1932:A; 1 (I), 1133B |
| FT-NMR : | 1(1), 694A | | 1 (2), 349A |
| Beilstein : | (+): 7, 153; I 101; II 128; III 861; (–): 7, 157; I 102; II 129; III 861; IV 214 | | 1, 388, IV 1666 |
| Merck : | 13, 1925 | 13, 4408 | 13, 2646; 12, 2685 |
| **Remarks** | | > Some perfumery uses geosmin: Patchouli; Earthy; Moss; Fern; Marine; (blends well with geosmin: Ambraketal; Ambrox; Calarene Epoxide; Patchouli Epoxide; Corps Atc.) > The (-)-enantiomer is the principal component of beet aroma. (J. Agric. Food Chem. 26, | |
| **Assignment of mould species** | | | |

### 8.1.1 MS spectra of MVOCs

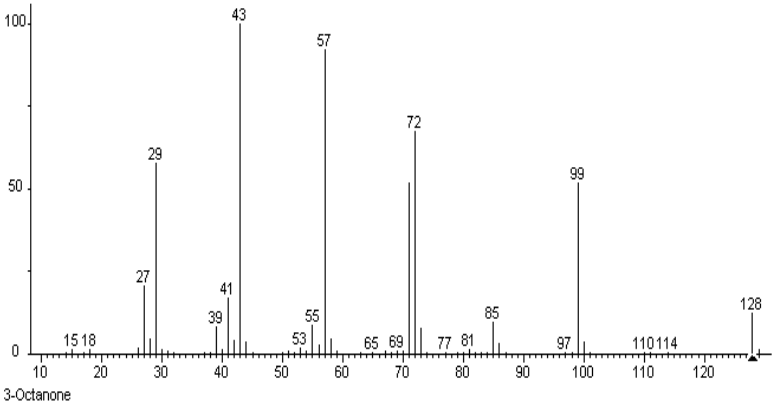




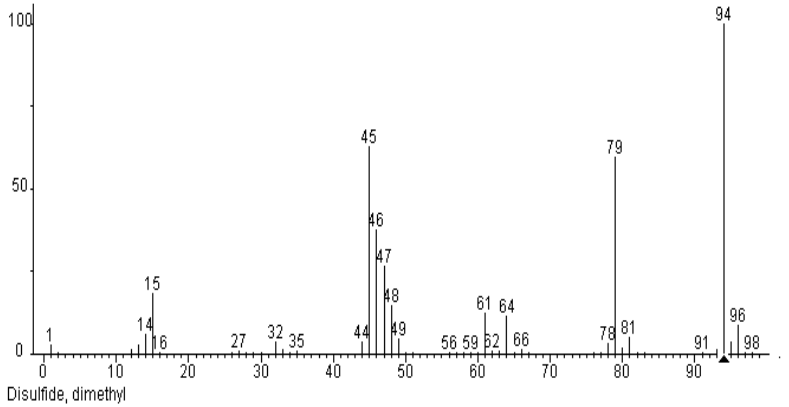

MVOC 8/9: Mass spectrum of fenchone



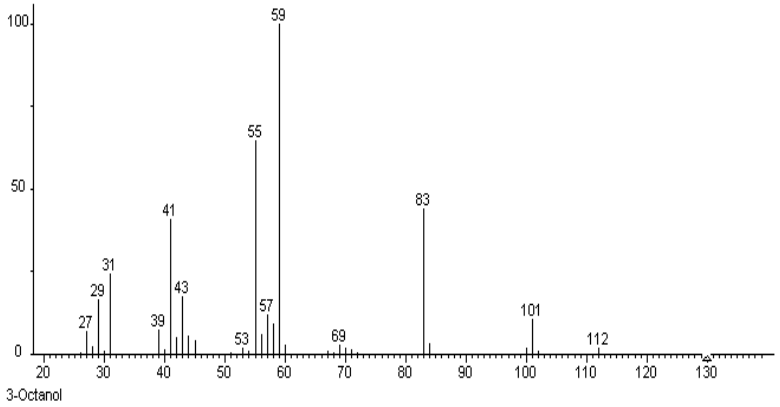

MVOC 10: Mass spectrum of heptan-2-one



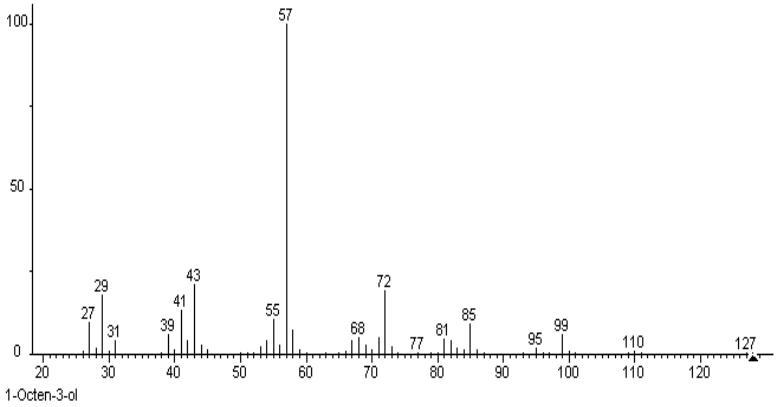

MVOC 11: Mass spectrum of octan-3-one



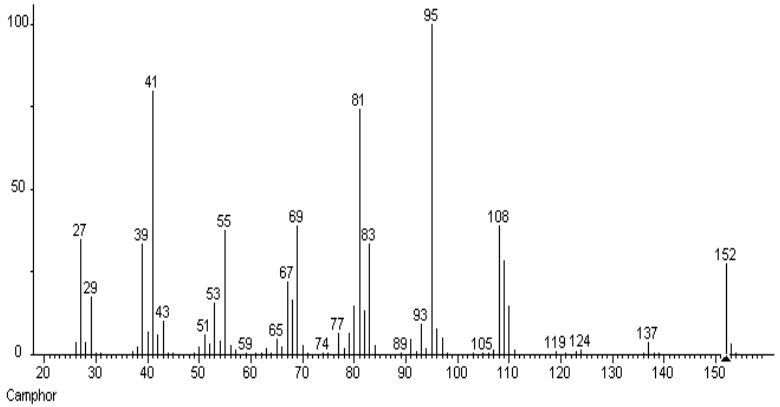

MVOC 12: Mass spectrum of methyl disulfide



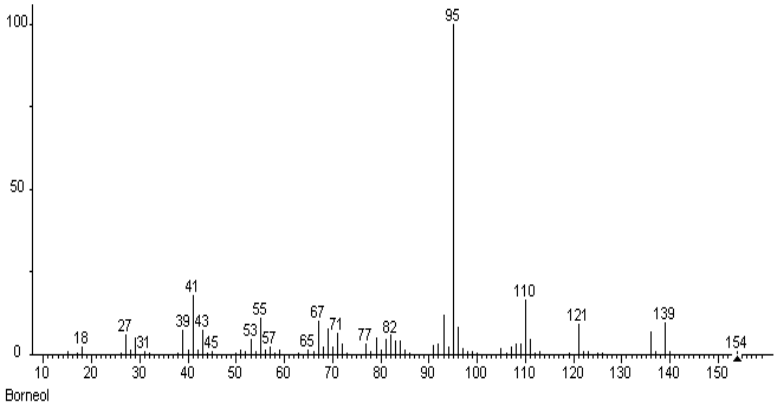

MVOC 13/14: Mass spectrum of octan-3-ol




MVOC 15/16: Mass spectrum of 1-octen-3-ol




MVOC 17/18: Mass spectrum of camphor




MVOC 19/20: Mass spectrum of borneol

MVOC 23: Mass spectrum of nonan-2-one



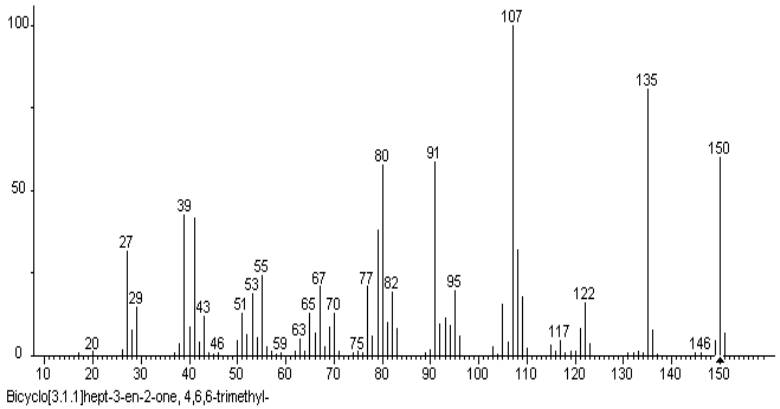

