Development and Validation of Novel Solventless Microextraction Techniques in Gas Chromatography

Dissertation

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Et Rheinisch Grundgesetz[1]

Artikel 1: *Et es wie et es.*

Artikel 2: *Et kütt wie et kütt.*

Artikel 3: *Et hätt noch emmer joot jejange.*

Artikel 4: *Wat fott es, es fott.*

Artikel 5: *Et bliev nix wie et wor.*

...

[1] zusammengetragen von Beikircher, K., in *Et kütt wie't kütt : das rheinische Grundgesetz*, Kiepenheuer und Witsch: 2001

Summary

Among the prior demands in current sample preparation for organic trace analysis are sensitivity, ease of automation and solvent exclusion. One approach to meet these demands has been the development of microextraction techniques, where the amount of extraction phase is very small compared to the sample phase. Today, microextraction techniques are used in current analytical methods from all fields. This thesis provides an overview on the recent developments in solventless microextraction techniques, with special emphasis on techniques providing full automation, starting from the first open-tubular trap techniques in the mid-1980s to recent devices utilizing sorbent packed needles. Different implementations of in-needle microextraction are discussed with their characteristic benefits, shortcomings and possible sampling modes. In this context, solid phase dynamic extraction (SPDE) was investigated for its applicability in quality control analysis of 196 German red wines. To that purpose, a fingerprinting database was created using commercial available chromatogram comparison software. 22 flavor relevant alcohols and esters have been quantified, also, to monitor the long term extraction performance of the SPDE needles, which showed constant results for up to 400 extractions using one extraction needle tip. A novel in-tube extraction (ITEX) device for headspace sampling has been evaluated for environmental and food analysis. To that end, five commercially available and six custom prepared sorbent traps have been evaluated for their extraction efficiency for over 50 analytes from different classes. They cover aromatics, heterocyclic aromatics, halogenated hydrocarbons, fuel oxygenates, alcohols, esters and aldehydes. During this course, the benefits of the use of adsorbent or absorbent materials, depending on the application, were shown, as well as the potential of mixed bed traps. Method detection limits in the low ng L^{-1}-range were achieved for compounds of importance for drinking water quality, which is much lower than demanded by regulatory limits and usually requires much more complex purge and trap systems. Furthermore, it was possible to discriminate the six beer varieties Alt, Helles, Kölsch, Pilsener beer, Schwarzbier and wheat beer and to assign 46 beers to these classes, just by analyzing volatile aroma constituents and applying Linear Discriminant Analysis. The governing parameters of the extraction and injection steps are discussed and the experiences from method development are summarized to give recommendations for the setting of proper extraction conditions, in order to minimize the experimental effort for future method development.

Kurzfassung

Zu den wichtigsten Anforderungen in der aktuellen organischen Spurenanalytik gehören Empfindlichkeit, leichte Automation und die Vermeidung von Lösemitteln. Ein Ansatz diese Ziele zu erreichen war die Einführung von Mikroextraktionstechniken, bei denen die Menge der Extraktionsphase, im Vergleich zur Probenphase, sehr klein ist. Heute werden Mikroextraktionstechniken in vielen analytischen Gebieten angewandt. Diese Arbeit bietet einen Überblick über die Entwicklung lösemittelfreier Mikroextraktionstechniken, mit vornehmlichem Schwerpunkt auf vollständig automatisierbare Techniken, beginnend bei den ersten Kapillartechniken Mitte der 1980er Jahre, bis hin zu aktuellen Ausführungen mit gepackten Nadeln. Die Verschiedenen Varianten werden in Bezug auf ihre charakteristischen Vor- und Nachteile und ihre Einsatzmöglichkeiten diskutiert. In diesem Zusammenhang wurde die Solid Phase Dynamic Extraction (SPDE) auf ihre Anwendbarkeit in der Qualitätskontrolle von 196 deutschen Rotweinen untersucht. Dazu wurde mit einer kommerziellen Vergleichssoftware eine Datenbank erstellt, mit der bekannte Proben identifiziert werden können. Weiterhin wurden 22 Geschmacksrelevante Alkohole und Ester quantifiziert und anhand dieser Daten die Langzeitstabilität der SPDE Nadeln untersucht. Mit einer Nadel konnten, bei konstanter Leistung, bis zu 400 Analysen durchgeführt werden. Eine neuartige In-tube Extraction (ITEX) Einheit wurde für die Umwelt- und Lebensmittelanalytik evaluiert. Dabei wurde die Extraktionseffizienz von fünf kommerziell erhältlichen und sechs speziell angefertigten Extraktionsnadeln anhand von über 50 Analyten verschiedener Klassen verglichen. Sie umfassten Aromaten, Heteroaromaten, halogenierte Kohlenwasserstoffe, Treibstoffzusätze, Alkohole, Ester und Aldehyde. Dabei wurden die anwendungsspezifischen Vorteile von Ab- und Adsorbtionsmaterialien und die Möglichkeiten gemischter Extraktionsphasen gezeigt. Für trinkwasserrelevante Analyten wurden Nachweisgrenzen im unteren ng L^{-1}-Bereich erzielt, die weit unter den erforderlichen Grenzwerten liegen und sonst nur mit deutlich komplexeren Purge & Trap Systemen erreicht werden. Weiterhin war es durch Messung von flüchtigen Geschmacksstoffen möglich, die sechs Biervarianten Alt, Helles, Kölsch, Pils, Schwarzbier und Weizen zu Unterscheiden und 46 Biere, durch lineare Diskriminanzanalyse, ihrer entsprechenden Variante zu zuordnen. Mit den Erfahrungswerten der Methodenentwicklung werden die entscheidenden Parameter der Extraktions- und Injektionsschritte diskutiert und Empfehlungen für geeignete Bedingungen gegeben, um den Entwicklungsaufwand für zukünftige Methoden zu minimieren.

