Needle Trap Sampling Thermal-Desorption Photoionization Time-of-Flight Mass Spectrometry for Analysis of Complex Gas Mixtures

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Dipl.-Chem. Juliane Kleeblatt

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1 Introduction & Motivation

Mass Spectrometry is one of the most important analytical techniques due to its real-time detection and comparable low detection limits. Therefore, numerous applications are possible, whether the samples are gaseous, liquid or solid. Using hard ionization techniques such as electron impact (EI, 70 eV) most organic molecules are ionized, but the fragmentation is mostly massive, because the electron energy is much higher than the substance-specific ionization energy. When ionizing complex sample mixtures with EI, the selectivity of the mass spectrometer is rather low without using e.g. an additional separation step such as gas chromatography mass spectrometry (GC-MS). Hence, the analytical method is limited to off-line detection. However, a soft ionization technique such as chemical ionization (CI), electrospray ionization (ESI) or photoionization (PI) is more suitable for direct analysis, because of having the advantage of less or even no fragmentation of the molecular ion. In combination with a powerful mass analyzer such as time-of-flight (TOF) tube it is possible to separate the molecular ion masses and assign it to sample components with different molecular masses.[1] Additionally, the opportunity is given to use the real-time MS method for on-line detection.

Hence, the combination of very soft ionization technique photoionization (PI) and time-of-flight mass spectrometry (TOFMS) is a promising on-line detection system for complex sample mixtures due to its fastness, universal ionization characteristics (single photon ionization, SPI) and substance-selectivity depending on selected ionization wavelength (Resonance-enhanced multiphoton ionization, REMPI). Because of already mentioned less fragmentation, only the molecular ion of the substance is accelerated in the flight tube. The ions are separated by means of their mass-to-charge ratios resulting in different flight times: small molecules are faster than larger ones. The usage of a reflectron time-of-flight mass spectrometer is preferable due to the suitability with pulsed lasers (used for photon generation) and the high mass resolution. A multichannel plate detector (MCP), consisting of several secondary electron multiplier channels on a plate, is very suitable to enhance the signal and to transmit the results to a data acquisition software.

In some application fields, e.g. drug detection, aerosol analysis, pyrolysis detection and breath gas analysis [2-4] an on-line method is most suitable because of the loss of analytes by diffusion, vaporization or unintentional adsorption during sample transport (when using an off-line technique).

For example, in the field of breath gas analysis GC-MS is an applied off-line technique having the advantage of identifying most of the major components. However, it is not possible to perform the method on-line, so that the long analysis time is disadvantageous. However, the application of on-line techniques increases within the research field. For selected ion-flow tube mass spectrometry (SIFT-MS) positive precursor ions (e.g. H_3O^+) are produced in an ion source, selected due to their mass-to-charge ratio and inserted to a helium flow. After introducing the sample gas into helium flow inside the tube, the sampling molecules react with the precursor ions and form characteristic products.[5] The analytical method can be used on-line, but only polar substances such as acetone and isoprene as well as unsaturated hydrocarbons can be detected. Aliphatic hydrocarbons are not measurable.[6] Another on-line technique, which is used for breath gas analysis, is the proton transfer reaction mass spectrometry (PTR-MS). The chemical ionization is performed by generation of H_3O^+ ions, which are mixed with the gas sample in a drift tube. The protonated molecules are analyzed using for example a quadrupole. Although the technique is more sensitive than SIFT-MS, it is limited due to the fact that only molecules with a higher proton affinity than water can be ionized. Furthermore, it is not possible to determine substances with same mass.[6] Already mentioned photoionization mass spectrometry was used for on-line detection of nonsmokers and smokers breath gas.[7]

For this thesis the determination of low-concentrated complex gas mixtures such as breath gas was done by a combination of fast PI-TOFMS and the pre-concentration method needle trap device (NTD). For successful coupling, the needle traps need to be thermal-desorbed and the desorbed substances need to be directly transferred into the mass spectrometer. Therefore, an injector of an gas chromatograph with a special SPME-GC inlet liner has to be installed and an optimization of needle trap material, desorption and transfer settings is necessary. For example, the selection of the adsorbent materials inside the needle traps is very important due to the substances that should be adsorbed and the less background signal that may occur by bleeding of adsorbents.

The analysis of breath gas was selected as first, demonstrative application of the needle trap device thermal-desorption photoionization time-of-flight mass spectrometry (NTD-TD-PI-TOFMS). As already mentioned for breath gas analysis it is of particulate interest to have a fast or rather on-line analytical technique providing rapid results. For safety reasons it is almost impossible to use the laser mass spectrometer in on-line mode directly on bedside (near subject). Hence, the idea was to use the needle traps not only for pre-concentration, but also to transfer the samples to the mass spectrometer which is located in a room next to the subject.

Besides the measurement of substances in breath gas of healthy, non-smoking and smoking persons, the aim of using NTD-PI-MS was to determine medically relevant compounds in patient's breath. Therefore, the selected substances need to be investigated for their photoionization properties (ionization energy, photoionization wavelengths-spectra) using high energetic radiation such as synchrotron at the facility BESSY II in Berlin. The results help to choose the best measurement wavelength or energy and therefore, to complete the measurement settings.

A completely different application shows the variability of applications of the new coupling: the analysis of ship engine exhaust. The influence on combustion products was tested when varying the fuel type (diesel fuel or heavy fuel oil), the engine load (20, 40, 60 or 80 kW) and the photoionization wavelength (248 or 266 nm). The idea was to measure almost exclusively aromatic and polycyclic aromatic substances in the gas phase and therefore, to find out, e.g. which fuel type is more suitable for ship engine operation due to less influence or rather less pollution of the environment.

2 Photoionization Mass Spectrometry

For less complicated data acquisition it is more favorable to use soft ionization techniques for investigation of complex samples. Hard ionization techniques such as electron ionization (EI) lead to massive fragmentation of the molecule because of high electron energy (70 eV). The most common soft methods chemical ionization (CI), electro spray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI) and matrix assisted laser desorption ionization (MALDI) only show less fragmentation and high signals for the molecular ion of the measured substance.

Another very soft ionization technique is the photoionization that is particularly suitable for measurement of gaseous samples with a complex composition. Depending on the photon energy, the molecules are excited and ionized if the individual molecule ionization energy is reached or alternatively exceeded. The ionization continuum can be reached using only one photon (see section single photon ionization) or by multiphoton ionization (for example see section resonance enhanced multiphoton ionization).
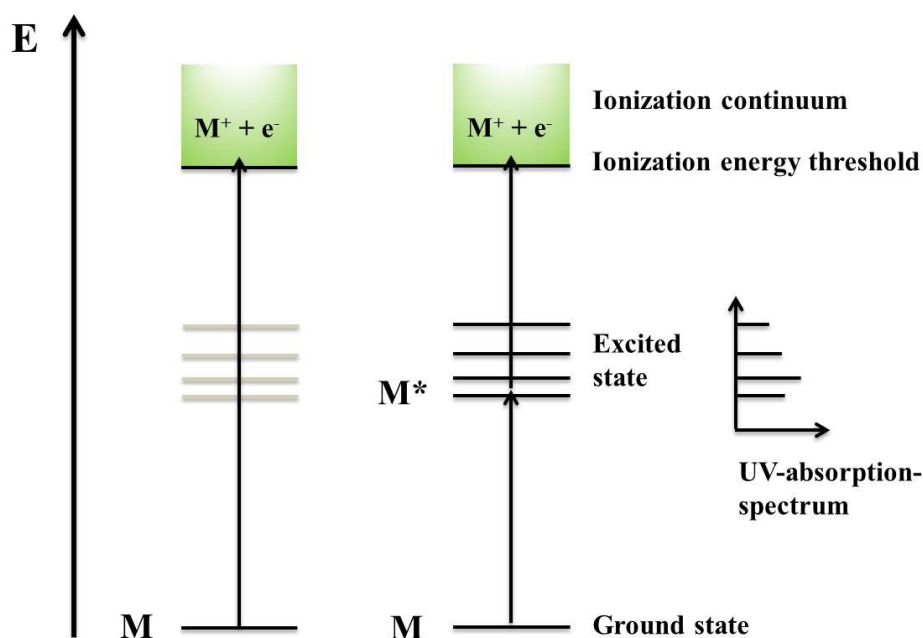
Figure 1. Photoionization schemes of Single Photon Ionization (SPI, left) and (1+1)-Resonance enhanced Multiphoton Ionization (REMPI, right).

2.1 Single Photon Ionization (SPI)

Single Photon Ionization (SPI) is based on the absorption of only one vacuum ultraviolet (VUV) photon (energy between 8 and 11 eV).[8, 9] If the used photon energy is equal or higher than the ionization energy of the molecule, the substance is ionized (*figure 1*). Because of low excess energy during process less or even no fragmentation of the molecule occurs. Also advantageously is the fact, that background gases such as nitrogen (IE = 15.6 eV), oxygen (IE = 12.1 eV), carbon dioxide (IE = 13.8 eV) and water (IE = 12.6 eV) are not ionized due to their high ionization energies (provided by NIST Chemistry WebBook). However, almost all organic and some inorganic compounds (ammonia, nitrogen monoxide and hydrogen sulfide) have ionization energies in the VUV region and therefore, the ionization technique is universally usable.[10-13]

The amount of ionized molecules, as a function of varying photon energy, shows the photoionization efficiency (PIE) and depends on the measured substance and the cross sections.[14] These photoionization cross sections (PICS) describe the interactions between the VUV photons and the sample molecules and is defined as an area with unit megabarn (1 Mb = 10^{-18} cm^2). If a molecule interacts with a photon having a higher energy than the IE of the substance, different processes are possible: on the one hand the direct ionization of the molecule. And on the other hand a superexcitation of the molecule, which can lead to the ionized molecule by autoionization or to neutral fragments by dissociation. The absorption cross section σ_a shows the probability of the absorption process for a certain photon, while the photoionization cross section σ_i reflects the probability of direct or autoionization of the molecule.[1, 15, 16] The photoionization quantum yield, the quotient of σ_i and σ_a, shows the competition between ionization of the molecule and the dissociation to neutral fragments. If the quantum yield is tending to zero, most of the molecules dissociate to neutral fragments. However, if each absorption of a photon leads to an ionized molecule, the photoionization quantum yield is 1.

The generation of VUV photons can be realized by different techniques.[17, 18] Deuterium and noble gas filled discharge lamps provide only low intensities and therefore, are rarely used for SPI. Often the third harmonic of Nd:YAG lasers in combination with a xenon filled cell (in total ninth harmonic) is used to produce pulsed photons with an energy of 10.5 eV (118 nm). VUV lamps or rather electron beam pumped rare gas excimer lamps (EBELs) are continuous, brilliant light sources with a high photon yield.[7, 19, 20] Furthermore, synchrotron radiation can be used for photon generation (see below).

2.2 Resonance Enhanced Multiphoton Ionization (REMPI)

Multiphoton ionization is based on the absorption of two or more photons of same or different energies to ionize the molecule.[21-25] Thereby, the energy of a single photon (4 to 6 eV) is lower than the ionization energy of the substance. The ionization is possible without exciting a certain intermediate state, but the efficiency of a photon in resonance with a transition state is much higher. Thus, resonance enhanced multiphoton ionization (REMPI, *figure 1*) is used more often than non-resonant version.

The classification of REMPI is done on the one hand by the amount of needed photons and on the other hand by the different photon energies (*figure 2*). Therefore, the ionization method is given by [x+y]-REMPI, with x for amount of photons used for reaching the excited state and y for amount of remaining photons for exceeding the ionization threshold. If only photons with same energy are used, it is called one-color-REMPI. However, it is also possible to irradiate during the lifetime of the excited intermediate state with different photon energy to ionize the molecule. Hence, it is a two-color process.
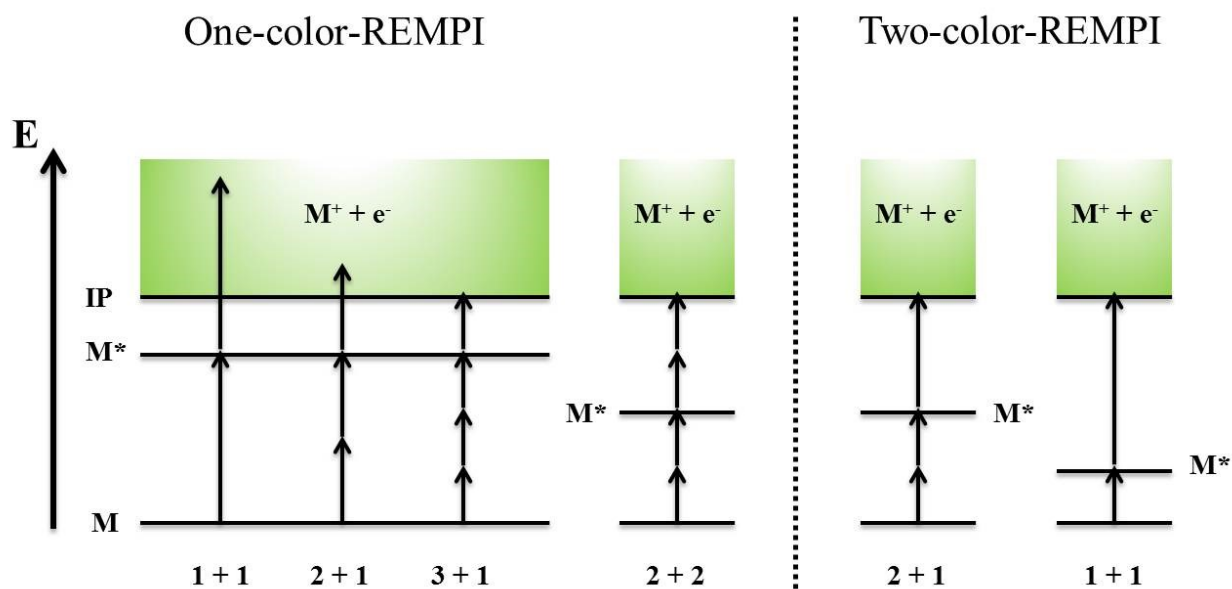
Figure 2. Classification of REMPI processes by amount of absorbed photons: [x+y]-REMPI and by energy of the used photons: one- or two-color-REMPI.