MVOC 27/28: Mass spectrum of alpha-terpineol



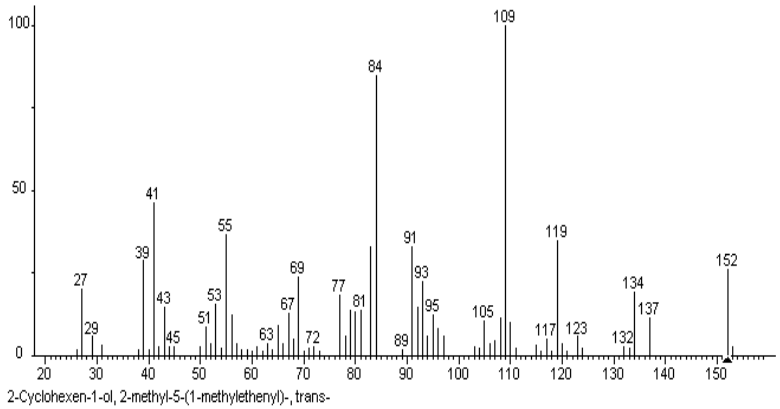

MVOC 21/22: Mass spectrum of linalool



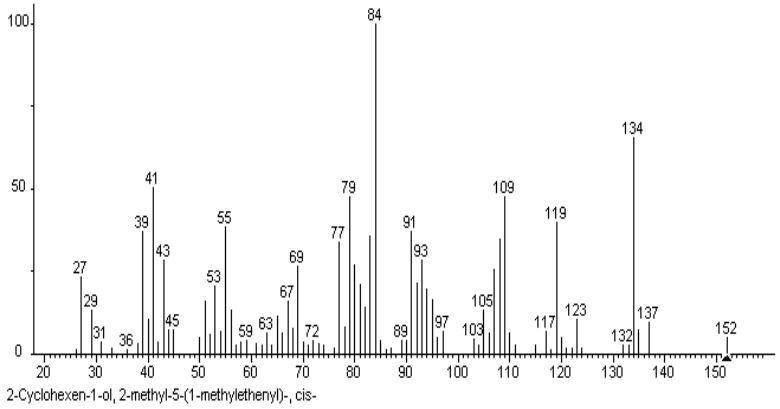

MVOC 24/25: Mass spectrum of terpinen-4-ol



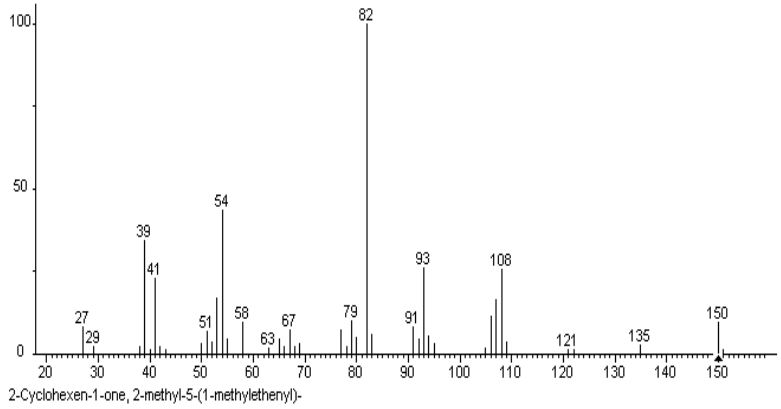

MVOC 29/30: Mass spectrum of verbenone



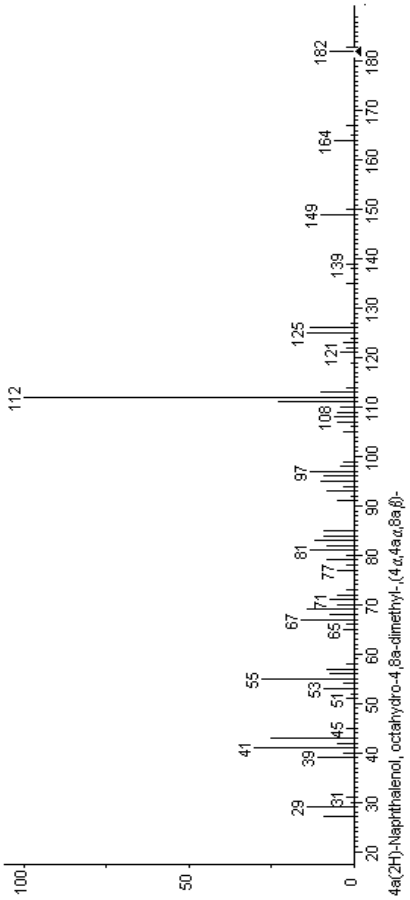

MVOC 31: Mass spectrum of trans-(-)-carveol



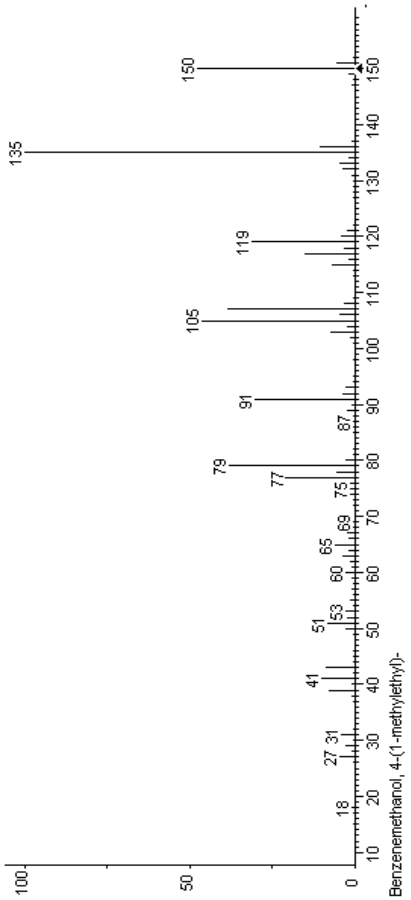

MVOC 32: Mass spectrum of cis-(-)-carveol




MVOC 33/34: Mass spectrum of carvone

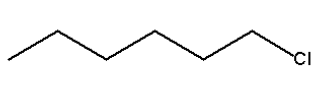
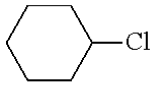


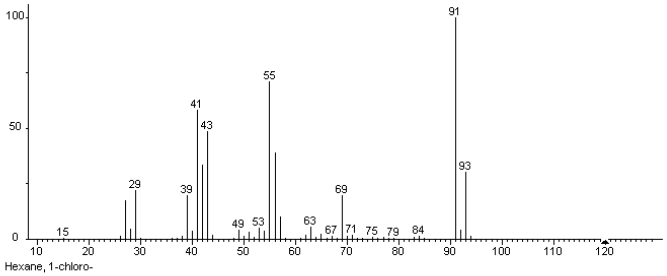

MVOC 35/36: Mass spectrum of geosmin



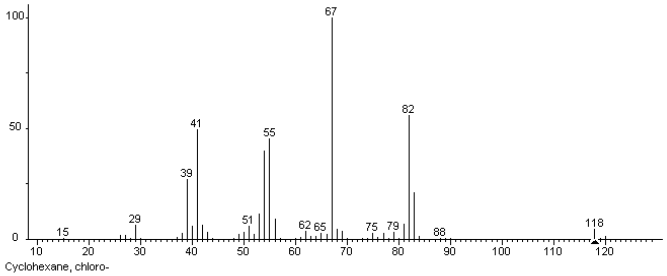

MVOC 37: Mass spectrum of cuminol

## 8.1.2 Standards

| *classification* | *Internal standard (ISTD)* | *Recovery standard (RSTD)* |
|---|---|---|
| **Trivial name** | **1-chlorhexane** | **cyclochlorhexane** |
| # | **ISTD** | **RSTD** |
| **Synonymous/ commercial name** | hexyl chloride | cyclohexyl chloride |
| **Systematic name** | 1-chlorhexane | cyclochlorhexane |
| **CAS** | 544-10-5 | 542-18-7 |
| **BRN** | 1731289 | 1900796 |
| **Molecular formula** | $C_6H_{13}Cl$ | $C_6H_{11}Cl$ |
| **Structure / chemical constitution** |  |  |