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1 Introduction and Scope

Parts of this chapter have been published in modified form in *Laaks, J.; Jochmann, M. A.; Schmidt, T. C., Solvent-free microextraction techniques in gas chromatography. Analytical and Bioanalytical Chemistry 2012, 402, 565-571,* © Springer-Verlag 2012 and *Jochmann, M. A.; Laaks, J.; Schmidt, T. C., Chapter 12: Solvent Free Injection Techniques in "Practical Gas Chromatography: A Comprehensive Reference" by Dettmer-Wilde, K.; Engewald, W. (Eds.) 2014,* © Springer-Verlag 2014

Sample preparation/pre-treatment is one of the most time consuming and laborious steps in analytical procedures; nonetheless, improvements in this field have been considered to be of less importance than separation and detection, for many years.[1, 2] The main purposes of sample preparation are (i) removal of matrix components interfering with separation and/or detection, (ii) converting the analyte to a suitable form for separation and (iii) enrichment of the analyte to increase sensitivity. Today, capillary gas chromatography (GC) coupled to mass spectrometry (MS) provides high separation efficiency with highly sensitive and selective detection, while at the same time, improvements in liquid chromatography columns and interfaces to MS, often render the second point unnecessary.

Consequently, many sample preparation protocols still rely on simple, classical techniques, such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE) for liquid samples or Soxhlet extraction for solid samples. These methods typically require high volumes of sample and organic solvent (from mL-range up to several liters), as well as repeated extractions for sufficient enrichment. The resulting large volumes of extracts necessitate further concentration by evaporation or distillation; which, together with clean-up steps, makes these methods laborious, time consuming and prone to analyte losses. The used high purity organic solvents are expensive, usually toxic, harmful to the environment (e.g. ozone layer destroying chlorinated solvents) and in the end substantial quantities of solvent waste have to be handled.[2-4]

New directives and guidelines demand ever decreasing limits of detection and quantification, which cannot be achieved by improved separation and detection capabilities alone. This put enrichment and matrix separation more into focus, because errors in this stage of the analytical process can hardly be corrected in the following steps. Therefore, sample preparation has become a prominent part of many studies on the trace-level determination of organic micro-contaminants in real-life samples.[1] The main observable trends in present sample preparation techniques are (i) higher extraction yields and better reproducibility, (ii)

higher sample throughput by lower processing time, (iii) on-line sample preparation, (iv) automation, (v) miniaturization and (vi) organic solvent reduction/exclusion.[2, 3, 5, 6] A considerable fraction among the multitude of newly developed sample preparation procedures is represented by microextraction techniques, which can combine several of the aforementioned properties in one device.

1.1 Microextraction Techniques

Although all microextraction techniques share one common feature, namely that the amount of extraction phase is small compared to the sample volume[7], a wide variety using many different approaches has been presented over the years. Some are merely downsized versions of the classical techniques like liquid-liquid extraction, which can be scaled down to use only a few dozen micro liters of solvent (liquid-liquid microextraction (LLME) or dispersive liquid-liquid microextraction (DLLME)[8]), down to a single drop of just about one microliter (single-drop microextraction (SDME))[9]. Similar formats have been developed for automated extractions like liquid-phase microextraction (LPME)[10] or organic solvent film extraction (OSF)[11]. Microextraction in packed syringe (MEPS)[12] is a scaled down SPE, using a sorbent filled barrel attached to a micro syringe, which can also be automated. Other procedures use membranes to separate the analytes from the matrix, like membrane extraction with sorbent interface (MESI)[13] or the hollow-fiber extraction syringe (HF-ESy)[14], for example. More information on modern sample preparation methods can be found in a review by *de Koning et al.*.[1] Recent reviews discuss liquid phase microextractions[15] or the role of surfactants[16] and new materials[17] to minimize solvent consumption.

The focus of this thesis is laid on solvent free microextraction techniques for gas chromatography, which utilize a liquid or solid sorbent for analyte extraction and preferably offer a high degree of automation. An advantage of these techniques is the reusability of the extraction device, which can be employed repeatedly after reconditioning of the sorbent. For some techniques, several hundred measuring cycles have been reported without significant loss of extraction efficiency.[18] They can be divided into three general groups, depending on the way the sorbent material is implemented. The first uses a sorbent coating on a mechanical support, the second a packed sorbent bed and in the third group, the sorbent is used as bulk phase.

1.1.1 Sorbent coating Based Techniques

First applications used capillaries with polymer coatings (such as polydimethylsiloxane (PDMS)) as sorptive phase for analyte extraction and have been developed in the mid 1980s.[19, 20] Similar approaches were presented later as open-tubular trapping (OTT), in-tube microextraction, in-tube solid phase microextraction or capillary microextraction (CME) and have been subject to several reviews.[6, 21-25]. However, this approach suffered from complex instrumental setups and unfavorable sampling conditions such as high pressure drops for long traps and limited sample flows[26], stimulating the development of more convenient techniques.

1.1.1.1 Solid Phase Microextraction

Probably the best-known microextraction technique is the solid phase microextraction (SPME), which was introduced in 1989 by *Belardi and Pawliszyn*.[27] It utilizes a fused silica fiber core, coated with a film of PDMS as extraction phase (**Figure 1.1 a**). It can be used for sampling either from the liquid phase, by direct immersion of the fiber into the sample, or for headspace analysis and combines sampling, enrichment and clean-up in one device. Sample injection is achieved by thermal desorption and can be performed in any conventional GC injection port. In this way, the amount of manual work is minimized and easy automation with common xyz-autosamplers is also possible.

Initial problems like the fragility of the fused silica fiber core and ghost peaks from septum particles, pushed into the injection port by the blunt needle, have been addressed by bendable metal fibers and septum less injection port seals. Today, a variety of several sorbent materials with different polarities and sorption mechanisms is commercially available. One remaining drawback is the limited extraction capacity, caused by the low volume of extraction phase on the fiber[28], which is, depending on the core diameter (d_c) and film thickness (d_f), between 0.2 µL (d_c = 0.1 mm, d_f = 7 µm) and 1.6 µL (d_c = 0.2 mm, d_f = 100 µm). Because thicker coatings prolong the extraction time by the limited diffusion inside the coating, and the length and diameter of the sorbent support are restricted by the in-needle design, different techniques have been developed to gain more extraction capacity.