The simplest and most efficient option is the [1+1]-REMPI. So, the first absorbed photon is in resonance with the transition between vibrationless ground state S_0 and the first excited state S_1 of the molecule. The second photon with same energy leads to the ionization because of exceeding the ionization energy of the substance.

The probability of the absorption of the first photon is specified by the absorption cross section σ_1 and depends on the molecule, photon energy and photon power density of the laser system. Theoretically the first absorption cross section can be determined from one-photon experiment with the limitation of not considering the vibronic and rovibronic states. The probability of absorption of a second photon is described by the cross section σ_2, which depends on the lifetime of the intermediate state. Although, it is not possible to measure the second absorption cross section, the calculation succeeds by calculation with the help of σ_1 and the laser pulse length. For ionization of the molecule it is of high importance that the lifetime of the intermediate state is longer than the laser pulse duration.[26]

Due to the substance-specific ionization, REMPI is more selective than SPI and it is possible to distinguish for example between two isomers by changing the photon energy.[27] The best ionizable substances are aromatic and polycyclic aromatic compounds.

2.3 Photoionization Time-of-Flight Mass Spectrometry (PI-TOFMS)

The sensitive, selective and soft photoionization is perfectly complemented by fast time-of-flight mass spectrometry.[28, 29] Therefore, the sample is transferred by a heated capillary directly into the ion source, where the molecules are ionized by the incoming photons. The formed ions are unplugged by the repeller and extractor plate, before attaining the flight tube and subsequently accelerated in an electric field. Depending on their mass-to-charge ratio they reach a specific velocity. Ions with lower masses are faster than those with higher masses. Because of the fact, that there are differences between the kinetic energies $E_{kin} = 1/2\ m_i v_i^2$ of the ions at the starting point, it is advisable to use a reflectron-TOF. Ions with lower kinetic energy pass this electrostatic field faster than ions with higher kinetic energies, which penetrate deeper into the reflectron. The differences in the kinetic energies of the ions are compensated. Subsequently the ions can be measured with a multi-channel plate (MCP) detector which has 10^5 to 10^6 secondary electron multiplier channels and is directly connected to the data acquisition.

2.4 Determination of Ionization Energies – Synchrotron Radiation

The determination of molecule ionization energies can be realized by using synchrotron radiation. The synchrotron and storage ring BESSY II (**B**erliner **E**lektronen**s**peicherring-Gesellschaft für **S**ynchrotronstrahlung mbH, Helmholtz-Zentrum Berlin) can be used to afford the needed synchrotron radiation (*figure 3*). In a first step electrons are generated by an electron gun. These charged particles are preaccelerated by a linear accelerator and subsequently injected to the synchrotron (circumference: 96 m), where they circulate several times controlled by deflecting magnets. Additionally, the electrons are further accelerated by so-called cavity resonators. When the electrons reached their final energy, they are injected to the storage ring (circumference: 240 m). There the particles circulate over hours and generate synchrotron radiation because they are forced to move in a curved path. The loss of energy that every electron suffers has to be offset by further cavity resonators. The experiments occur at the edge of the storage ring where the synchrotron radiation can be taken from so-called beam lines. For measurements in energy range between 5 and 35 eV (7 to 12 eV sufficient for IE determination) the beam line U125/2-10m-NIM is used.[30]
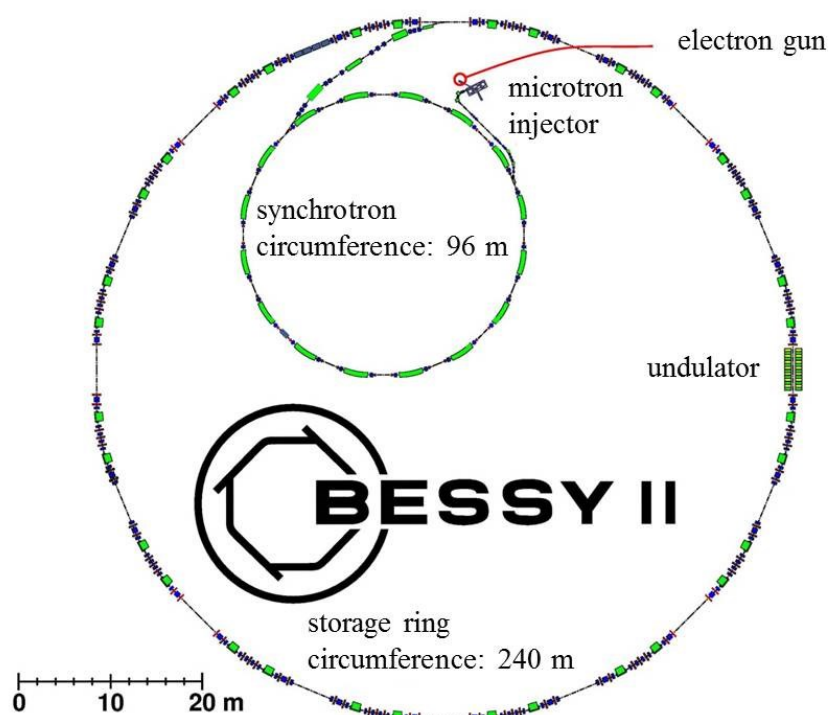
Figure 3. Scheme of the synchrotron and storage ring BESSY II at the Helmholtz-Zentrum Berlin (modified from reference).[31]

During the measurement campaign at BESSY II the already mentioned photoionization efficiency (PIE) diagrams were obtained during variation of the photon energy in range between 7.1 and 11.9 eV. Because of the not continuously given molecule flow into the ion source, an additional installed electron ionization source was used for checking the amount of incoming substance and afterwards for correction of the measured data. For insertion of the sample into the ionization chamber a heatable direct probe inlet was used. The substance to be measured was conveyed in a small glass tube and placed in the inlet. Additionally, inside the ion source a photodiode measured the current of the incoming photons. Higher photon energies arising from higher spectral diffraction orders were filtered out using a LiF window (11.9 eV cut off). The separation and detection of the ions occurred in a Reflectron-TOF-mass spectrometer.[2] The detailed description is given in ***publication 1*** (annex).

2.5 REMPI Wavelengths-Spectra

Comparable to the PIE diagrams for VUV region it is possible to determine the ionization opportunity when using photon energies below the IE of the molecule. The results are REMPI wavelengths-spectra which inform about best measurement energy/wavelength.[32]

Additionally, the different transitions between the possible states or rather intermediate states are determinable by the REMPI wavelength spectra. If a molecule is excited by an electronic excitation (4 to 5 eV), the Π electrons are shifted to a Π^* antibonding molecular orbital. Due to the energy of the excitation a weakening of molecule bonds are possible and therefore, the distance between the atomic nuclei can change. An electronic transition between the vibrationless ground state S_0 to the first excited singlet state S_1 is designated as 0-0 transition without vibronic and rovibronic excitation.[21, 33]

If the ionization energy of the determined substance is known, the half of this energy (IE/2) or rather wavelength can be complemented in wavelengths-spectra. A response at higher wavelength is not possible, when ionizing with only two photons.

For investigation of REMPI wavelengths-spectra a tunable laser system consisting of a Nd:YAG laser, a third harmonic generator (THG), an optical parametric oscillator (OPO) and a second harmonic generator (SHG) was used (see section 3.2 Measurement Setup). The wavelength is ranging between 218 and 345 nm, even though most organic substances are not ionizable above 300 nm due to higher molecule ionization energy compared to sum energies of both used photons.

3 NTD-TD-PI-TOFMS

Photoionization time-of-flight mass spectrometry is often used in combination with an on-line sampling procedure. However, a pre-concentration method has the advantage of adsorption of even low concentrated (ppt range) substances and therefore, provide the detection of these. For example, for solid phase extraction (SPE) sorbent material packed cartridges are used, through which the sample flows and extraction takes place. In most cases, the required large sampling volume is a significant problem. Hence, at the end of the 1980s *Pawliszyn* and coworkers developed an alternative method for adsorption of substances from only small sample amounts.[34] The solid phase microextraction (SPME) is more practical due to the simplified sample preparation, thus, the combination of sampling, extraction and desorption step.[35] Therefore, it was already applied in different research fields.[36-43] The thin sorbent coated fiber is located inside a stainless steel needle and is pulled out for extraction and desorption. The compared to SPE rapid, solvent-free sampling method can be used for liquid, gaseous and solid samples. Additionally, it is advantageous that desorption takes place directly in a GC inlet. However, a disadvantage can be seen in limited coating thickness and equilibrium conditions (e.g. temperature). Therefore, it is not possible to enhance the amount of analyte beyond the substance concentration that is obtained when equilibrium is reached. Because of long equilibration time (mostly between 20 and 30 minutes) it makes no sense to couple SPME technique with PI-MS compared to very fast on-line sampling. More useful is the combination of needle trap device (NTD) and photoionization mass spectrometry. Hence, the advantages of NTD, setup of the coupling and the setting optimization are discussed in detail in the following sections.

3.1 Needle Trap Device (NTD)

Needle trap devices represent the advancement of the mentioned SPME technique. The needles are more robust due to keeping the sorbent packed in a thin stainless steel needle (*figure 4*) instead of severe coated SPME fiber.[44, 45] Compared to the time-consuming extraction process using solid phase microextraction, substances are adsorbed when flushing the gaseous sample or sample headspace through the packed needle. Therefore, a single use syringe is connected (Luer Lock connection, *figure 4*) and syringe plunger is pulled back.