| $M_W$ (amu) | 120.0706 | 118.0549 |
| **SR** ($cm^3\ min^{-1}$) | 27.4 | |
| **Appearance, colour, odour etc. (physical state at 20 °C)** | colourless crystal clear liquid | colourless, crystal clear liquid |
| **t.-o. conc.** ($\mu g\ m^{-3}$) | | |
| **mp** (°C) | -94 | -43 |
| **bp** (°C) | 133…134 | 141…143 |
| **Solubility** ($g\ L^{-1}$) | | |
| **Water solubility** ($g\ L^{-1}$) | insoluble | $2\ 10^{-5}$ |
| **Vapour pressure (Pa)** | 5300 at 55 °C | 2010 |
| **ρ** ($g\ cm^3$) | 0.877 | 0.997 |
| $n^{20}_D$ | 1.4194 | 1.4626 |
| $[\alpha]^{20}_D$ | % | % |
| **Hazard indications** | | irritant liquid |
| **R-phrases** | 10 | 10-36/37/38 |
| **S-phrases** | 23-24/25 | 26-36 |
| **Swiss poison class** | | |
| **selected supplier** | Fluka Chemie GmbH (Buchs, CH); product #: 24771; purity: ≥ 99.5 % (standard for GC, GC) | Fluka Chemie GmbH (Buchs, CH); product #: 24160; purity: ≥ 98.0 % (GC) |
| supplier remark: | limited shelf life, expiry date! | % |
| **Literature** FT-IR : | 2(I), 86D, 1(I), 58B | 2(I), 147D, 1(I), 103D |
| FT-NMR : | 2(1), 61D, 1(1), 86A | 1(1), 152A |
| Beilstein : | 1, 143; I 51; II 109; III 388; IV 349 | 5, 21; I 8; II 11; III 37; IV 48 |
| Merck : | 13, 2163; 12, 2196; 11, 2144 | 13, 2761; 12, 2801; 11, 2738 |
| **Remarks** | | |