Despite its initial limitations and the availability of more sophisticated techniques, SPME is still widely used, because of its simplicity and versatility. Over the past 20 years, several hundred applications for SPME have been published in all analytical fields, covering environmental and food analysis, as well as biological and medical applications. Several

books[29-31] and reviews[3, 5, 7, 23, 32] are available, also. However, the transfer to national standard procedures for environmental and drinking water monitoring is still only beginning, because of the time consuming standardization process.[33-35]

1.1.1.2 Solid Phase Dynamic Extraction

One approach to increase the extraction capacity of SPME was to place short pieces of capillary GC columns inside a syringe needle, which was called inside needle capillary absorption trap (INCAT).[36, 37] A similar technique was commercialized in 2000 as the "magic needle" by Chromtech (Idstein, Germany) or solid phase dynamic extraction (SPDE)[38], where the coating is applied directly to the inner surface of a stainless steel needle (see **Figure 1.1 b**). In this way, about 4.5 µL PDMS can be immobilized as a 50 µm thick coating in a 56 mm long needle.[28] Besides the higher extraction phase volume, SPDE also offers more mechanical stability than SPME while maintaining the variability of possible extraction phase materials and easy autosampler integration. Sampling is performed by repeated pumping of the sample solution (liquid phase (LP)) or the sample headspace (HS) through the needle using a gastight syringe. The extraction conditions, like flow and number of pumping cycles, must be kept constant for all corresponding measurements because the extraction process is usually stopped before equilibrium is reached. This hampers the use of SPDE and related techniques, when no autosampler is available. Another possible disadvantage of SPDE arises from the length of the coating in the needle; a significantly varying temperature profile over the inlet depth of some GC injection port designs can result in incomplete desorption of analytes.

SPDE and INCAT have not been as well accepted as SPME, only about 30 applications have been published in the 15 years since the first presentation, but they also cover several different analytical tasks (see **Table 1.1**). The first application of SPDE by *Lipinski* was the extraction of halogenated pesticides from water.[39] Although no problems were reported, it remains the only application of SPDE with extraction directly from the liquid phase, until now. Other early works include the analysis of drugs of abuse from ground hair samples with an on-coating derivatization of amphetamines[38], cannabinoids[40] and other designer drugs[41]. Another core area of applications is food analysis, where *Bicchi* tested HS-SPDE for the extraction of dried rosemary leaves, green and roasted coffee, banana fruits and wines[28]; wine must[42] and wine[18, 43, 44] were the topic of other applications. An unusual application was the analysis of pheromones from elephant urine.[45] Further details are discussed in previous reviews on this topic.[46, 47]

Table 1.1 List of previous INCAT and SPDE applications (if not stated else, all extractions were performed from the headspace)

Method	Sorbent	Matrix	Analytes	LOD[a]	LOQ[a]	Ref.
INCAT	5%-Phenyl/PDMS Carbon	Air, Water	BTEX	-	-	[36]
INCAT	Carbon	Water	BTEX	-	-	[37]
INCAT	Carbon	Petroleum, Water	BTEX, VOCs	-	-	[48]
INCAT	Polyaniline/Hexagonally ordered silica	Water	PAHs	1 ng L^{-1}	-	[49]
LP-SPDE	PDMS	Water	Halogenated pesticides	0.3 ng L^{-1}	-	[39]
SPDE	10%-Carbon/PDMS	Hair samples	Amphetamines/ Synthetic designer drugs	30 mg g^{-1}	110 mg g^{-1}	[38]
SPDE	10%-Carbon/PDMS	Hair samples	Cannabinoids	90 mg g^{-1}	440 mg g^{-1}	[40]
SPDE	10%-Carbon/PDMS	Hair samples	Drugs of abuse	6 µg g^{-1}	25 µg g^{-1}	[41]
SPDE	10%-Carbon/PDMS	Food matrices	β-pinene, isoamyl acetate, linalool	-	-	[28]
SPDE	WAX, PDMS, 10%-Carbon/PDMS, cyanopropyl-phenyl/PDMS	Water	Alcohols	4 ng L^{-1}	-	[44]
SPDE	-	Elephant urine	Insect pheromones	-	-	[45]
SPDE	PDMS	Water	Furan, benzene, toluene	0.17 µg L^{-1}	-	[50]
SPDE	PDMS, 10%-Carbon/PDMS	Soft drinks	BTEX	0.03 ng L^{-1}	-	[51]
SPDE	10%-Carbon/PDMS	Water	Chlorinated VOCs	12 ng L^{-1}	-	[52]
SPDE	PDMS	Air	Toluene	-	-	[53]
SPDE	10%-Carbon/PDMS	Snow water	BTEX, aldehydes	19 ng L^{-1}	53 ng L^{-1}	[54]
SPDE	10%-Carbon/PDMS	Snow/Ice water	Alkylated benzenes, monoterpenes, chlorinated VOCs	-	-	[55]
SPDE	Polypyrrole	Water	PAHs	2 ng L^{-1}	-	[56]
SPDE	10%-Carbon/PDMS	Serum, urine	γ-hydroxybutyric acid	160 µg L^{-1}	-	[57]
SPDE	10%-Carbon/PDMS	Grape must	Aroma compounds	-	-	[42]
SPDE	PDMS	Barley, malt, beer	*trans*-2-nonenal	5 ng L^{-1}	15 ng L^{-1}	[58]
SPDE	-	Air	Biogenic VOCs	-	-	[59]
SPDE	TiO_2-coating	Urine, water	Desomorphine, desocodeine	250 µg L^{-1}	-	[60]
SPDE	10%-Carbon/PDMS	Elephant urine	Volatile urinary chemicals	-	-	[61]
SPDE	Molecularly imprinted polymer	Urine	Amphetamine, methamphetamine, ecstasy	12 µg L^{-1}	40 µg L^{-1}	[62]
SPDE	Molecularly imprinted polymer	Water	Triazine herbicides	2.6 µg L^{-1}	-	[63]
SPDE	10%-Carbon/PDMS	Blood	n-heptane metabolites	6 µg L^{-1}	-	[64]
SPDE	WAX (PEG)	Wine	Aroma compounds	0.1 µg L^{-1}	-	[18]
SPDE	PDMS	Citrus essential oil	Limonene, linalool, γ-terpinene	6.8 ng abs.	22.6 ng abs.	[65]
SPDE	10%-Carbon/PDMS	Wine	Aroma compounds	-	-	[43]

[a] only the lowest limit of detection/quantification is given for mixtures of multiple analytes

1.1.1.3 Stir Bar Sorptive Extraction

Several techniques utilizing coated stir bars or glass rods have been developed to gain significantly larger volumes of extraction phase, for example stir bar sorptive extraction (SBSE, **Figure 1.1 c**, commercialized by Gerstel as "Twister")[66], high capacity headspace sorptive extraction (HSSE, **Figure 1.1 d**)[26] or dual twister[67]. The Twister is available as magnetic glass stir bar (2 mm *o.d.*) with lengths of 1 cm or 2 cm, with coating thicknesses of 0.5 mm and 1 mm, resulting in sorbent volumes of up to 315 µL. These techniques lose the advantage of complete automation, as the stir bar or rod has to be manually removed from the sample to put it into a desorption tube, sometimes rinsing and drying steps are also necessary. The sample introduction to the GC requires a special thermodesorption unit or inlet system, which is able to change the tube (containing the rod or bar) automatically, otherwise this has to be performed manually, too. Until now, only PDMS and polyethylene glycol (PEG) coatings are commercially available.