Subsequently the sample is pushed back again through the needle. The process can be repeated as often as necessary and therefore, leads to a higher concentration of substances adsorbed. However, the limitation is the breakthrough volume of the substances depending on the used adsorbent(s) and the sorbent volume inside the needle trap. Depending on the application different adsorbent materials such as carbon or porous polymer adsorbents are filled into the needles.[46] Because of their hydrophobic surface carbon adsorbents are highly suitable for samples containing undesired water (e.g. breath gas and exhaust). The often used non-porous carbons Carbopack and Carbotrap are categorized according to their particle size (naming it with capitals e.g. Carbopack B). Porous carbons or molecular sieves such as Carboxen are divided by the pore and particle size and get a number as additional designation (e.g. Carboxen 1000). The best-known synthetic material or rather polymer adsorbent material for NTDs represents Tenax, which is based on 2,6-diphenyl-p-phenylene. However, silicon-based polymers such as polydimethylsiloxane (PDMS) and other specialized polymers are also used in needle traps.[47] It is possible to fill in only one type of adsorbent or to use multibed fillings, which provide better chances to adsorb a great amount of different substances out of a complex sample mixture. Therefore, the company PAS Technology (Magdala, Germany) sells needle traps containing three different adsorbents (respectively 1 cm; *figure 4*). The sorbent materials have to be arranged in correct order, thus the strength of the adsorbents increases from the tip to the Luer connection. The tip of the needle trap can be shaped in different ways (e.g. partially ground, straight or conical) and the opening is located directly at the end (tip hole) or in the form of a side hole.
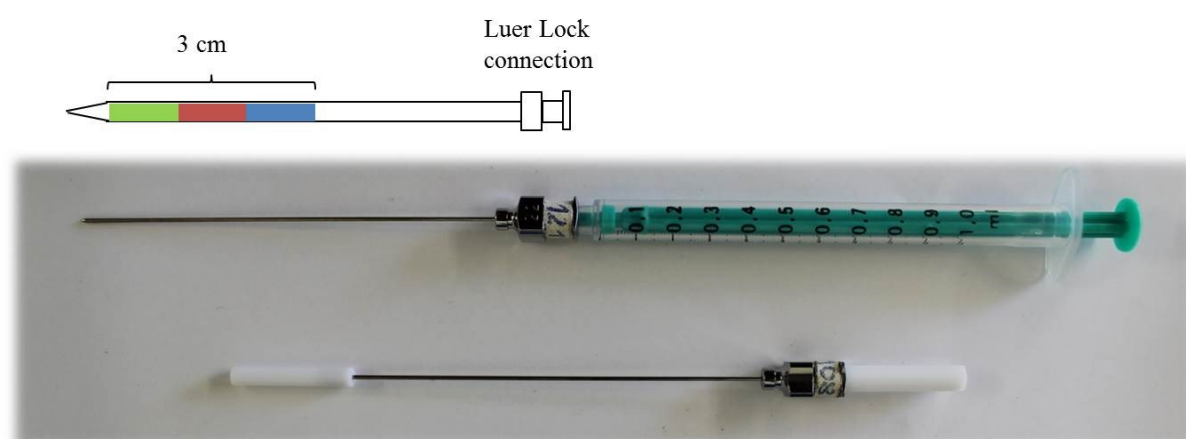
Figure 4. Scheme of multibed needle trap device (above) and example for application with 1 ml single use syringe and for storage with two Teflon caps.
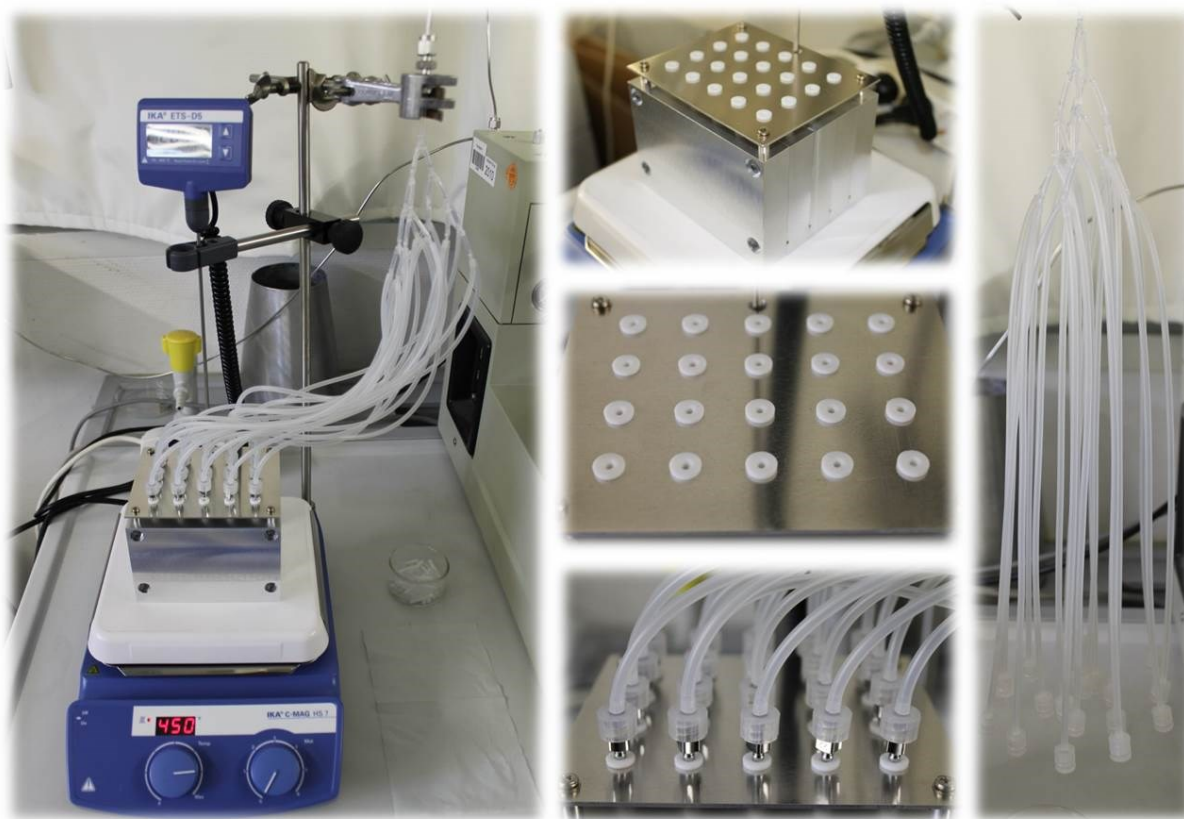
Figure 5. System (completely shown on left side) for conditioning of the NTDs, in detail the home-made aluminum heating block (middle) and the tubes for flushing the needles by helium flow (right).

The first conditioning of the needle trap devices is essential for elimination of contaminations from production process and depends on the used adsorbent materials. For realization of this step a ceramic heating plate (C-MAG HS7 IKAMAG, IKA) was utilized to heat a home-made solid aluminum block with 20 drilled holes for the NTDs (*figure 5*). The temperature was controlled by a contact thermometer (ETS-D5) measuring inside the block (same hole depth). The needles were connected (Luer Lock) to flexible tubes for flushing it by a continuous helium flow. The additional installed plate on the aluminum block protects the female Luer Lock connections of the tubes and the Teflon rings ensure, that the needles remain in place. For the used PDMS/Carbopack B/Carboxen 1000 needle traps the conditioning temperature was 290 °C. Before first use the needles were heated for 12 hours and before every usage it was repeated for 30 min. As shown in *figure 4* each NTD can be sealed with two Teflon caps for storage and in-between usage.

3.2 Measurement Setup

The measurement setup consists of an Agilent/HP 5890 gas chromatograph for desorption of the needle traps and a coupled photoionization time-of-flight mass spectrometer (*figure 6*). The dimension of the used SPME-GC inlet liner (Supelco, 78.5 mm × 6.5 mm × 0.75 mm) fits perfectly for the utilized 22 gauge, 7 cm long NTDs. After desorption the molecules are directly transferred by a helium flow into the ion source. Therefore, an up to 250 °C heated fused silica capillary (BGB Analytik AG, phenyl/methyl deactivated, 250 μm ID, 350 μm OD, length 2.8 m) is installed between gas chromatograph and mass spectrometer.
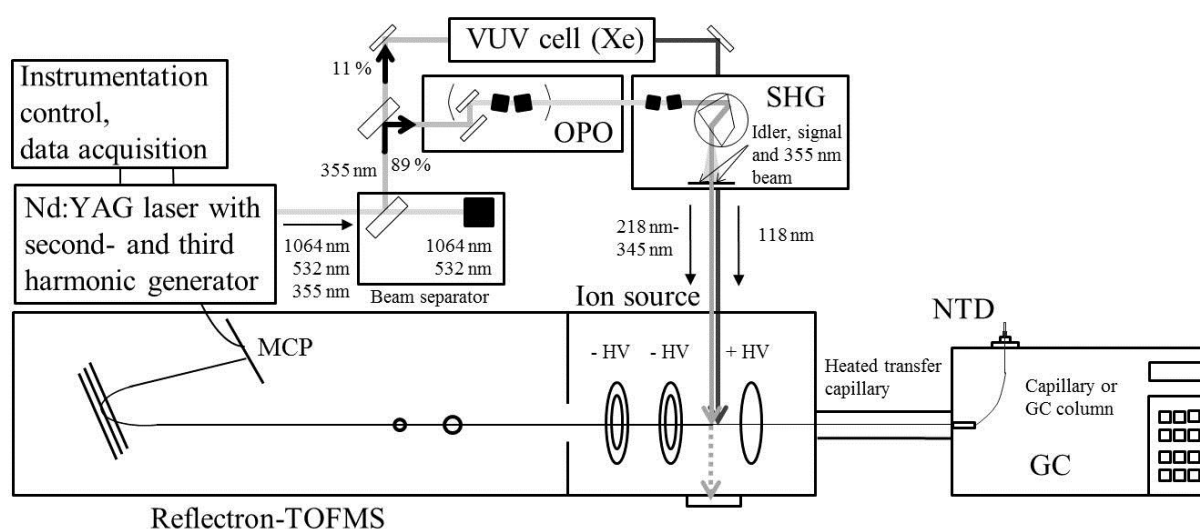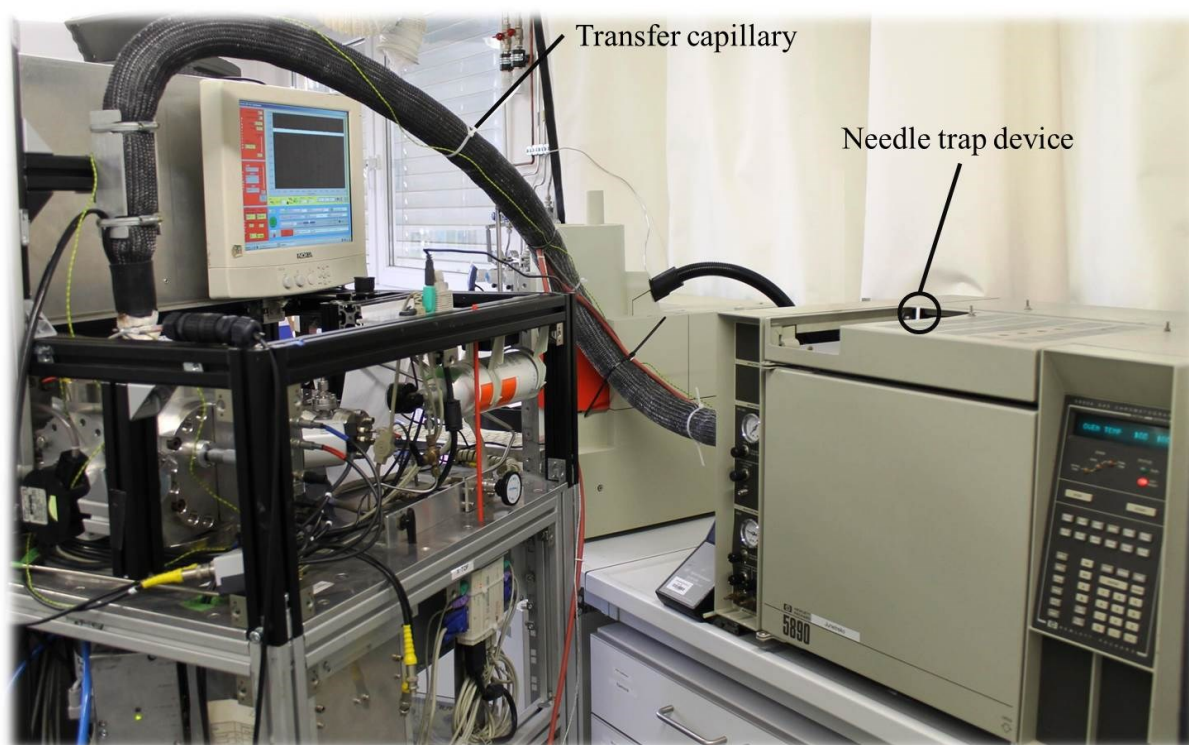

Figure 6. Scheme of complete measurement setup of new coupling NTD-TD-PI-TOFMS.

The ionization method of the mass spectrometer can be varied.[48, 49] The fundamental wavelength of the used neodymium doped yttrium-aluminum-garnet (Nd:YAG) laser (Continuum Surelite III, Santa Clara, USA, repetition rate 10 Hz) is frequency tripled with the help of a third harmonic generator (THG) and the resulting beam (355 nm) is divided into two parts. The remaining radiation consisting of the fundamental and the frequency doubled beam is stopped by a beam dump directly behind the laser. Eleven percent of the 355 nm beam is used to pump a gas cell filled with xenon and thus, to frequency triple the beam again. The result is a beam of VUV photons with a wavelength of 118 nm (10.5 eV) for single photon ionization.

The remaining part of 89 percent can be used to pump an optical parametric oscillator (OPO, GWU-Lasertechnik GmbH, Erftstadt, Germany). This unit contains two non-linear beta-bariumborate (BBO) crystals that cleave the input energy into a signal and idler energy. With respect to the conservation of energy the sum of the output frequencies is equal to the input. The wavelength is changed by varying the angle of the two non-linear crystals. Subsequently the produced photons are frequency-doubled in the second harmonic generator (SHG) and the signal is separated from idler and pump energy by a prism. Tunable UV laser pulses in a wavelength range between 218 and 345 nm result.

The beam of the respectively used photoionization technique is directly focused underneath the effusive molecular beam gas inlet. The incoming gaseous molecules are ionized and subsequently captured by electrostatic acceleration fields in the ion source of a reflectron time-of-flight mass spectrometer (Kaesdorf, Munich, Germany). After separation in the flight tube the ions are detected by a multichannel plate (MCP) detector. Data analysis was performed by home written LabView and MATLAB software tools. In *figure 7* the whole system is shown on a photograph.

Figure 7. Photo of complete measurement setup of new coupling NTD-TD-PI-TOFMS.

Furthermore, it is possible to upgrade the connection between gas chromatograph and mass spectrometer using an additional chromatographic column. Thus, it is possible to identify the measured substances by comparison with pure substances. Even though the fastness of NTD-TD-PI-TOFMS is lost, the selectivity of the ionization method is enhanced by a third dimension – the retention time. Therefore, a DB-5MS capillary column (250 µm ID; 0.25 µm film thickness; J&W Scientific/Agilent Technologies, Santa Clara, California, USA) was used. In case of REMPI measurements 10 m length and a short temperature program was perfect: 40 °C for 1 min, 12 °C/min to 250 °C for 1 min. For SPI measurements a longer column (30 m) and a different temperature program (30 °C for 1 min, 10 °C/min to 250 °C for 1 min) was chosen, due to the rather volatile compounds ionizable by single photon ionization.