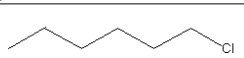
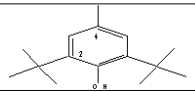


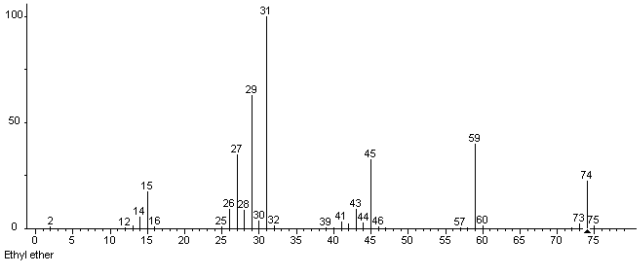

Mass spectrum of 1-chlorhexane



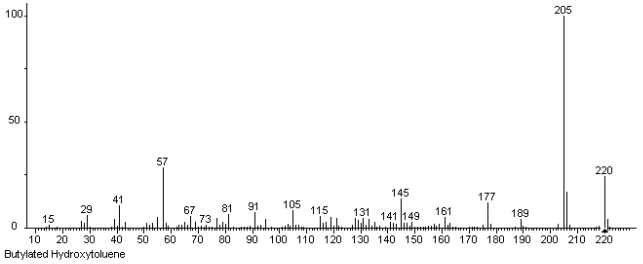

Mass spectrum of cyclochlorhexane

# 8.1.3 Solvent

| Trivial name | diethyl ether | butylhydroxytoluene (BHT) |
|---|---|---|
| Synonymous/ commercial name | ethyl ether, ethyl oxide, ether | 2,6-di-tert.-butyl-4-methylphenol; 2,6-di-tert-butyl-p-cresol; 2,6-di-tert-butyl-1-hydroxy-4-methylbenzene; 1-Hydroxy-4-methyl-2,6-di-tert-butylbenzene; 4-hydroxy-3,5-di-tert-butyltoluene; 4-methyl-2,6-di-tert-butylphenol; |
| Systematic name | diethyl ether | 2,6-bis(1,1-dimethylethyl)-1-hydroxy-4- methyl benzene |
| CAS | 60-29-7 | 128-37-0 |
| BRN | 1696894 | 1870662 |
| Molecular formula | $C_4H_{10}O$ | $C_{15}H_{24}O$ |
| Structure / chemical constitution |  |  |
| $M_W$ (amu) | 74.1224 | 220.1827 |
| SR ($cm^3\ min^{-1}$) | colourless crystal clear liquid; characteristic sweet, ether odour (detectable at 0.33 ppm); hygroscopic/air-light sensitive | clear, colorless powder |
| Appearance, colour, odour etc. (physical state at 20 °C) | 0.0001 | |
| t.-o. conc. ($\mu g\ m^{-3}$) | | |
| mp (°C) | -116.3 | 68…72 |
| bp (°C) | 34.6 | 265 |
| Solubility ($g\ L^{-1}$) | 0.069 | methanol: 0.1; ethanol: 0.1; vegetable oils: soluble |
| Water solubility ($g\ L^{-1}$) | 58700 | insoluble |
| Vapour pressure (Pa) | 0.23 (at 20 °C) | < 1.3 |
| ρ ($g\ cm^3$) | 0.71 | 1.048 |
| $n^{20}_D$ | 1.3526 | |
| $[\alpha]^{20}_D$ | % | |
| Hazard indications | Extremely flammable; harmful liquid | harmful liquid |
| R-phrases | 12-19-22-66-67 | 22-36/37/38 |
| S-phrases | 9-16-29-33-46 | 26-36 |
| Swiss poison class | 4 | 3 |
| selected supplier<br><br>supplier remark: | J.T.Baker (Deventer, NL); product #: 8254; purity: ≥ 99.5 % (Baker analyzed, GC)<br><br>Stabilized with 7 ppm BHT | stabilized of diethyl ether |
| Literature FT-IR : | 2(I), 302B; 1(I), 203A | 2(II), 1873A; 1(I), 1094D |
| FT-NMR : | 1(1), 317A | 1(2), 285A |
| Beilstein : | 1, 314; I 158; II 311; III 1289; IV 1314 | 6, III 2073; IV 3511 |
| Merck : | 13, 3840; 11, 3762 | 13, 1547; 12, 1583; 11, 1548 |
| Remarks | MAK: 1200 mg $m^3$; LD50(oral, rat): 1215 mg kg-1; LC50 (inhalation, rat): 73000 ppm (V) per 2h; Testing in humans: no sensibilization; Narcotic | > BHT is a phenolic antioxidant. It has been shown to inhibit lipid peroxidation. It causes lung injury and promotes tumors in mice which may be due to a metabolite of BHT, 6-tert-butyl-2-[2'-(2'-hydroxymethyl)-propyl]-4-methylphenol.<br>> Metabolites of BHT have also been reported to induce DNA strand breaks and internucleosomal DNA fragmentation (a characteristic of apoptosis) in cultured cells.<br>> In rats, a single intraperitoneal injection of BHT (60 mg/kg body mass) results in a significant increase in nuclear DNA methyl transferase activity in the liver, kidneys, heart, spleen, brain and lungs.<br>> Incubation of alveolar macrophages with BHT significantly reduced the level of TNF-a which may explain the mechanism by which this antioxidant reduces inflammation.<br>> Preincubation of aspirin-treated platelets with BHT inhibits the secretion, aggregation, and protein phosphorylation induced by protein kinase C activators. BHT was also found to inhibit the initiation of hepatocarcinogenesis by aflatoxin B1. |