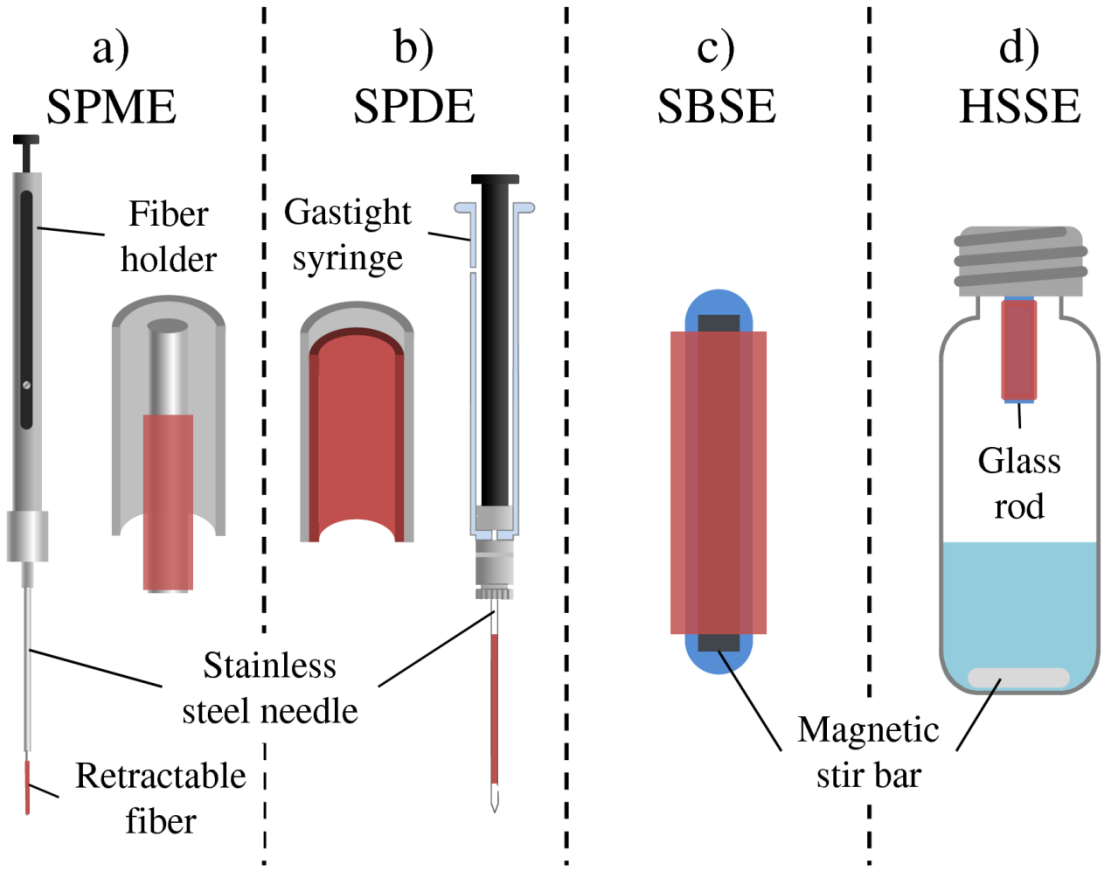

Figure 1.1 Important coating based extraction techniques: a) Solid phase microextraction, b) Solid phase dynamic extraction, c) Stir bar sorptive extraction, d) Headspace sorptive extraction; sorbent coating represented in red

These methods are primarily used for sampling of low volatile compounds like pesticides, polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) directly from the liquid phase.. While the total number of published applications is in the hundreds, only few applications utilizing extractions from the sample headspace are available[68]. More information can be found in recent reviews.[69-71]

1.1.2 Packed Sorbent Techniques

A common constraint of the methods mentioned before is the limitation to liquid polymer coatings, with PDMS being the most frequently used but poly(acrylate) (PA) or PEG also being common for more polar compounds. Some adsorbents that cannot be applied as a coating are available embedded in PDMS, for example activated carbon, Carboxen/Carbopack or divinylbenzene (DVB). While liquid polymers act as absorbents, enriching the analytes by partitioning into the extraction phase, only adsorption to the surface takes place with solid packing materials. Because the number of sorption sites is limited, competition effects or displacement of analytes might occur at higher concentrations.[29] On the other hand, adsorption is typically stronger than absorptive interactions, allowing the efficient trapping of more volatile compounds. To enhance the range of available extraction phases to the complete set of standard sorbents, well known from gas analysis or purge and trap systems, several techniques utilizing a packed sorbent bed inside a needle or tube have been developed. Another advantage of packed sorbent techniques is their versatility. In addition to the wide range of available sorbent materials, mixed bed traps can be prepared effortlessly by combining sorbent materials with different sorption capacities and affinities in order to achieve optimal conditions for each analytical task.

The sorbents can be placed either directly inside the needle, as in the needle microconcentrator[72] the needle trap (NT, see **Figure 1.2 a**)[73] and the fiber-packed needle (FPN)[74] (**Figure 1.2 b**) or in a larger diameter tube attached to a needle, like the cylindrical microconcentrator[72] and the in-tube extraction (ITEX)[75] (**Figure 1.2 c**). Of those, only the NT and ITEX have found further prevalence, as they have been commercialized by PAS Technology (Magdala, Germany) and CTC Analytics (Zwingen, Switzerland), respectively. Both implementations have specific advantages and shortcomings. While the devices with in-needle packings can be inserted directly into the GC injection port for thermal desorption, the in-tube devices require an external heater around the packing. The in-needle systems on the other hand show the same sensitivity to temperature gradients in the injector as SPDE and the amount of sorbent is limited by the smaller inner diameter. Several sampling modes are

possible: (i) open system sampling (exhaustive sampling) with a syringe pump or purge gas supply, (ii) closed system sampling (equilibrium sampling) from a vial by aspirating and dispensing headspace gas with a syringe or (iii) passive time weighted average sampling. Each design favours certain sampling modes and applications.