3.3 Method Optimization

The filling of the needle trap devices was tested due to the background signals measured with PI-TOFMS. Thereby, the bleeding of polymers such as tenax poses problems because of the excellent ionization of fragments by REMPI. Most suitable adsorbents are carbons as mentioned above (e.g. Carbopack and Carboxen) and silicon-based polymers (e.g. PDMS). The used triple-bed NTDs with conical tip are filled with respectively 1 cm of polydimethylsiloxane (100/120 mesh), Carbopack B (60/80 mesh) and Carboxen 1000 (60/80 mesh). For optimization of parameter settings (e.g. split, head pressure) care was taken to ensure Gaussian bell-shaped peaks. Therefore, the split mode was most suitable with a split of 1:2.5 and a head pressure of 50 kPa. Desorption of the needle traps was done at a temperature of 290 °C with respect to the most sensitive adsorbent (PDMS). The possibility to flush the needles with helium while desorbing in inlet gave no increase of the measured signals.

The evaluation of coupling both techniques was done using a standard gas mixture containing inter alia benzene, toluene and 1,2,4-trimethylbenzene from Linde AG (Pullach, Germany). More details and the results of LOD and LOQ calculation are presented in ***publication 2*** (see annex).

4 Applications

Due to fast, simple sampling technique combined with the sensitive and selective photoionization method many applications are conceivable for NTD-TD-PI-TOFMS. The following sections will discuss the usage for breath gas analysis and investigation of exhaust from ship diesel engine operated on different settings.

4.1 Breath Gas Analysis

In recent years the breath gas analysis became more important due to the fact that sampling is non-invasive compared with taking blood samples. Moreover, the technique is repeatable as often as necessary and an on-line monitoring of breath gas becomes possible. The main goal of research is to find biomarkers for earlier diagnosis of diseases e.g. cancer. However, problems can be seen in the low concentration (ppb to ppt range) of substances in breath gas. Thus, the limits of detection of the used analytical technique have to be accordingly low. Furthermore, the application on bedside has to be realizable due to the welfare of the patient.

Breath gas analysis is only possible because of the gas exchange in the lungs. The branches or bronchioles have small bags, the alveoli, at their ends. Due to the blood capillaries, surrounding the millions of alveoli, gas exchange between blood and inhaled air is feasible. While incoming O_2 diffuses through the alveolar membrane, in blood present CO_2 diffuses into the alveoli. Of particular interest for breath gas analysis is the exchange in the alveoli of other volatile organic substances.

The interesting history of breath gas analysis already began with studies of Hippokrates (460-375 BC) who recognized the relation between breath odor and illnesses. He postulated different smells in conjunction with the diagnosed disease. The functionality of breathing was first observed by Antoine-Laurent Lavoisier (1743-1794) and his wife in 1789. Lavoisier recognized the exchange of inhaled oxygen with exhaled carbon dioxide based on experiments with guinea pigs and later also humans. The modern era of breath gas analysis first started with measurements of urine and breath by Linus Pauling in 1971. Using cooling trap (isopropanol and dry ice) the breath gas was sampled, subsequently desorbed and detected with GC-FID. The result was a chromatogram showing more than 300 different substances.[50-52]

Meanwhile the composition of breath gas is well known. Nitrogen does not participate in respiration and therefore, is exhaled unchanged. The body does not use the complete inhaled oxygen, thus, it is exhaled partly together with exchanged carbon dioxide. Furthermore, water vapor, rare gases and volatile organic compounds (VOCs) are exhaled. These volatile substances can be of endogenous or exogenous origin. Endogenous means that the compound is naturally produced in the body e.g. in metabolism. Exogenous VOCs are foreign substances in the body that are ingested by food or breath. Taken medications also belong to this group. Volatile organic compounds that can be found in breath gas are for example aldehydes such as acetaldehyde, propanal, butanal and hexanal as well as ketones such as acetone, which is produced by lipolysis. Further VOCs in breath gas are alkanes, e.g. ethane and pentane, which have been associated with inflammatory diseases. Isoprene is known from biosynthesis of cholesterol. Also existing volatile organic compounds are ethanol and aromatic compounds such as benzene, styrene and phenol. Increased concentrations of sulphurous substances such as dimethyl sulphide and carbon disulphide indicate liver failure or are associated with rejection of transplants. Nitrogen monoxide is increased in breath gas of persons suffering from respiratory diseases e.g. asthma.[53, 54]

The sampling of breath gas can be divided in off- and on-line techniques. A downward trend for off-line method represents the use of Tedlar bags which are blown up by the subject.[55] Problems occur during transport to laboratory because of diffusion through the wall and adsorption at the inside of the bag. Often pre-concentration methods as already mentioned (sorbent traps, SPME and NTD) are used in combination with GC-MS.[56-59] On-line techniques such as proton transfer reaction mass spectrometry (PTR-MS) and photoionization mass spectrometry are great applicable for direct breath gas analysis.[60, 61]

Describing the new coupling NTD-TD-PI-TOFMS as quasi on-line technique refers to the possibility of placing device in an adjoining room and directly desorb the sampled needle traps. The measurement takes only a few seconds and the selectivity of photoionization methods simplifies the fast interpretation even without gas chromatographic separation. If searching a special substance e.g. drugs, the wavelength with best photoionization response can be determined using synchrotron radiation or a tunable laser system for multiphoton ionization (see next section).

4.1.1 Photoionization Properties of Medically Relevant Substances

For planned measurements of medically relevant substances in breath gas the following candidates were chosen and the photoionization properties investigated. First selected substance was amantadine, which is used as influenza prevention. Diazepam acts as a tranquilizer, while etomidate is an intravenous short-acting hypnotic. The indole alkaloid *N,N*-dimethyltryptamine acts as a powerful hallucinogen and ketamine finds use as intravenous narcotic. Mescaline paralyzes the central nervous system and methadone is used as powerful analgesic. The hypnotic propofol is described in detail in the next section.

The REMPI wavelengths-spectra of these substances (*figure 8*) were detected using the tunable laser-OPO-system described above and the ionization energies (*figure 9*) were determined at BESSY facility. The results are discussed in detail in *publication 1* (see annex). Propofol is the most interesting substance due to successful determination in breath gas of a mechanically ventilated pig (see next section).
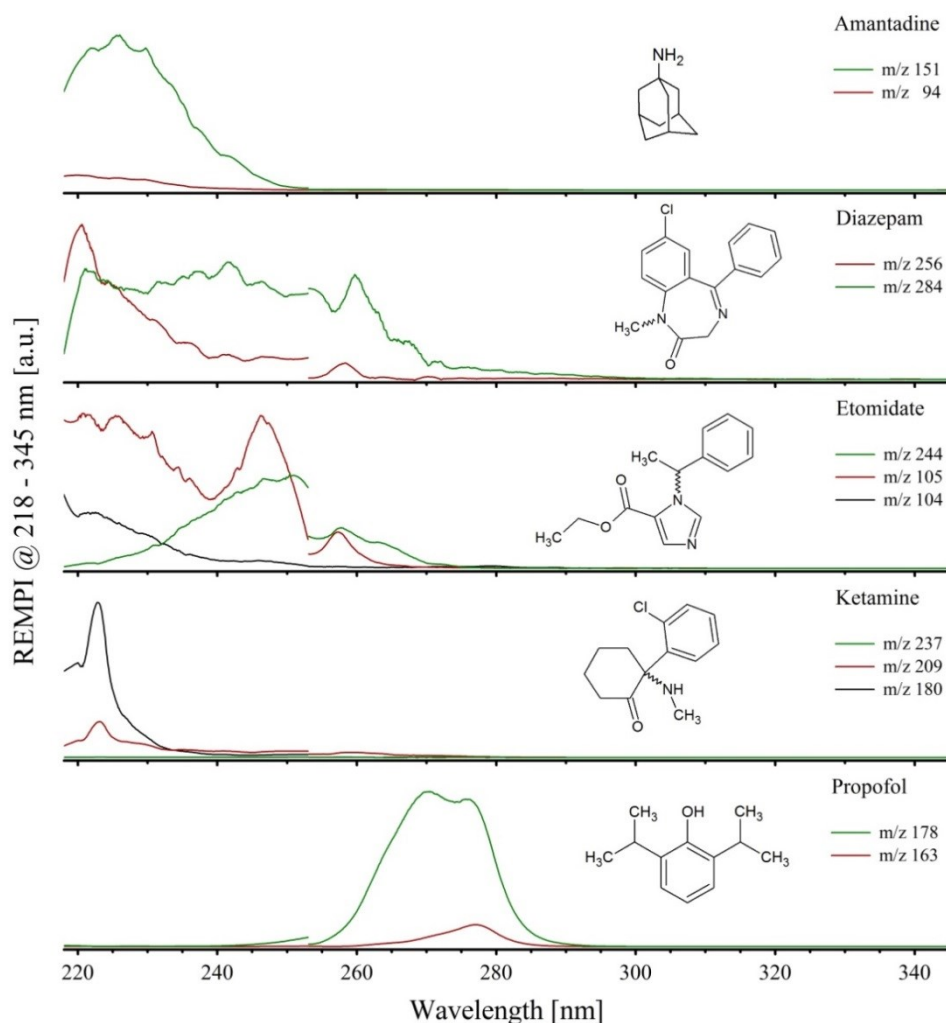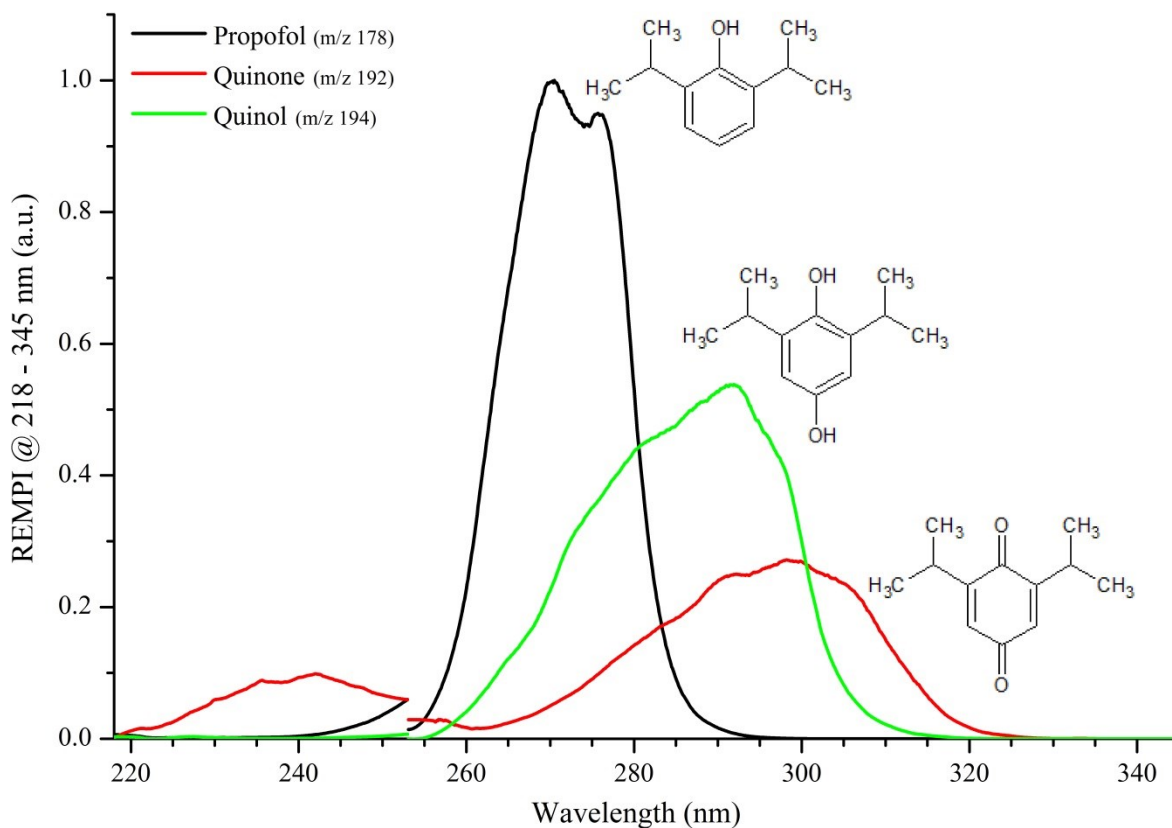