Mass spectrum of diethyl ether




Mass spectrum of butylhydroxytoluene (BHT)

## 8.2 Grob-test

Evaluation of column quality and capillary separation was performed for non-enantioselective as well as enantioselective phases with a modified Grob-test as described by Jaus (Jaus, 2000). This universally applicable test mixture for GC capillary columns, originally introduced by Grob Jr. et al. (Grob et al., 1978), was applied under standardized temperature program conditions (see Tab. 8.2). It provided information about separation efficiency and adsorption of (polar) compounds. Additionally, it was useful to decide when column and system maintenance was needed.

The applied modified polar test mixture consisted of 12 + 3 compounds that differed in structure and polarity: Decane, undecane, nonanal, 1-octanol, D(-)-2,3-butanediol, 2,6-dimethylaniline, 2-ethylhexanoic acid, 2,6-dimethylphenol, dicyclohexylamine and the methylesters of decanoic, undecanoic, dodecanoic, tetradecanoic, hexadecanoic and octadecanoic acid (E10, E11, E12, E14, E16 and E18) (Tab. 8.1).

The separation efficiency of the capillaries was expressed by separation numbers (Trennzahlen, TZs). Fatty acid methylesters (FAME) were chosen for the TZ determination by Grob et al. (Grob et al., 1978) since their polar and apolar parts compensated each other and consequently their retention was widely independent from the polarity of the stationary phase. TZs were calculated according to equation (15):

$$TZ_{E_X/E_{X+1}} = \frac{\Delta t}{w_{bx} + w_{bx+1}} - 1 \qquad (15)$$

where $\Delta t$ is the time difference between the consecutive peaks of the FAME $E_X$ and $E_{X+1}$ (s) and $w_{bx}$ and $w_{bx+1}$ is the peak width at half height of the concerned peak $E_X$ and $E_{X+1}$ (s)

(compare Fig. 8.23). In case of a good symmetrical peak shape of the fatty acid methylesters, a $TZ_{E10/E11}$ or $TZ_{E11/E12} \geq 15$ and a $TZ_{E16/E18} \geq 20$ were considered as sufficient for a good separation.

**Tab. 8.1: Composition of the modified polar Grob-test mixture consisting of 12 + 3 compounds including six fatty acid methylesters (FAME) (Jaus, 2000)**

| # | compound | concentration in cyclohexane p.a. (mg $L^{-1}$) | formular manufacturer (product number & quality) | CAS registry number |
|---|---|---|---|---|
| 1 | 2,3-butanediol | 53 | $CH_3CH(OH)CH(OH)CH_3$ Fluka (#18970; > 99.0 % (GC)) | [513-85-9] |
| 2 | dicyclohexylamine | 31 | $(C_6H_{11})_2NH$ Fluka (#36620; > 99.5 %) | [101-83-7] |
| 3 | 2,6-dimethylaniline | 32 | $(CH_3)_2C_6H_3NH_2$ Fluka (#39520; > 98.0 % (GC)) | [87-62-7] |
| 4 | 2,6-dimethylphenol | 32 | $(CH_3)_2C_6H_3OH$ Fluka (#41345; > 99.0% (GC) | [576-26-1] |
| 5 | 2-ethylhexanoic acid | 38 | $CH_3(CH_2)_3CH(C_2H_5)COOH$ Fluka (#03300; > 98.0 % (GC)) | [149-57-5] |
| 6 | nonanal | 40 | $CH_3(CH_2)_7CHO$ Aldrich (#N30803; > 95 %) | [124-19-6] |
| 7 | 1-octanol | 36 | $CH_3(CH_2)_7OH$ Fluka (#74850; puriss.; > 99.5 % (GC)) | [111-87-5] |
| 8 | undecane | 29 | $CH_3(CH_2)_9CH_3$ Fluka (#94000; ≥ 99.8 % (GC)) | [1120-21-4] |
| 9 | Decane | 28 | $CH_3(CH_2)_8CH_3$ Acros (#AC11187-0020; ≥ 99 %) | [124-18-5] |
| 10 | nC10-FAME (E10) | 42 | $CH_3(CH_2)_8COOCH_3$ Fluka (#21479; ≥ 99.5 % (GC)) | [110-42-9] |
| 11 | nC11-FAME (E11) | 42 | $CH_3(CH_2)_9COOCH_3$ Fluka (#94118; ≥ 99.8 % (GC) | [1731-86-8] |
| 12 | nC12-FAME (E12) | 41 | $CH_3(CH_2)_{10}COOCH_3$ Fluka (#61689; > 99.5 % (GC)) | [111-82-0] |
| 13 | nC14-FAME (E14) | 41 | $CH_3(CH_2)_{12}COOCH_3$ Fluka (#70129; ≥ 99.5 % (GC)) | [124-10-7] |
| 14 | nC16-FAME (E16) | 41 | $CH_3(CH_2)_{14}COOCH_3$ Fluka (#76159; ≥ 99.5 % (GC)) | [112-39-0] |
| 15 | nC18-FAME (E18) | 41 | $CH_3(CH_2)_{16}COOCH_3$ Fluka (#85769; ≥ 99.5 % (GC)) | [112-61-8] |

Generally, the HP 5890 Series II (Hewlett Packard, Palo Alto, USA) gas chromatograph was used for the modified Grob-test (Grob et al., 1978; Jaus, 2000). The injector temperature was 220 °C and detector temperature was 250 °C. Hydrogen (99.995 %) at a flow rate of 30 mL $min^{-1}$ and synthetic air at a flow of 300 mL $min^{-1}$ were applied for the FID. The dead

time was determined with propane and adjusted to a linear gas velocity of 40 cm $s^{-1}$ (at 100 °C) for hydrogen as carrier gas. Split injections of 1 µL were performed manually with a split ratio of 30:1. The standardized conditions for the Grob-test are listed in Tab. 8.2.