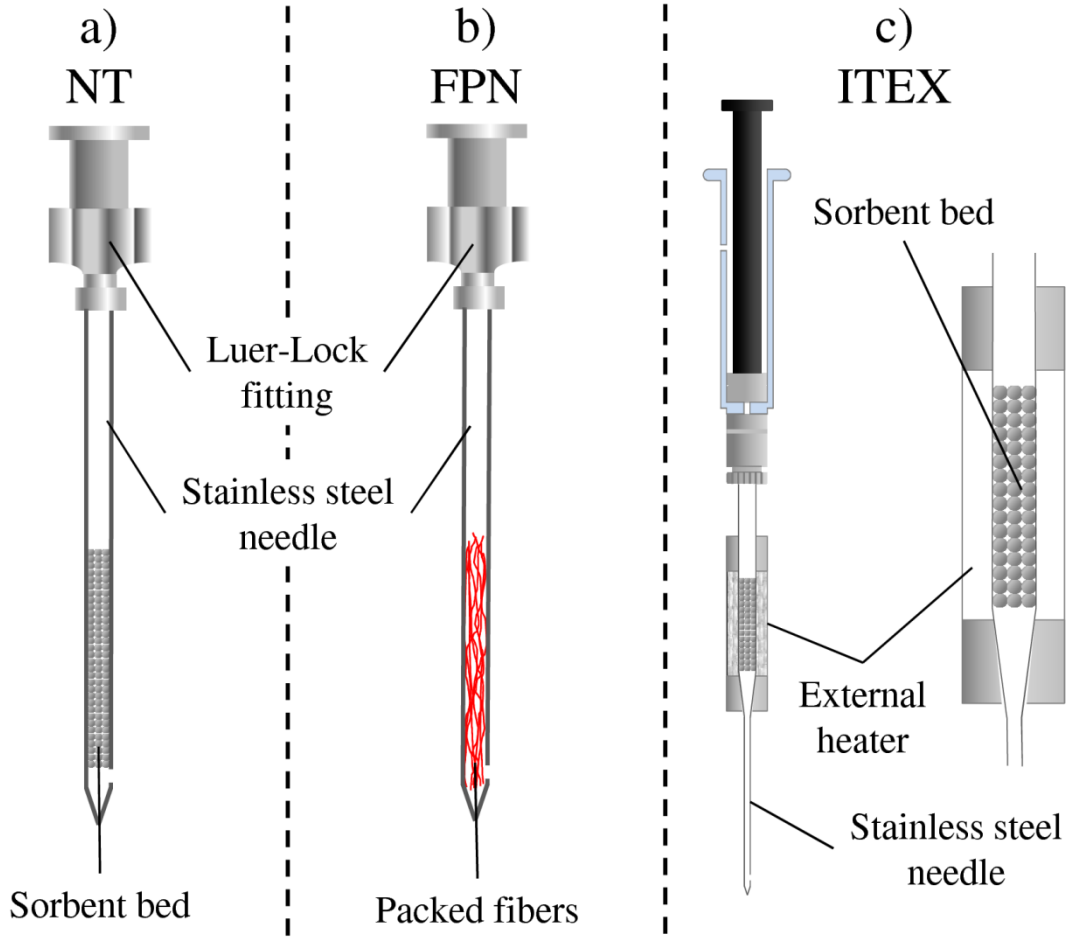

Figure 1.2 Common packed sorbent techniques: a) Needle trap, b) Fiber-packed needle, c) In-tube extraction

1.1.2.1 Needle Trap

The NT-device typically consists of a 22-gauge (0.72 mm *o.d.*, 0.41 mm *i.d.*) stainless steel needle with a conical tip and side port hole, but other needle diameters (e.g. 23-gauge, 0.34 mm *i.d.*) and tip configurations are also possible. The sorbent bed is held in position either by a quartz wool- or spiral steel-plug and the bed length can vary between 7 mm[73] and 30 mm[76], resulting in bed volumes of 0.6 µL to 4 µL. Most applications (**Table 1.2**) use standard sorbents like Carbopack X, Carboxen 1000, Tenax, DVB or HayeSep Q, while some investigate new materials like copolymers of methacrylic acid/ ethylene glycol dimethacrylate[77, 78], gold wire for amalgamated mercury extraction[79], nanosilica[80] or carbon nanotubes[81, 82]. The fiber-packed needle is a peculiar implementation of the NT principle,

utilizing PDMS coated filaments as sorbent material, which allows sampling from the liquid phase, too.

The NT has been promoted for exhaustive sampling (sampling mode (i)) since its presentation, while sampling modes (ii) and (iii) should be continued using SPME, which was introduced by the same group. Consequently, about two thirds of the publications on the NT-device use exhaustive sampling, which can be performed in the field with syringe pumps or vacuum sampling systems. In this way the device may take advantage of the automated exchange of field loaded needles by an autosampler back in the laboratory. Only few applications are using sampling modes (ii) and (iii). Applications and fundamentals of NT-devices have also been discussed in several reviews.[6, 83]

Table 1.2 Applications of sorbent packed needle techniques

Method	Sorbent	Matrix	Analytes	LOD[a]	LOQ[a]	Ref.
Needle Microconcen-trator	-	Tobacco smoke	Benzene, toluene	-	-	[72]
NT	None (quartz wool filter)	Airborne particles and aerosols	PAHs	-	-	[84]
NT	Carboxen 1000	Gas	BTEX	0.23 ngL^{-1}	-	[73]
NT	Methacrylic acid/ ethylene glycol dimethacrylate	Gas	Acetone, ethyl acetate, hexane, toluene	-	-	[77]
NT	Carbopack X	Water	BTEX	50 ng L^{-1}	80 ng L^{-1}	[85]
NT	DVB, Carboxen 1000, Tenax, Davison silica gel	Gas	BTEX, C_6-C_{15}	-	-	[86]
NT	DVB, Carboxen 1000	Gas	BTEX	-	-	[87]
NT	DVB	Water	BTEX	1 µg L^{-1}	-	[88]
NT	DVB	Water	Formic acid, acetic acid	87.2 µg L^{-1}	-	[89]
NT	Carboxen 1000	Gas	BTEX	0.6 µg L^{-1}	2.02 µg L^{-1}	[90]
NT	Gold wire	Gas	Mercury	0.2 pg m^{-3}	-	[79]
NT	DVB	Mosquito coil smoke, airborne particles	VOCs	-	-	[91]
NT	Carboxen 1000/ Carbopack X/ Tenax	Breath gas	Breath biomarkers	1.9 ng L^{-1}	-	[76]
NT	Carboxen 1000/ Carbopack X/ Tenax	Breath gas	Breath biomarkers, propofol	-	-	[92]
NT	Carbopack X	Water	BTEX	10 µg L^{-1}	-	[93]
NT	DVB/ Carboxen	Aerosols, smoke	PAHs	-	-	[94]
NT	Carbon nanotube-sol-gel	Water	PAHs	1 ng L^{-1}	-	[81]
NT	Oleic acid grafted nanosilica	Water	PAHs	2 ng L^{-1}	-	[80]
NT	Carbopack X, Tenax TA	Gas	BTEX, VOCs	2 ng L^{-1}	7 ng L^{-1}	[95]
NT	Carboxen 1000/Tenax TA	Water	BTEX, VOCs	10 ng L^{-1}	70 ng L^{-1}	[96]
NT	DVB/Carboxen 1000	Pump oil, solid PAHs	BTEX, PAHs	-	-	[97]
NT	DVB/Carbopack X/Carboxen 1000, PDSM/Carbopack X/Carboxen 1000, DVB/Carboxen 1000,	Gas	C_1-C_{10} aldehydes, BTEX, VOCs	0.22 ng L^{-1}	0.74 ng L^{-1}	[78]