Figure 8. REMPI wavelengths-spectra of amantadine, diazepam, etomidate, ketamine and propofol.
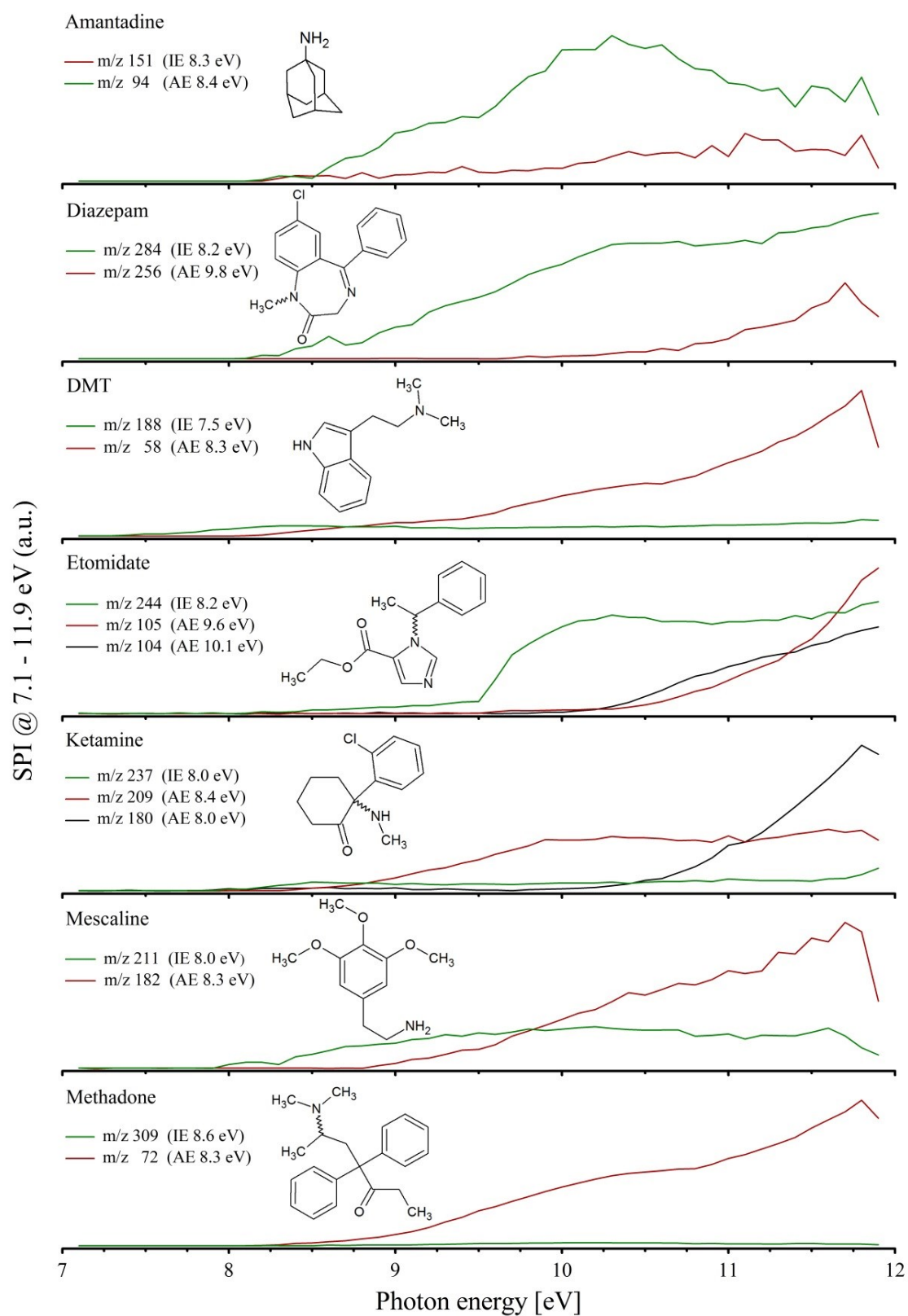
Figure 9. Photoionization efficiencies (PIE) of amantadine, diazepam, *N,N*-dimethyltryptamine (DMT), etomidate, ketamine, mescaline and methadone measured at BESSY facility.

## 4.1.2   Results for Mechanically Ventilated Pig under Propofol

2,6-Diisopropylphenol (propofol) is an intravenous sedative hypnotic agent which is used for induction of anesthesia as well as for maintaining the narcosis by continuous intravenous injection.[62-65] Propofol is metabolized very well in the human body (high total body clearance). One way for elimination of the molecule is the conjugation of itself to a propofol glucuronide. Another metabolism leads to the intermediate phase I metabolites 2,6-diisopropyl-1,4-benzoquinone and 4-hydroxypropofol. The 4-hydroxypropofol is further conjugated to 1- quinol glucuronide, 4-quinol glucuronide and 4-quinol sulphate.[66-68] These three glucuronides and the sulphate are excreted with urine. Besides propofol presence in breath gas it is also interesting to search for intermediate phase I metabolites 2,6-diisopropyl-1,4-benzoquinone and 4-hydroxypropofol. Therefore, the metabolites were independently synthesized and purified (because of high acquisition costs) for subsequently investigation of REMPI wavelengths-spectra using device as described above.

Figure 10. REMPI wavelengths-spectra of 2,6-diisopropylphenol (propofol) and its phase I metabolites 2,6-diisopropyl-1,4-benzoquinone and 4-hydroxypropofol.

The resulting REMPI spectra show best measurement properties for propofol, quinone and quinol in higher wavelengths range (see ***figure 10***). For determination of these substances in breath gas of a mechanically ventilated pig under surgery the best ionizing wavelength (276.9 nm) was selected for measuring propofol and the metabolites at same time.

The anesthesia of the animal model (pig, 25.6 kilos) was inducted by 100 mg propofol (1%), 0.15 mg Fentanyl and 20 mg Nimbex. Subsequently the intravenous infusion of propofol (2%) was maintained with a flow of 5 ml/h for about 8 hours. Four breath gas samples with a sampling volume of 20 ml were taken over a period of one hour using a t-piece connected into expiration tube. The averaged and inspiration subtracted result is shown in ***figure 11***.
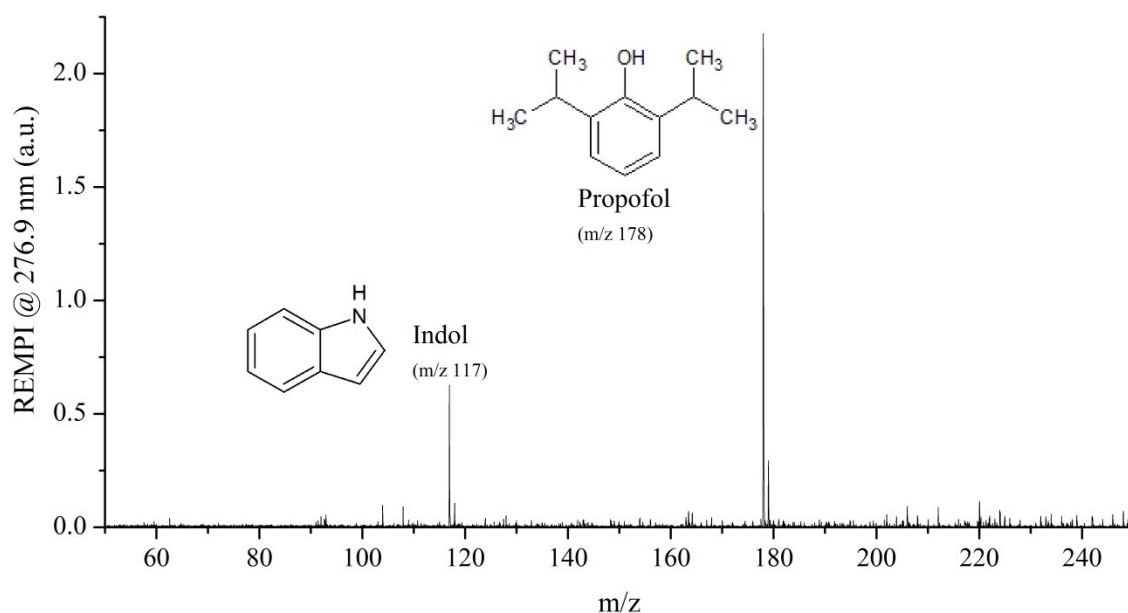

Figure 11. Averaged mass spectrum for breath gas of mechanically ventilated pig (inspiration subtracted) measured with REMPI at 276.9 nm.

While it was possible to measure propofol in the breath gas of the animal model, the metabolites were not detected using this wavelength. Maybe if choosing the appropriate wavelength with highest signal for quinone and quinol there is a chance to prove the existence of these substances in breath under sedation with propofol. However, *Harrison et al.* already reported on PTR measurements of propofol and its phase I metabolites in breath gas of patients undergoing surgery.[69]

4.1.3   Human Breath Gas Sampling

For determination of substances in breath gas of healthy non-smoking and smoking subjects a u-shaped device was equipped with a replaceable mouthpiece (*figure 12*). The capnometer (Capnogard EtCO2 Monitor, Novametrix Medical Systems INC.) was coupled for alveolar sampling. That means only breath gas that was involved in gas exchange in the alveoli and not the dead volume (in mouth, throat, trachea) was adsorbed on needle traps. The NTD is connected to a 1 ml single use syringe and subsequently punctured through a septum directly into the breath gas flow. A sampling volume of 30 cycles or rather 30 ml was chosen.
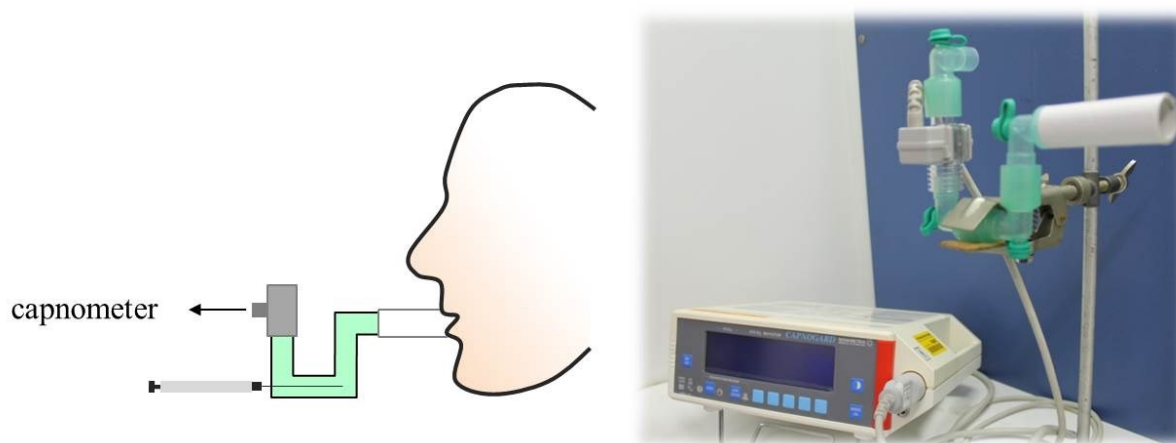

Figure 12. Alveolar sampling of healthy human using a capnometer to control the CO_2 concentration in breath gas.

All subjects (two female and three male, one smoker) had been instructed not to eat and brush their teeth one hour before sampling. The breath gas of the smoker was sampled directly after smoking and after one hour without smoking.