**Tab. 8.2: Standardized Grob-test conditions (Grob et al., 1978; Jaus, 2000)**

| column length (m) | temperature program | column head pressure (kPa) |
|---|---|---|
| 15 | 40 °C (2 min) → 3.3 °C/min → 220 °C | 46 |
| 20 | 40 °C (2 min) → 2.5 °C/min → 220 °C | 58 |
| **25** | **40 °C (2 min) → 2.1 C/min → 220 °C** | **72.5** |
| **30** | **40 °C (2 min) → 1.3 °C/min → 220 °C** | **86** |

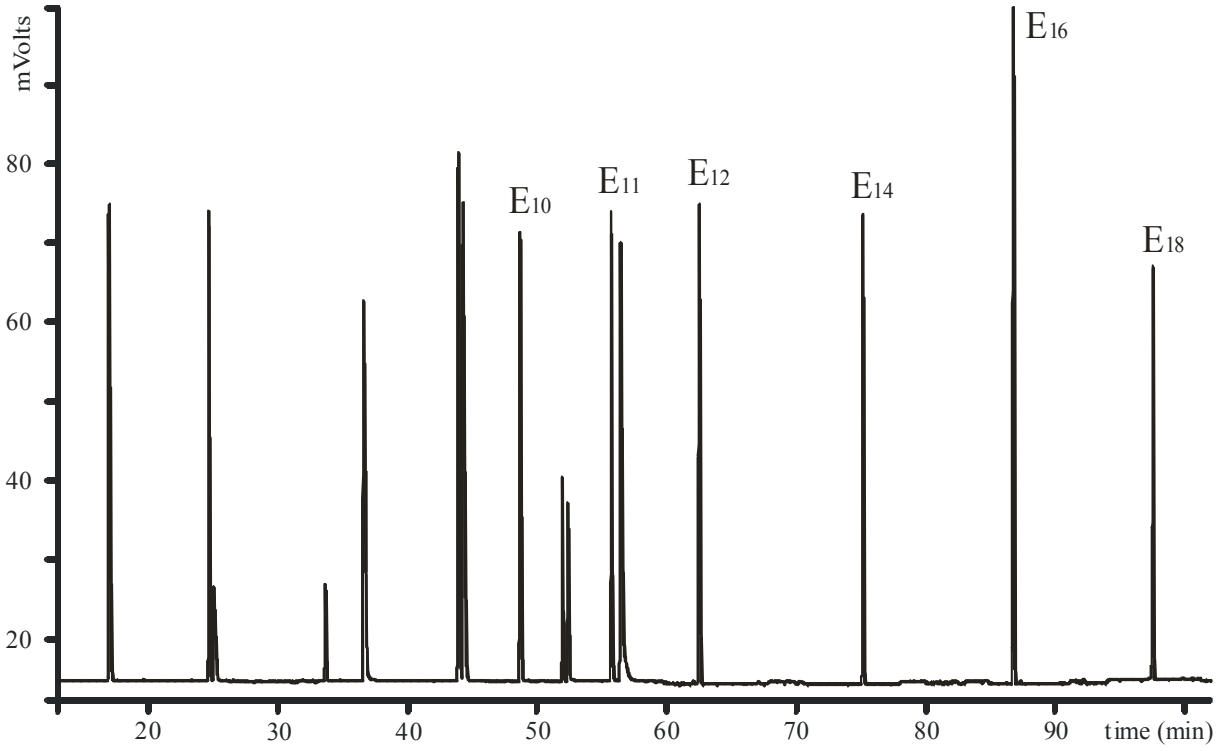
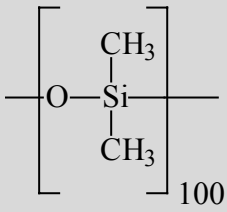
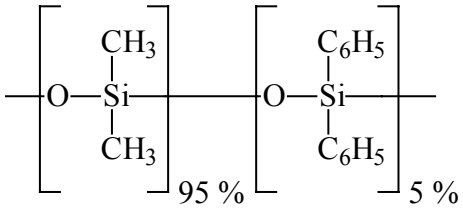
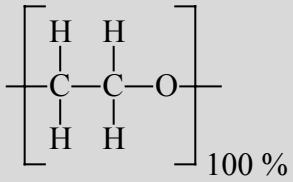



**Fig. 8.22: Grob-test on a Supelco β-DEX 120 capillary (see Tab. 4.7) using FID detection (see Tab. 8.2); determine Trennzahl (TZ): $TZ_{E10/E11} = TZ_{E11/E12} = 34$, and $TZ_{E16/E18} = 27$**

## 8.3 Overview of applied non-enantioselective capillary columns

For the non-enantioselective separation of the 22 selected MVOCs three different stationary phases were tested. All these columns were obtained from Agilent Technologies (Schweiz) AG (Basel, Switzerland).