Method	Sorbent	Matrix	Analytes	LOD[a]	LOQ[a]	Ref.
	PDMS/Carboxen 1000, methacrylic acid/ ethylene glycol dimethacrylate copolymer					
NT	Haysep Q	Gas	Bed bug related pheromones	-	-	98
NT	Carbopack X/Carboxen 1000	Gas	Breath VOCs	-	-	99
NT	DVB	Water	Formic acid, acetic acid	-	-	100
NT	Carboxen 1000/Tenax TA	Blood	BTEX, VOCs	0.02 µg L^{-1}	0.2 µg L^{-1}	101
NT	Carboxen 1000, DVB	Gas	BTEX,	-	-	102
NT	Single wall carbon nanotubes/silica composite	Gas	Halogenated VOCs	1 ng L^{-1}	5 ng L^{-1}	82
NT	DVB, DVB/Carboxen 1000	Garlic	Derivatized thiols	11 µg L^{-1}	0.1 µg L^{-1}	103
NT	Methacrylic acid/ ethylene glycol dimethacrylate copolymer	Breath gas	n-C_1-C_{10} aldehydes	-	-	104
NT	Tenax TA/Carbopack X/Carboxen 1000	Breath gas	Volatile blood and breath constituents	0.012 nmol L^{-1}	-	105
NT	Polystyrene/DVB	Water	Phenolic compounds	-	0.4 µg	106
NT	DVB	Water	Formic acid, acetic acid	1 mg L^{-1}	-	107
NT	DVB	Ground coffee	Coffee aroma compounds	-	-	108
NT	Multi walled carbon nanotubes/silica composite, PDMS	Gas	Volatile organohalogen compounds	0.01 µg L^{-1}	-	109
NT	DVB/Shincarbon ST/carbon molecular sieve	Water	BTEX, volatile organohalogens	0.01 µg L^{-1}	0.03 µg L^{-1}	110
NT	DVB/Carboxen 1000	Gas	Limonene, α-pinene, acetone	4 ng L^{-1}	12 ng L^{-1}	111
NT	Tenax TA	Gas	Insect pheromones	3 ng m^{-3}	-	112
FPN	PDMS	Gas	Aldehydes	1.2 ng L^{-1}	3.6 ng L^{-1}	113
FPN	PDMS	Gas	Smoking related volatiles	1.2 ng L^{-1}	-	114
LP-FPN	PDMS, 50%-Phenyl/PDMS	Water	PAHs, phthalates	-	-	115
LP-FPN	PDMS	Water	Bisphenol A	-	-	116
FPN	PDMS	Gas	Ethylene oxide	1.8 ng L^{-1}	5.4 ng L^{-1}	117
LP-FPN	PDMS	Water	Alkylbenzenes (C_6-C_{12}), *n*-alkanes (C_8-C_{12})	-	-	118
INCAT	Porapack Q/aluminum oxide	Water	BTEX	19 ng L^{-1}	52 ng L^{-1}	119

[a] only the lowest limit of detection/quantification is given for mixtures of multiple analytes

1.1.2.2 In-tube Extraction

The ITEX trap features a 30 mm high sorbent bed, which is fixed between two quartz-wool plugs in a 54 mm long tube with 2.6 mm *i.d.*; the resulting bed volume of about 160 µL is much higher than for in-needle techniques. The tube is combined with a 41 mm long and 0.72 mm *o.d.* needle with conical tip and side port for septum penetration and surrounded by an electric heating system for thermal desorption. Typically, the tube will be connected to a gastight syringe, placed in an autosampler, allowing full automation of sampling mode (ii), while the other modes are less favorable and manual operation is not intended.

The specialization on one sampling mode and the larger amount of extraction phase, which results in a higher extraction capacity and sensitivity, make ITEX a good tool for the headspace analysis of volatile to semi volatile compounds in trace concentrations. This is also reflected in the published applications, which cover environmental, food and forensic analysis (**Table 1.3**); the number is still limited, because ITEX is a relatively new technique. The achievable detection limits for the analysis of BTEX and halogenated VOCs are in the range of purge and trap systems, which are more complex and prone to contamination by sample matrix.[75, 120] *Zapata et al.* developed a method, which uses very small sample amounts (20 µL of 1:10 diluted sample) to achieve quantitative trapping of beer and wine aroma compounds without saturation of the sorbent by ethanol.[121] The method was also adapted for multiple headspace (MHE) analysis of wine samples.[122] *Rasanen et al.* presented a method for the analysis of methyl derivatives of glycolic- and formic acid, which can be used as markers for an ethylene glycol or methanol poisoning, even when the toxic alcohols are no longer detectable, due to delayed sampling after the ingestion.[123] Owing to the novelty of the technique, it is discussed in few reviews.[83, 124, 125]