4.1.4   Comparison between non-smoking and smoking subjects

Breath gas samples of non-smoking and smoking subject were analyzed by using SPI (118 nm) and REMPI (266 nm) as ionization methods. *Figure 13* and *14* show the resulting mass spectra for smoker directly after and 1 hour after smoking as well as averaged mass spectrum of non-smoker, all corrected by blank sample subtraction.
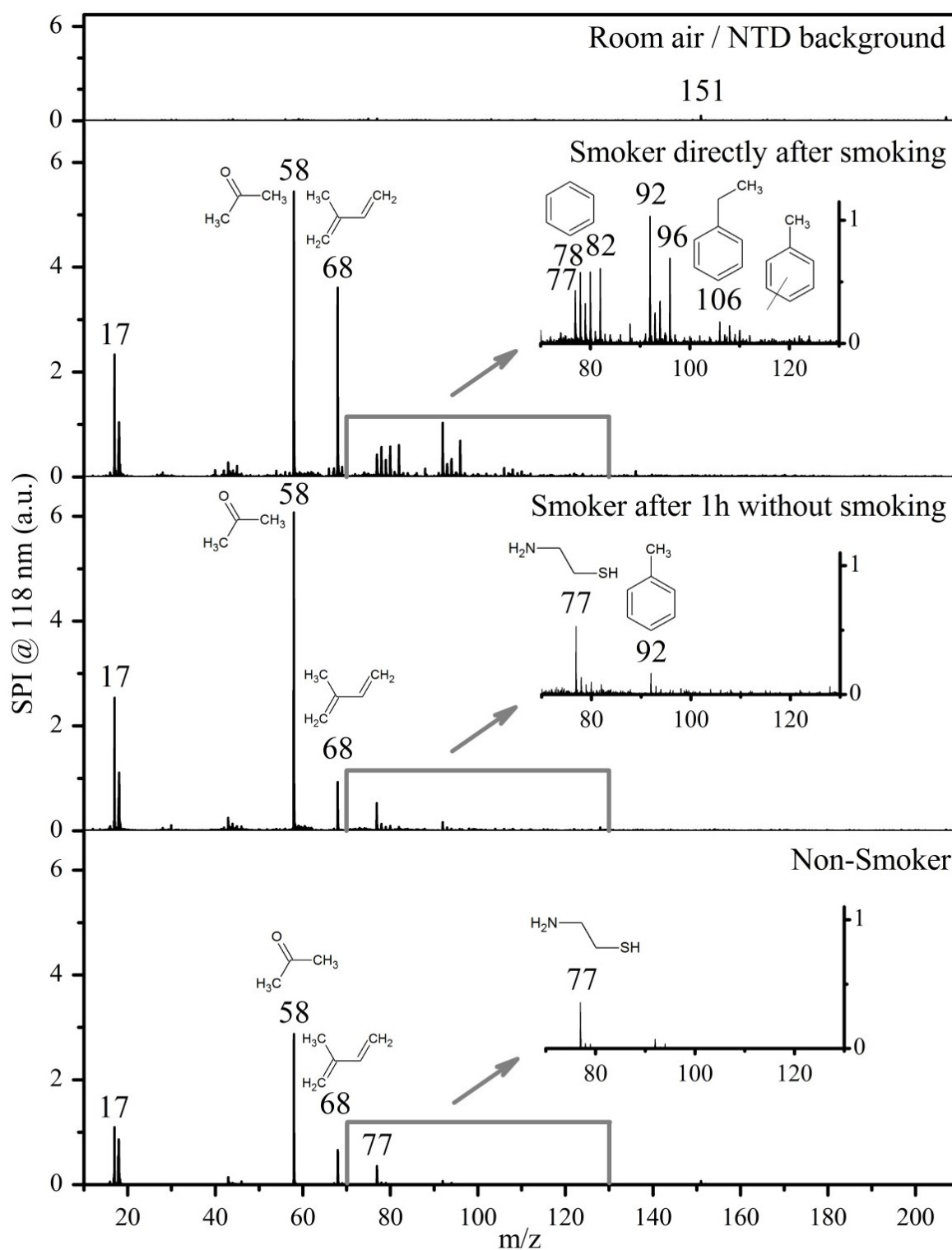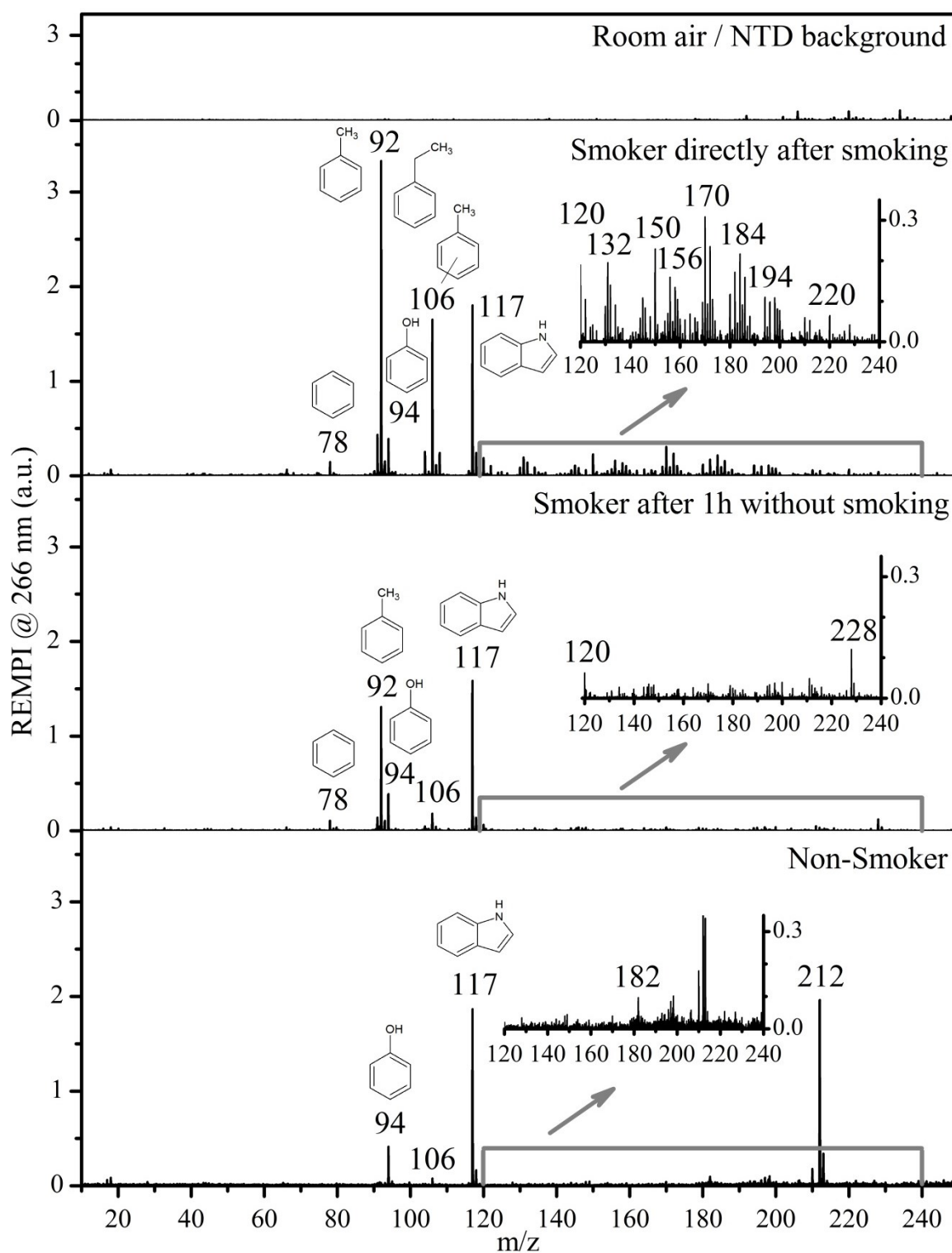
Figure 13. SPI mass spectra (118 nm): Comparison between breath gas of a non-smoking subject (female, 30 years old) and breath gas of a smoking person (male, 49 years old) directly after and 1 hour after smoking.

In *figure 13* the mass spectrum of the non-smoking subject (female, 30 years old) shows volatile substances such as acetone, isoprene and cysteamine as well as small amount of acetaldehyde. Same substances are also occurring in the breath gas of the smoking subject and are already described in literature as endogenous compounds, thus, substances produced in human metabolisms. For example acetone (m/z 58), is a decarboxylation product of acetoacetate (dextrose metabolism and lipolysis) and acetaldehyde (m/z 44) derives from ethanol metabolism in liver.[70, 71] Most of the measured compounds were confirmed by additional gas chromatographic separation (*figure 15*) in comparison with pure substances. Therefore, e.g. cysteamine (m/z 77), which is rarely described in literature as endogenous breath gas compound, was identified by SPI-TD-GC-TOFMS.[72] Furthermore, a lot of exogenous substances were found in the smoker's breath gas mainly directly after smoking a cigarette, because of inhaling the smoke. The amount of these substances, e.g. benzene (m/z 78), toluene (m/z 92) and benzaldehyde (m/z 106) is decreasing when smoking is stopped for longer time. In mass spectrum after 1 hour without smoking most of the exogenous substances are no longer existing or rather below the limit of detection of the method. Peaks occurring at m/z 82 and m/z 96 were not identified by gas chromatographic step but can be assigned to smoking related substances or rather marker methyl furan and 2,5-dimethylfuran sufficiently documented in literature.[73-76]

For REMPI measurements same relation between non-smoker and smoker directly and 1 hour after smoking can be observed (*figure 14*). While smoker's breath directly after inhaling the smoke shows a lot of aromatic substances of exogenous origin, the concentration of these compounds is decreased 1 hour later and breath gas of the non-smoking subject contains fewer compounds measurable with REMPI-TOFMS. Besides endogenous compounds such as phenol (m/z 94, can additionally be of exogenous origin) and indol (m/z 117) the odor of the smoking person contains for example benzene, toluene, styrene (m/z 104), ethylbenzene (m/z 106), 1,2,4-trimethylbenzene (TMB, m/z 120) and the xylene isomers (m/z 106). These substances are associated with lung diseases such as chronic obstructive pulmonary disease (COPD) or lung cancer. In *figure 15* the results of the smoker's gas chromatographic breath gas measurements (directly after smoking, REMPI and SPI compared) are shown.

Differences between the photoionization methods can be seen in ionization of different breath gas compounds. Only if using both methods a more precisely picture of the contained substances is build. Further details are presented in *publication 2*.

Figure 14. REMPI mass spectra (266 nm): Comparison between breath gas of a non-smoking subject (female, 30 years old) and breath gas of a smoking person (male, 49 years old) directly after and 1 hour after smoking.
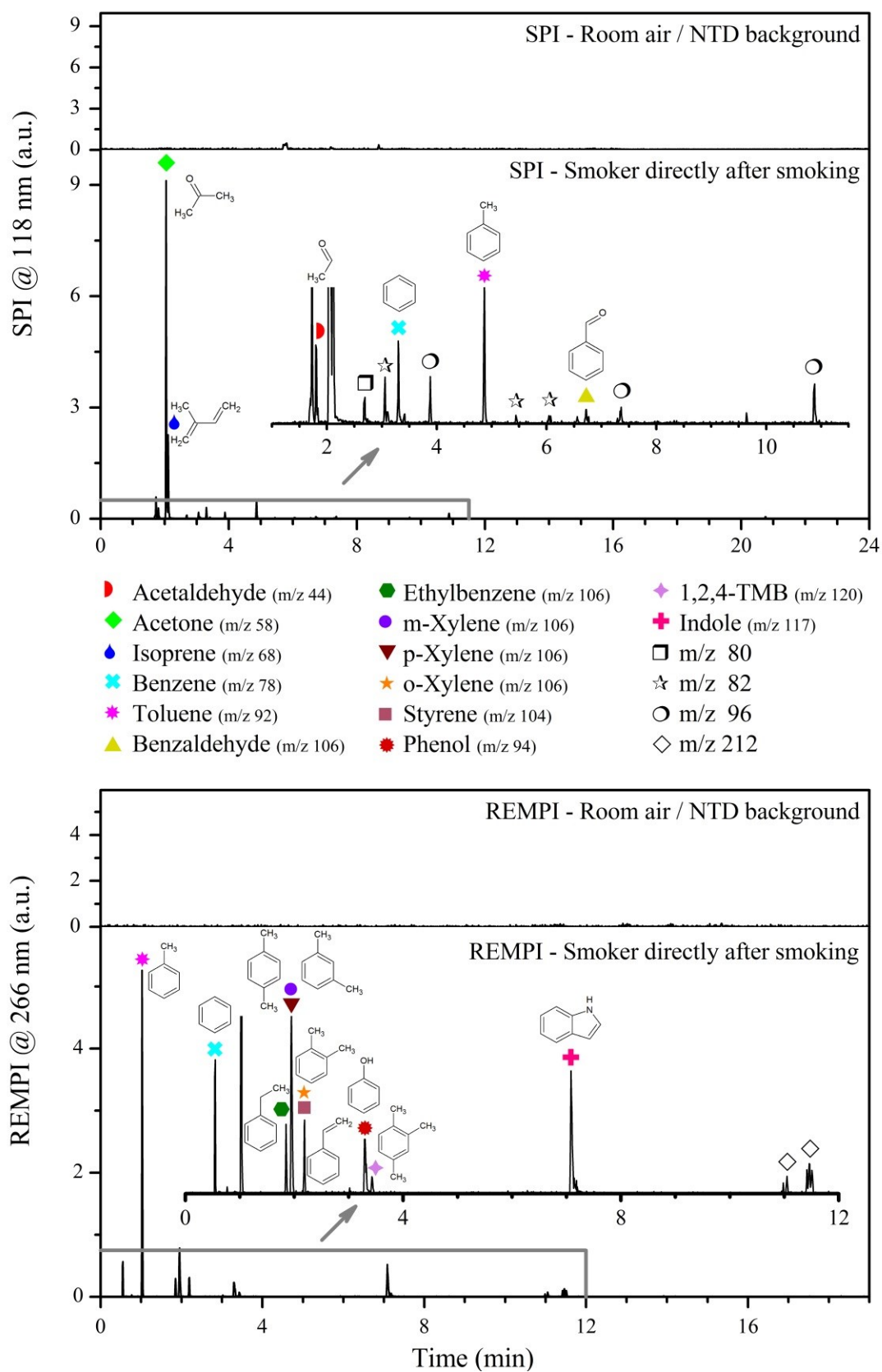
Figure 15. Chromatograms for SPI (118 nm) and REMPI (266 nm): Smoking subject (male, 49 years old) directly after smoking a cigarette.