**Tab. 8.3: Overview of selected and tested columns for non-enantioselective MVOC separation obtained from Agilent Technologies (Schweiz) AG (Basel, Switzerland)**

| column notation (part no.) | stationary phase | characteristic: column length (m) x ID (mm) x film thickness (µm) and temperature limits (°C) |
|---|---|---|
| HP-1 (HP-19091Z-433) | 100 % dimethyl polysiloxane  | 30 m x 0.25 mm ID x 0.25 µm film thickness with a temperature range from –60 °C to 325/350°C |
| HP-5 MS (HP-19091S-433) | 5 % diphenyl 95 % dimethyl polysiloxane special bonded and cross-linked  | 30 m x 0.25 mm ID x 0.25 µm film thickness with a temperature range from –60 °C to 325/350 °C |
| HP-INNOWax (HP-19091N-133) | polyethylene glycol (PEG) | 30 m x 0.25 mm ID x 0.25 µm film thickness with a temperature range from 40 °C to 260/270 °C |
| DB-Wax (J&W-122-7032) |  | 30 m x 0.25 mm ID x 0.25 µm film thickness with a temperature range from 20 °C to 250/260 °C |
| tandem column: HP-5 (HP-19091J-233) + HP-INNOWax (HP-19091N-202) | 5 % diphenyl 95 % dimethyl polysiloxane and polyethylene glycol (PEG) | 30 m x 0.25 mm ID x 1.00 µm film thickness with a temperature range from –60 °C to 325/350 °C coupled with a 25 m x 0.2 mm ID x 0.4 µm film thickness with a temperature range from –40 °C to 260/270 °C |

## 8.4 Separation of (chiral) compounds

### 8.4.1 Separation factor α

The separation factor α represents the relative interaction (e.g. solubility) between each of the solutes and the stationary phase, and can be used to express the relative intermolecular forces and the magnitude of their similarity or difference. The degree to which two compounds (1 and 2) are separated is a function of a) the ratio of their retention times $t_{R1}$ and $t_{R2}$, and b) the sharpness of the peaks (dependent on the theoretical plates N of the chromatographic column). The ratio of adjusted retention times ($t'_R$) of two components is termed the separation factor α. It is measured by the time or distance between the maxima of the two concerning compound signals. Its value can be determined straight from the chromatogram. The separation factor is defined as ratio of the retention factors $k_1$ and $k_2$ or a ratio of the distribution constants $K_{C1}$ and $K_{C2}$ of the eluted compounds (or enantiomer).

$$\alpha = \frac{K_{C2}}{K_{C1}} = \frac{k_2}{k_1} = \frac{\frac{t_{R2} - t_M}{t_M}}{\frac{t_{R1} - t_M}{t_M}} = \frac{\frac{t'_{R2}}{t_M}}{\frac{t'_{R1}}{t_M}} = \frac{t'_{R2}}{t'_{R1}} \qquad (16)$$

**Eq. (16): Definition of the separation factor α, where $t_R$ is the retention time of the compound 1 (2), $t_M$ is the retention time of the non-retained compound, $K_C$ is the distribution constant and k is the retention factor of compound 1 (2).**

The retention factor k is the time the solute spends in the stationary phase relative to the time it spends in the mobile phase.

By convention, α is never less than 1.0, so that the function of the second (or more retained) solute is always used as the numerator. If α equals 1, then the peaks have the same retention time and co-elute. In other words, solute pairs with large α values can be separated even on

low resolution columns, but as α approaches unity, columns with increasingly larger numbers of theoretical plates N are required to achieve separation. (Jennings et al., 1997; McNair and Miller, 1998)

### 8.4.2 Resolution ($R_S$)

The separation factor α is a useful measure of relative peak position in the chromatogram. This function, however, is not adequate to describe the degree to which two compounds (1 and 2) are resolved since it does not contain any information about peak widths. The separation of two peaks in a chromatogram is defined by the resolution ($R_S$) that is the ratio between the separation of the two peak maxima ($\Delta t_R$) and their average width at base (Eq (17)) (following the recommendation of the ASTM and IUPAC). In case of enantiomeric pairs, the term chiral resolution ($cR_S$) is used. This definition, however, requires extrapolation to determine the widths of peaks at the base. If peaks are assumed to be Gaussian, the peak width at half height ($w_h$) is used (Eq. (18)) since peak width at base ($w_b$) is equal to four times the standard deviation σ of the peak and peak width at half height is equal to 2.354 σ (Jennings et al., 1997; Poole, 2003; Sandra, 1989).

$$R_S = 2\frac{\Delta t_R}{w_{b1} + w_{b2}} \tag{17}$$

with $w_b = 1.699 w_h$

$$R_S = \frac{2\left(t_{R2} - t_{R1}\right)}{1.699\left(w_{h1} + w_{h2}\right)} = 1.177\frac{\Delta t_R}{\left(w_{h1} + w_{h2}\right)} \tag{18}$$

**Eq. (17) & Eq. (18): Definition of peak resolution ($R_S$), where $\Delta t_R$ is the absolute difference in retention time of the two peaks, $w_{h1(2)}$ is the peak width of 1(2) at half-height (2.354 σ) and $w_{b1(2)}$ at base (4σ).**

$R_S$ is a dimensionless number while all measurements have the same units.

The larger the value of the resolution, the better the separation. For two peaks of similar height $R_S = 1.0$ corresponds to a valley separation of about 94 % and is generally considered as adequate separation.

An optimal (chiral) resolution is achieved by definition if $cR_S \geq 1.50$. Here a 99.73 % separation of two peaks, a so-called "baseline" separation, is realized. Asymmetry, tailing, or gross discrepancies between the size of the two peaks, however, can cause complications (Mosandl, 1995).