Table 1.3 Applications of sorbent packed tube techniques

Method	Sorbent	Matrix	Analytes	LOD[a]	LOQ[a]	Ref.
Cylindrical Microconcen-trator	-	Tobacco smoke	Benzene, toluene	-	-	72
ITEX	Tenax TA	Water	BTEX, halogenated VOCs	29 ng L^{-1}	-	75
ITEX	Tenax TA	Blood, Urine	Ethylene glycol, glycolic acid, formic acid, VOCs	3 mg L^{-1}	10 mg L^{-1}	123
ITEX	⅔ Tenax GR/⅓ Carbosieve S III	Water, soft drinks	BTEX, halogenated VOCs	1 ng L^{-1}	-	120
ITEX	Tenax TA	Petroleum source rock extracts	Aliphatic hydrocarbons	-	-	126
ITEX	Tenax TA	Blood, Urine	Formic acid	-	-	127
ITEX	Tenax TA	Torreya grandis extract	Odor compounds	-	-	128
ITEX	Bond Elut ENV	Beer, wine	Acetaldehyde, ethyl acetate, ethyl propanoate, diacetyl, ethyl butyrate, isobutanol, isoamyl acetate	5 µg L^{-1}	-	121
MHE-ITEX	Tenax TA, Bond Elut ENV	Wine	Aroma compounds	10 ng L^{-1}	-	122
ITEX	Tenax TA	Sea buckthorn berries, juice	Aroma compounds	-	-	129
ITEX	Multi walled carbon nanotubes	Water	BTEX, naphthalene	2 ng L^{-1}	-	130

[a] only the lowest limit of detection/quantification is given for mixtures of multiple analytes

1.1.3 Bulk Sorbent Techniques

A low-cost alternative to other microextraction techniques is the use of silicone rods (SR) or tubes (ST), which can be acquired in different qualities from several suppliers by the meter. The benefits of PDMS for this application are the stability towards water, temperature and a broad range of organic solvents. While standard SRs and STs can contain filler materials like silicic acid esters, phenyl-vinyl-methyl polysiloxane or chalk[20, 131], pure PDMS rods tailored especially for analytical use are also available (e.g. Sorb-Star by IMT GmbH, Vohenstrauß, Germany; SR with 20 mm length and 2 mm diameter).

Detailed information an SR and ST extraction can be found in a comprehensive review by *van Pinxteren et al.*.[20] The standard tubing or rods are cut to the desired length and then weighted, to assure a constant extraction phase volume, which is for typical applications between 8 µL and 635 µL, but extremes of 14.7 mL have also been used.[132] Before analytical use, the material has to be cleaned by solvent rinsing or thermal bake-out, to remove impurities from production and storage or to avoid carryover between measurements. The application is similar to SBSE or HSSE, with extraction mainly direct from the liquid matrix, but headspace extractions are also performed. Sample injection is performed either by thermodesorption in the GC or by solvent back-extraction and liquid injection with GC-compatible solvents. Because of the low cost of SRs and STs and the easy identification of PDMS degradation products by mass spectrometry, long-term stability is less important, also allowing the use of solvents causing considerable swelling of the silicone.

Prieto at al. compared the performance of polyethersulphone (PES), polypropylene and Kevlar as alternative low cost materials with PDMS and found PES to have better extraction efficiencies than PDMS for both polar and non-polar compounds.[133]

1.2 Fundamental Principles of Microextraction Techniques

Three main subjects have to be considered regarding the aforementioned microextraction techniques: the sampling system, the sorption type (mechanism and sorbents) and the sampling strategy. The sampling system may consist of two or three phases, the sorption mechanisms for GC applications typically are either adsorption or absorption and the sampling strategies can be distinguished by static and dynamic sampling. The characteristics of these three subjects will be discussed in the following.

1.2.1 Sampling Systems

The simplest possible system for microextraction techniques is the 2-phase system, where the sorbent is brought in direct contact with the sample matrix and analyte distribution is limited to these two phases. This applies for gas analysis and liquid phase sampling, while direct extraction from solids is unpractical due to the small contact area and slow diffusion. In a 3-phase system, the analytes distribute between the sample phase and the gas phase and between the gas phase and the sorbent. This procedure, usually referred to as headspace sampling, is limited to solid and liquid samples, because two gas phases would intermix and therefore again result in a 2-phase system. Typical combinations of sampling systems and common microextraction techniques can be found in **Table 1.4** and **Figure 1.4**. A special case is the headspace analysis of solid, adsorptive samples, where the analyte release to the gas phase can be slow and concentration dependent. This system can be changed to a partitioning system, with extended linear range, by the addition of a suitable solvent which can act as modifier/displacer. This is called *surface modification method*, when the amount of solvent is small enough that only the surface of the sample is wetted or *suspension approach*, when the solvent amount is large enough to separate from the solids. In this case, we have a 4-phase system, with the solid phase from which the analytes have been eluted and which usually has no further influence, the new liquid phase containing the analytes, the gas phase and the extraction phase.[134]

Table 1.4 Typical microextraction techniques for different sampling systems

	2-Phase system	**3-Phase system**
Gaseous sample	NT, SPME	-
Liquid sample	SPME, SBSE, SR/ST, SPDE, FPN	SPME, HSSE, SR/ST, SPDE, NT, ITEX
Solid sample	-	SPME, HSSE, SPDE, NT, ITEX

1.2.2 Sorption

1.2.2.1 Adsorption

Adsorption relies on interactions of active groups of the sorbent material with analyte molecules, which can vary from weak *van-der-Waals* forces to strong ionic interactions, depending on the sorbent and analyte combination. Diffusion of analyte molecules into the material is restricted by the glassy or crystalline structure and can be neglected for analytical use, limiting the available active sites to the sorbent surface. To compensate this, adsorbents usually are manufactured as porous materials to gain a high specific internal surface area, which can be over 1000 $m^2 g^{-1}$ for activated carbons. The limitation of sorption sites can lead

to problems in quantitative analysis under equilibrium conditions, when the analyte mass is high (either due to too high concentration or too large sample amount). The sorption isotherm is non-linear and competition of different compounds for sorption sites may occur, which can cause the displacement of weak binding molecules by stronger retained ones (target or matrix compounds). This leads to variations in the extracted analyte amounts in equilibrium and hence to wrong quantification results.[135] To avoid this, adsorbent materials are often used in non equilibrium breakthrough sampling conditions, which will be discussed in **Section 1.2.3.2**.