4.2 Exhaust of Ship Diesel Engine

Due to increasing pollution of our seas, coastal areas and atmosphere by growing ship traffic it is of particular interest to change parameters such as fuel type, speed and engine load to reduce emissions and therefore, to reduce the entry of harmful substances into the environment. For example, the CO_2 emission can be reduced up to 70 % if the ship's speed is halved.[80] Other measures can be the usage of bigger vessels to transport more containers by lower fuel consumption per container or the replacement of fuel oils by alternative fuels such as liquefied natural gas (LNG). Additionally, it is important to filter the ship exhaust due to produced particulate matter (PM) and nitrogen oxides as well as sulfurous compounds. The aim of these measures is to reduce emission of ship engine combustion products and to reduce or rather to avoid influences of emitted substances on environment, climate, composition of atmosphere and last but not least health of all living beings.[81]

When burning fossil oil in an engine, not only the main components of combustion carbon dioxide and water are produced. Because of partial combustion or only by emission of the unburned fuel several harmful substances are released: CO, SO_2, NO_X, N_2O, NH_3, non-methane volatile organic compounds (NMVOCs), polycyclic aromatic hydrocarbons (PAH) and particulate matter.[82-85] In focus of the following described measurements is the gaseous phase of exhaust and especially the PAH. These organic substances consist of two or more condensed benzene rings, also five-membered rings are possible. Due to anthropogenic influence (e.g. industry) and small amount natural disasters (e.g. forest fires, volcanic eruptions) PAH are ubiquitous in the atmosphere.[86, 87] The increasing presence of these substances represents a risk, because most of the polycyclic aromatic hydrocarbons are classified as verifiable mutagenic and carcinogenic.[87, 88]

During measurement campaign of the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE) exhaust samples were determined using a single-cylinder diesel research engine with an injection system, using a common rail system with system pressures up to 1,300 bar (*figure 16*). The engine was operated using two different fuel types: diesel according to German DIN standard EN 590 and heavy fuel oil. One running cycle was about two hours, changing the engine load always at the same time. After starting the engine and warming phase the engine was driven on full load (80 kW) for 24 minutes. Subsequently the load was reduced to 75 % (60 kW) and held for one hour. Next 18 min the engine load was 50 % or 40 kW followed by the last section (18 min) on 25 % engine load.
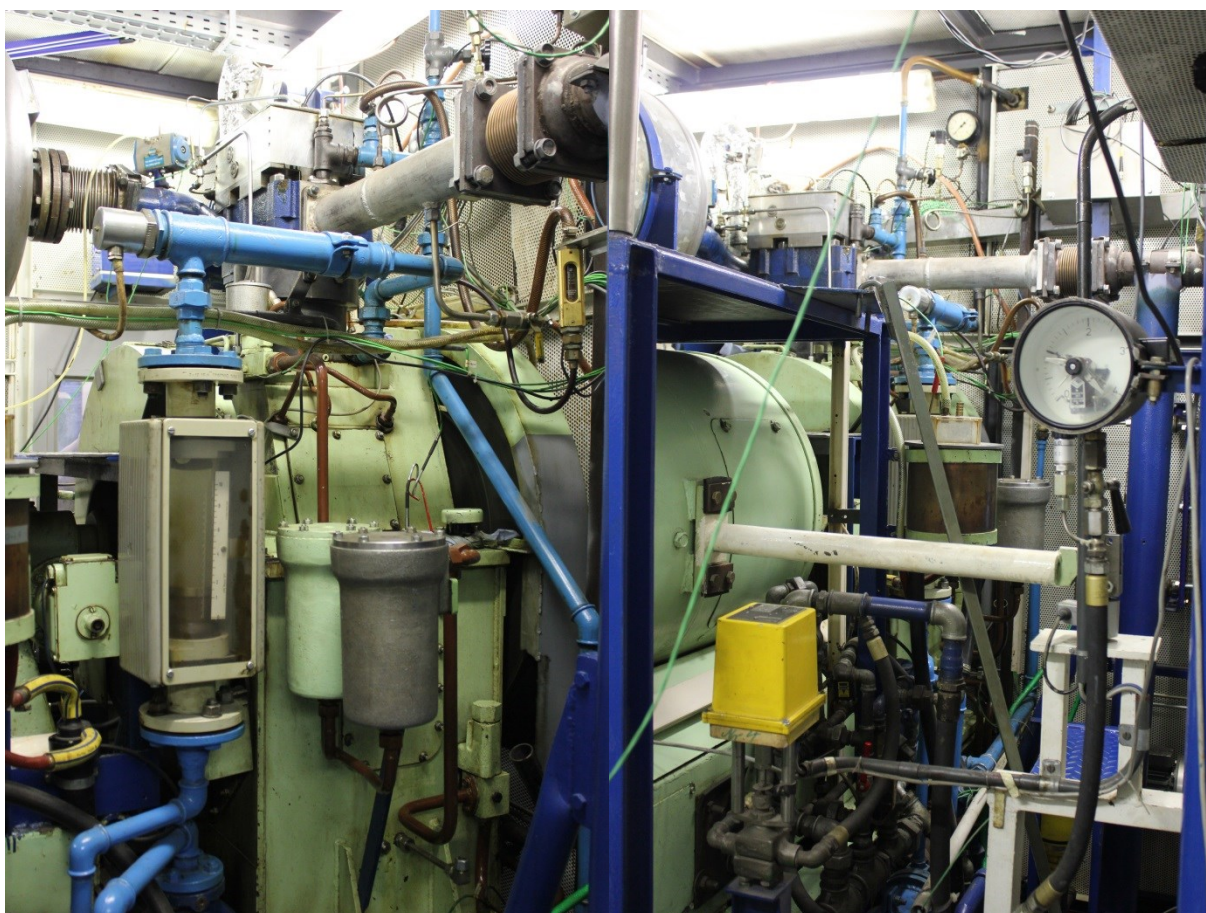
Figure 16. Photos of the used single-cylinder diesel research engine at the Institute of Piston Machines and Internal Combustion Engines in Rostock.

4.2.1 Exhaust Sampling

After passing the crystal filter behind the engine the hot exhaust was transferred by a heated tube through a Fourier transform infrared (FT-IR) spectrometer with a glass filter inside. Then the exhaust flowed along in a non-heated PTFE tube with a connected stainless steel t-piece after approximately 5 m. Thus, the cooled exhaust was sampled through a septum inside the t-piece using needle trap device coupled with a 1 ml syringe (*figure 17*). When signals of FT-IR spectrometer were stable after changing the engine load respectively three samples (sampling volume 15 ml) were taken. While diesel fuel exhaust was directly sampled, the HFO exhaust was diluted by a nitrogen flow (1:2) because of excessive water condensation inside the tube. Additionally, for HFO exhaust measurements the sampling volume had to be reduced from 15 ml to 10 ml because of higher concentration and better REMPI response of the phenanthrenes (see results).
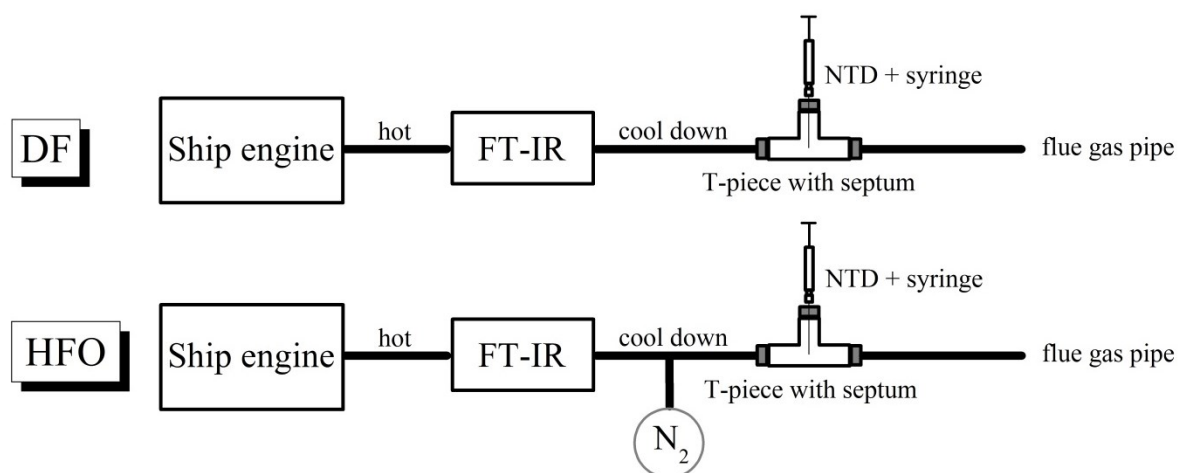
Figure 17. Exhaust sampling during HICE measurement campaign using T-Piece with BBO-Septum.

4.2.2 Results

Respectively three samples (under same combustion conditions) were averaged to one mass spectrum. For comparison of diesel and heavy fuel oil exhaust, mass spectra measured with REMPI at a wavelength of 266 nm and an engine load of 50 % are shown in *figure 18*. The differences are obvious: while diesel exhaust gas contains aromatic substances in a mass range between m/z 75 and 220, HFO exhaust shows higher molecular compounds from m/z 90 to 280. Major compounds in DF emission are naphthalene, benzofuran and respectively their alkylated homologues as well as the homologue row of benzene. These substances also appeared in former measurements of headspace above a diesel sample.[89] Thus, the compounds reach the exhaust partly as unburned products of the fuel. Furthermore, the polycyclic aromatic hydrocarbons phenanthrene, pyrene and fluorene as well as their alkylated isomers are measurable in the exhaust of marine engine when operated with diesel fuel. Other aromatic compounds such as biphenyls, styrenes, dibenzofuranes and acenaphthenes appear in the DF exhaust. Major compounds in HFO exhaust gas are phenanthrenes and naphthalenes. Despite of exhaust dilution by nitrogen, compounds with higher mass-to-charge ratio appear with higher signals than in diesel exhaust. In contrast to diesel fuel exhaust, more PAH such as chrysenes and benz(a)anthracenes as well as higher alkylated naphthalenes, acenaphthenes, phenanthrenes and pyrenes are detected. PAH with four or more fused benzene rings are predominantly found in the particle phase, thus, the signals are rather small.[90]
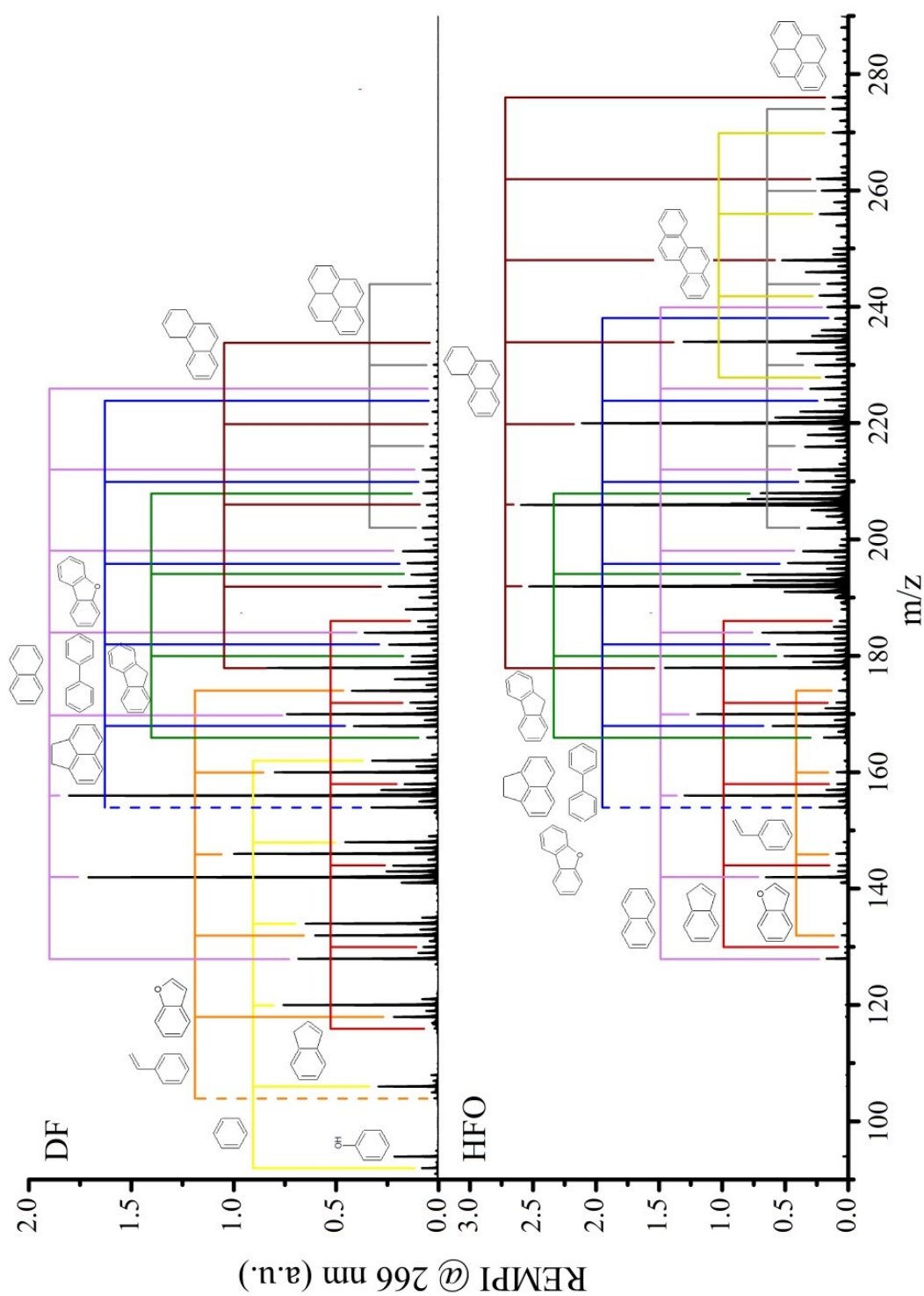
Figure 18. REMPI mass spectra (266 nm): Comparison between diesel fuel (DF) and heavy fuel oil (HFO) exhaust when ship engine was operated on 50 % load.