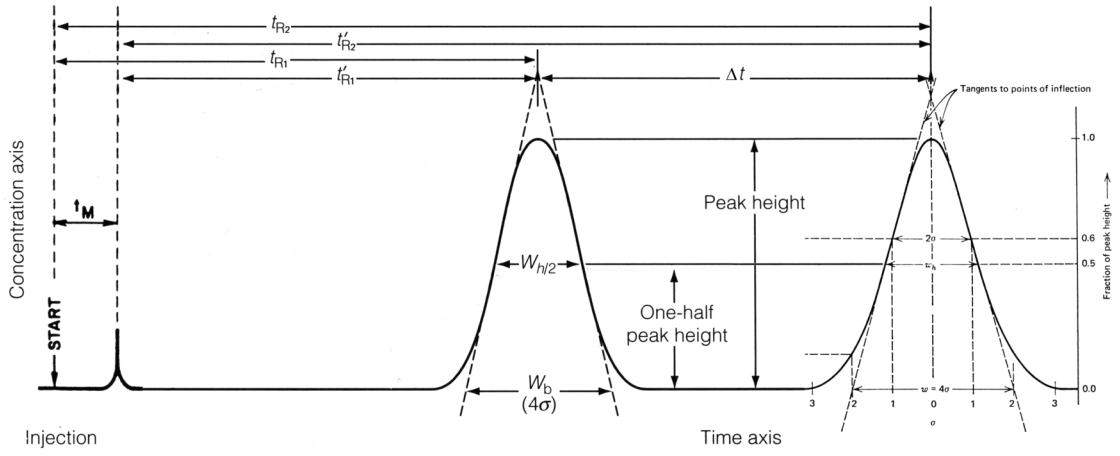



**Fig. 8.23: Determination of the chiral resolution ($cR_S$) of enantiomeric pairs following the IUPAC nomenclature of chromatographic resolution (Ettre et al., 1996), $t_M$ = retention time of unretained compound, $t_R$ = retention time, $t'_R$ = adjusted retention time, $w_h$ = peak width at half height, $w_b$ = peak width at base width of the first (1) and the second (2) eluted compound – Tangents are drawn to the inflection points in order to determine the widths of the peaks at their bases (Kealey and Haines, 2002; Miller, 1988).**

## 8.5 Definition of relative retention time

The relative retention time (RRT) is defined by the ratio of the adjusted retention time ($t'_{R,analyte} = t_R$ - hold-up time of a non-retained compound ($t_M$)) of the analyte over the adjusted retention time ($t'_{R,ISTD}$) of the internal standard (Kolb, 1999).

$$RRT = \frac{\left(t_{R,analyt} - t_M\right)}{\left(t_{R,ISTD} - t_M\right)} = \frac{t'_{R,analyt}}{t'_{R,ISTD}} \tag{19}$$

For graphical details compare Fig. 8.23, if $t_{R1}$ is set equal to $t_{R,ISTD}$ and $t_{R2}$ is replace by $t_{R,analyte}$.

# Table of figures

# Index of tables

# Curriculum Vitae

| | |
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| Name: | Sven Heekmann |
| Date of birth: | November 7th, 1971 |
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| Citizenship: | German |

| | |
|---|---|
| 1978…1982 | United primary school in Hochheide (Duisburg, Germany). |
| 1982…1983 | Comprehensive secondary school "Franz-Haniel-Gymnasium" (Duisburg, Germany). |
| 1983…1984 | Pre-secondary school in Meckelfeld (Seevetal**,** Germany). |
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| 1988…1989 | King City Secondary School (Ontario**,** Canada). |
| 1989…1992 | Comprehensive secondary school "Gymnasium Meckelfeld" (Seevetal**,** Germany). |
| 1992…1999 | Studies in chemistry at the Carl von Ossietzky University Oldenburg (Oldenburg (Oldb.)**,** Germany). |
| July 1999…April 2000 | Diploma thesis in physical (analytical) chemistry at the **Carl von Ossietzky University Oldenburg (Oldenburg (Oldb.), Germany)** under the supervision of **Prof. Dr. Werner Butte**. |
| April 2000…March 2005 | Doctoral thesis studies in organic analytical chemistry at the **University of Basel (Basel, Switzerland)** under the supervision of **Prof. Dr. Michael Oehme** with an independent project of the Swiss National Science Foundation (SNSF). |
| Since April 2005 | Analytical scientist for Lonza Ltd., Visp, Switzerland. |

During my education at the University of Basel I attended courses with the following lecturers (in alphabetical order):

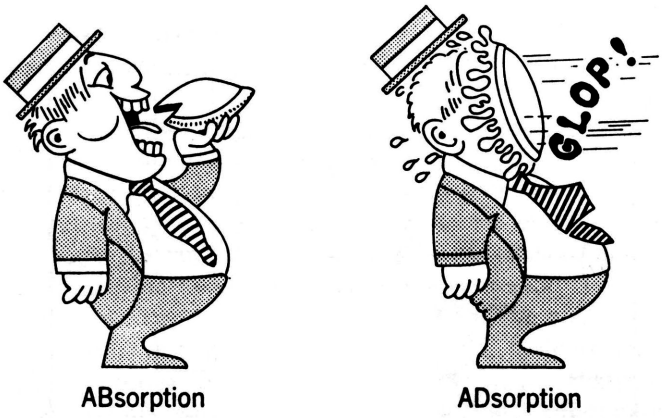
C. Brutschin; Prof. Dr. T.A. Bickle, Prof. Dr. F. Dolder, Prof. Dr. B. Ernst, Prof. Dr. W. Flückiger, PD Dr. H. Gampp, PD. Dr. G. Gescheidt, Prof. Dr. P. Hauser, PD Dr. A. Herrmann, Dr. D. Keller, PD Dr. M. Kunz, Prof. Dr. J. Meier, Prof. Dr. M. Oehme, Prof. Dr. U. Séquin.

# Eidesstattliche Erklärung

«Ich erkläre, daß ich die Dissertation „NON-ENANTIOSELECTIVE AND ENANTIOSELECTIVE DETERMINATION OF MICROBIAL VOLATILE ORGANIC COMPOUNDS AS TRACER FOR HUMAN EXPOSURE TO MOULD GROWTH IN BUILDINGS“ nur mit der darin angegebenen Hilfe verfaßt und bei keiner anderen Universität und keiner anderen Fakultät der Universität Basel eingereicht habe».

Basel, den 06. Juni 2006

Sven Heekmann




*„Small differences makes life interesting!“*
*Comical illustration of the difference between absorption (partition) and adsorption (Miller, 1988)*