The gas chromatographic results for diesel fuel exhaust (***figure 19***) show the isomers of the methylated substances and the differences between the used REMPI wavelength – 248 nm and 266 nm. The signal of the respective substance depends on the probability of ionization at the chosen wavelength, for example, the methylated benzenes (m/z 92, 106, 120 and 134) are better measurable using 266 nm. Toluene (m/z 92), the xylene isomers (m/z 106) as well as ethylbenzene (m/z 106) and 1,2,4-trimethylbenzene (m/z 120) were identified using pure substances. Phenol (m/z 94, better response with 266 nm) and styrene (m/z 104, better measurable at 248 nm) were also clearly assigned to signals of exhaust samples. For methylated styrenes as well as benzofuran and its homologues (m/z 118, 132 and 146), which appear at same m/z, gas chromatogram shows several peaks. The assignment is possible by using REMPI wavelengths-spectra of the appropriate substances or by checking the retention times of the pure substances. At m/z 116 occurs only one peak clearly in chromatogram measured with 248 nm, probably assignable to indene.
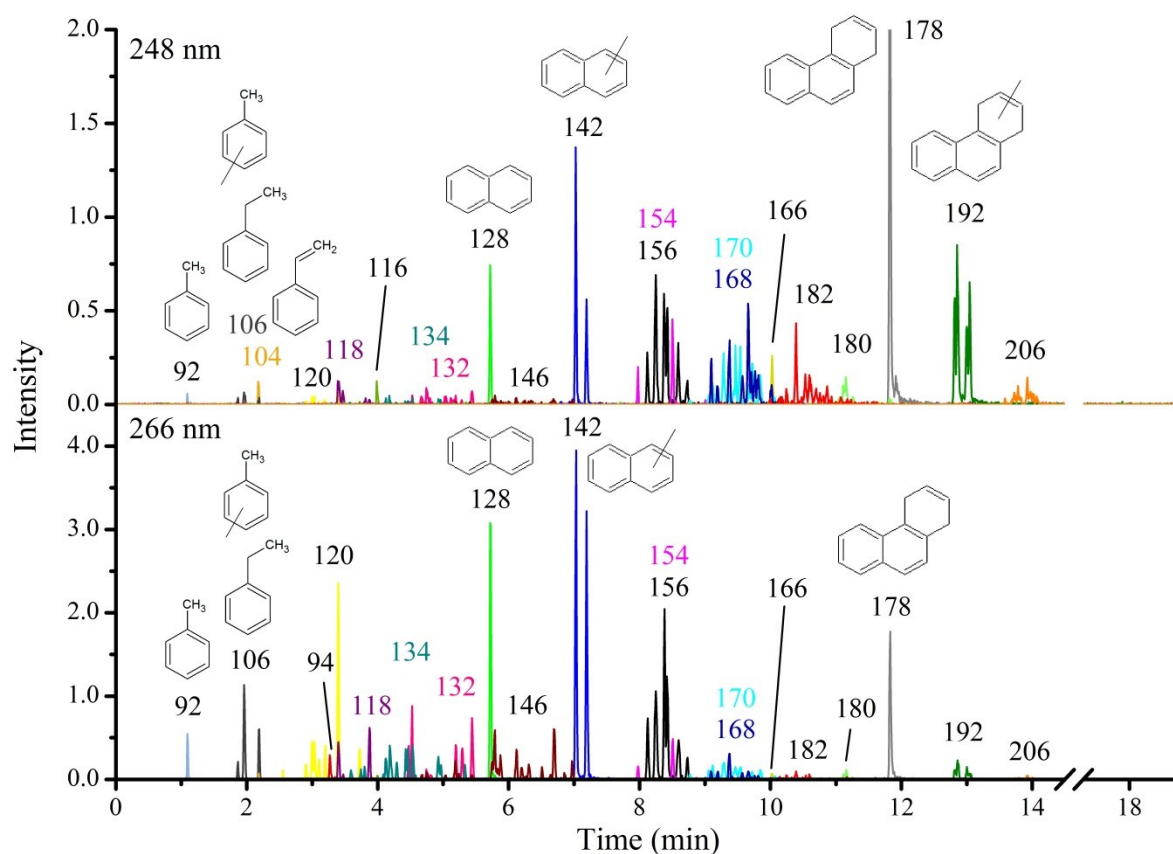
Figure 19. Chromatograms for comparison between REMPI wavelengths 248 and 266 nm: Diesel fuel exhaust when ship engine was operated on 50 % load.

The only peak at m/z 128 was clearly identified as naphthalene and is best ionized by REMPI 266 nm.[91] Also the methylated naphthalenes are better measurable using this wavelength. It was possible to assign the later eluting peak for m/z 142 to 1-methylnaphthalene. The occurring signals at m/z 156 distinguish in their relation to each other when changing the ionizing wavelength. Therefore, it is possible to assign the respective isomers by using the REMPI wavelengths-spectra. Better REMPI response using 248 nm show the substances at m/z 166 and 180, which are likely the polycyclic aromatic hydrocarbon fluorene and its single methylated isomers. Also better measurable with 248 nm are the compounds with a mass-to-charge ratio of 154 - probably biphenyl and acenaphthene - as well as the methylated compounds and the dibenzofurans (m/z 168 and 182). Phenanthrene (m/z 178) was successfully identified by pure substance measurement. Only when ionizing with 248 nm a small peak occurs, that can be assigned to anthracene in agreement with earlier published data showing no ionization at 266 nm.[91] Several not completely separated peaks appear at masses m/z 192 and 206 – probably methylated phenanthrenes/(anthracenes). ***Publication 3*** presents more details.

5 Summary & Outlook

Summarizing, the combination of pre-concentration method needle trap device and the thermal desorption of the needles in a photoionization time-of-flight mass spectrometer stands out for a great applicable technique. The sampling takes only a few minutes and the measurement is already done in less than ten seconds. Optional it is possible to exchange the fastness of the method for a third dimension (retention time in addition to mass of molecular ion and ionization wavelength), when implementing a gas chromatographic step.

First applications on breath gas and ship engine exhaust were successful and show the great variety of measurable samples.

The measured substances in breath gas of non-smoking and smoking subjects distinguish depending on the used photoionization method. With SPI (118 nm) it is possible to measure endogenous, more volatile compounds as acetone, acetaldehyde, isoprene and cysteamine. Additional measurable exogenous compounds in smoker's breath are benzene, toluene and benzaldehyde. Endogenous substances measured in breath gas using REMPI (266 nm) are aromatic compounds, such as phenol and indole. The breath gas of the smoker shows directly after smoking exogenous substances such as benzene, toluene, ethylbenzene, styrene, xylenes and 1,2,4-trimethylbenzene.

In preparation of measuring medically relevant substances in breath gas of patients, some selected compounds were determined due to their ionization properties. The ionization energies of amantadin, diazepam, *N,N*-dimethyltryptamine, etomidate, ketamine, mescaline and methadone were investigated at synchrotron device BESSY II at Berlin. Furthermore, it was possible to create REMPI wavelengths-spectra of amantadine, diazepam, etomidate, ketamine and propofol. Using these results and therefore, the best measuring wavelength, it succeeded to detect propofol in the breath gas of a mechanically ventilated pig undergoing surgery. The attempt to detect etomidate in breath gas of patients, which had an induction of narcosis with this hypnotic, has failed for the moment. The other substances were not searched in breath gas by now. For future measurements it is important to use a more powerful laser mass spectrometer in combination with needle traps. Additionally, a supersonic jet inlet instead of an effusive molecular beam gas inlet should be used for production of thinner and higher signals and therefore, better limits of detection.[92, 93]

Results of direct sampling and subsequently measurement of marine engine exhaust gas depended on used fuel type, engine load and ionization wavelength. Ionizing with resonance enhanced multiphoton ionization, aromatic substances and polycyclic aromatic hydrocarbons occur in different compositions. Because of higher cancerogenic or mutagenic potential of higher molecular PAH, which are contained in HFO exhaust, diesel fuel should be preferred when deciding between the two fuel types. Further projects should include new, alternative fuels such as biodiesel or liquefied natural gas.

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A Annex

List of Figures

Abbreviations

AE	Appearance Energy
APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photoionization
BBO	Beta-Bariumborate
BESSY	Berliner Elektronenspeicherring-Gesellschaft für Synchrotronstrahlung mbH
CI	Chemical Ionization
DMT	*N,N*-Dimethyltryptamine
EBEL	Electron Beam pumped Rare Gas Excimer Lamp
EI	Electron Ionization (formerly known as Electron Impact)
ESI	Electro Spray Ionization
GC	Gas chromatography
IE	Ionization Energy
Laser	Light Amplification by Stimulated Emission of Radiation
LNG	Liquefied Natural Gas
LOD	Limit of Detection
LOQ	Limit of Quantitation
MALDI	Matrix Assisted Laser Desorption Ionization
MCP	Micro Channel Plate
Nd:YAG	Neodym Yttrium-Aluminium-Granat
NIM	Normal Incidence Monochromator

NTD	Needle Trap Device
OPO	Optical Parametric Oscillator
PAH	Polycyclic Aromatic Hydrocarbons
PDMS	Polydimethylsiloxane
PI	Photoionization
PICS	Photoionization Cross Sections
PIE	Photoionization Efficiency
PTFE	Polytetrafluoroethylene
PTR	Proton Transfer Reaction
REMPI	Resonance Enhanced Multiphoton Ionization
SHG	Second Harmonic Generation
SPE	Solid Phase Extraction
SPI	Single Photon Ionization
SPME	Solid Phase Microextraction
TD	Thermal-Desorption
THG	Third Harmonic Generation
TOFMS	Time-of-Flight Mass Spectrometry
VOC	Volatile Organic Compound
VUV	Vacuum Ultraviolet

Publications

Publication 1

Investigation of the Photoionization Properties of Pharmaceutically Relevant Substances
by Resonance-Enhanced Multiphoton Ionization Spectroscopy and
Single-Photon Ionization Spectroscopy Using Synchrotron Radiation

by

Juliane Kleeblatt, Sven Ehlert, Jasper Hölzer,

Martin Sklorz, Jan Rittgen, Peter Baumgärtel,

Jochen K. Schubert, Ralf Zimmermann

Juliane Kleeblatt was involved in sample preparation and implementation of measurements during measurement campaign at BESSY II facility. She performed the data analysis and the preparation of the manuscript. The work of Juliane Kleeblatt to this publication accounts to approximately 75 %.

Publication 2

Detection of Gaseous Compounds by Needle Trap Sampling and
Direct Thermal-Desorption Photoionization Mass Spectrometry:
Concept and Demonstrative Application to Breath Gas Analysis

by

Juliane Kleeblatt,

Jochen K. Schubert,

Ralf Zimmermann

Juliane Kleeblatt implemented all breath gas measurements. She performed the data analysis and the preparation of the manuscript. The work of Juliane Kleeblatt to this publication accounts to approximately 90 %.

Publication 3

Needle Trap Sampling Thermal-Desorption Resonance Enhanced Multiphoton Ionization
Time-of-Flight Mass Spectrometry for Analysis of Marine Diesel Engine Exhaust

by

Juliane Kleeblatt, Benjamin Stengel, Christian Radischat,

Johannes Passig, Thorsten Streibel, Olli Sippula, Rom Rabe,

Horst Harndorf, Ralf Zimmermann

Juliane Kleeblatt was involved in preparation and implementation of measurements during measurement campaign. She performed the data analysis and the preparation of the manuscript. The work of Juliane Kleeblatt to this publication accounts to approximately 80 %.

Conference Contributions

J. Wulf, M. Sklorz, W. Miekisch, J.K. Schubert and R. Zimmermann (2010): Analysis of volatile organic compounds in breath gas using PI-TOF-MS. *Poster* at Interdisciplinary Scientific Seminar, Rostock.

J. Kleeblatt (2010): Analyse von Substanzen im Atemgas – Entwicklung der Atemgasanalytik und ihre gegenwärtige Bedeutung in der Medizin. *Oral Presentation* at lecture series "Kurs auf die Wissenschaft"of Interdisciplinary Faculty, Rostock.

J. Kleeblatt (2011): Investigation of medically relevant Substances using PI-TOF-MS. *Oral Presentation* at Conference "Spectroscopy – Detective in Science", Rostock, 15th to 17th June 2011.

J. Kleeblatt (2012): Analysis of volatile organic compounds in breath gas using Photoionization Mass Spectrometry. *Poster* at Evaluation Workshop of Department Life, Light and Matter, Rostock.

J. Kleeblatt, M. Sklorz and R. Zimmermann (2012): Investigation of medically relevant Substances using Photoionization Time-of-Flight Mass Spectrometry. *Poster* at Analytica Conference, Munich, 17th to 20th April 2012.

J. Kleeblatt, M. Sklorz, J.K. Schubert and R. Zimmermann (2013): Investigation of medically relevant Substances using PI-TOFMS. *Oral Presentation* at Conference of Deutsche Gesellschaft für Massenspektrometrie, Berlin, 10th to 13th March 2013.