The standard adsorbents used for thermal desorption in microextraction techniques can be categorized in two groups, inorganic carbon based materials and porous organic polymers. The first group can be subdivided into activated carbon, carbon molecular sieves and graphitized carbon blacks, which differ in their structure and functional groups. The surface of activated carbon possesses several functional groups like hydroxyl-, carbonyl-, and carboxylic functions, which allow non-specific and specific interactions,[136] but polar analytes like alcohols might be irreversibly adsorbed by hydrogen bonds.[137] Carbon molecular sieves consist of amorphous carbon and layers of condensed aromatic rings, which gives them a well defined pore size distribution and high specific surface area. Although the surface can contain traces of metals and salts, adsorption is mainly based on non-specific interactions and they can be used to trap highly volatile hydrocarbons and even methane, but they are not suitable for the sampling of reactive analytes, which can be degraded on the catalytic surface.[138-140] Graphitized carbon blacks are formed of planar graphite layers, which results in a lower specific surface area of about $5 - 500\ m^2\ g^{-1}$ and lower sorption strength. They are typically used for the analysis of $C_3 - C_{20}$ hydrocarbons. Adsorption is caused by *van-der-Waals* forces (dispersion and induction) and their hydrophobicity allows sampling of VOCs in humid conditions without additional drying agents.[141] A common feature of these inorganic carbon materials is that they can withstand desorption temperatures of more than 400 °C without significant degradation.

The most used porous organic polymers in microextraction applications are DVB copolymers (sometimes under the trade names Chromosorb or Porapak) and Tenax TA. They consist of polymeric building blocks and are very pure substances, due to the controlled manufacturing process. A negative side effect of this is the limited temperature stability (compared to inorganic adsorbents) and possible depolymerisation, which results in increased background signals in chromatography; Tenax for example is known to produce artifacts like aldehydes

and ketones.[139] The specific surface area is also smaller than for activated carbon or carbon molecular sieves, especially for Tenax TA with only 35 $m^2 g^{-1}$. The polymers are therefore best used to sample hydrocarbons from C_7 upwards, or in combination with stronger sorbents to enlarge the breakthrough volume.

An overview of common sorbent materials for thermal desorption is given by *Dettmer and Engewald*[139] and new materials for the sampling of polar compounds are discussed by *Fontanals et al.*[21].

1.2.2.2 Absorption

In absorption, the analyte molecules partition into the extraction phase and can diffuse into the whole volume of the extractant during the extraction time, when the film thickness or particle size is small enough. The analytes are solvated in the extraction phase like in an organic solvent, without competition or displacement effects and the equilibrium conditions do not vary, until the extracted amount is large enough (a few percent of the sorbent mass) to modify the properties of the sorbent phase.[3] This is hardly the case for analytical purposes, as enrichment techniques are typically used for trace analysis. Absorptive interactions are weaker than adsorption on active surfaces, which makes the trapping of highly volatile analytes difficult; on the other hand, this allows lower desorption temperatures and shorter desorption times, which minimizes the degradation of unstable analytes [20, 139] and reduces sorbent degradation.

Absorbents are polymeric materials which are used above their glass-liquid transition temperature (T_g). While they are hard and brittle below this temperature, they change to a rubbery, liquid like state above it, allowing the analytes to partition into the material. The most used partitioning material for analytical purposes is PDMS, which is not only used in many microextraction techniques, but also as stationary phase in capillary columns for gas chromatography. It possesses several beneficial characteristics: the glass transition temperature is very low (T_g = -127 °C), it is highly hydrophobic and shows little swelling in water, it is inert to many chemicals and degradation products are easily identified by mass selective detectors.[20, 131] The selectivity can be adjusted with additives like phenyl or cyanopropyl-phenyl (often named 1701) for INCAT and SPDE[44] or is used as support for adsorbent materials like activated carbon, Carboxen or DVB in SPME. Another absorbent for the extraction of more polar analytes is PA, which is used as a crystalline coating in SPME, that turns liquid at desorption temperatures. As the exact type of the used PA polymer is not

publicly available, no information on the T_g can be given, because values ranging from -36 °C to 120 °C can be found in literature for different side chains. Despite the crystalline structure, the primary extraction mechanism is absorption, but the diffusion coefficient in PA is about one order of magnitude lower than for PDMS.[135] A third absorbent, which is also popular for the analysis of polar compounds, is PEG. There is also no information on the molecular weight of the PEG used in coatings for SPME, SPDE and SBSE, but the maximum T_g within a range of molecular weights from 10^1 to 10^6 is -17 °C[142] and the melting point is between 50 °C and 67 °C for molecular weights from 4000 to 20000. Because of the low melting point and good water solubility, PEG is often embedded in PDMS, to avoid the detachment of the coating.

1.2.3 Sampling Strategies

1.2.3.1 Static sampling

In static sampling, the whole amount of sorbent and sample are brought together at the start and remain unaltered until the end of the extraction time. The analytes will then begin to distribute between extractant and sample phase towards equilibrium. The equilibrium concentrations are defined by the distribution constant, which is K_{ES} in the case of absorption:

Equation 1.1

$$K_{ES} = \frac{C_E}{C_S} = \frac{m_E}{m_S} \times \frac{V_S}{V_E}$$

where C, m and V are the analyte concentration, mass and phase volume, with the indices E for the extractant and S for the sample phase, respectively. The distribution constant K_{ES}^{S} for adsorption is defined as:

Equation 1.2

$$K_{ES}^{S} = \frac{S_E}{C_S}$$

with S_E being the adsorbent surface concentration of adsorbed analytes.[3] The distribution constants reflect the physicochemical composition of the extraction phase and can be determined chromatographically or by estimation with polyparameter-linear free energy relations (pp-LFERs).[143] As thermodynamic constants, they depend on, e.g., temperature, pressure, sample pH and salt content; but they are not affected by mixing procedures like shaking or stirring, which are often applied to speed up the extraction process.

When the sampling time is sufficiently long, equilibrium will be reached (although the extraction is often stopped before, to save time) and the resulting mass of extracted analyte in a 2-phase system can be calculated according to:

Equation 1.3

$$m_E = \frac{C_0 V_E V_S K_{ES}}{V_E K_{ES} + V_S}$$

where C_0 is the initial concentration of the analyte in the sample. A more convenient way to estimate the extraction efficiency is to calculate the analyte recovery:

Equation 1.4

$$R = \frac{m_E}{m_0} = \frac{1}{\frac{\beta}{K_{ES}} + 1}$$

with m_0 being the initial analyte mass and β the phase ratio V_S/V_E. Following **Equation 1.4**, only two parameters control the recovery, the distribution constant and the phase ratio. As mentioned before, the distribution constant is depending on the analyte, the extractant and the sample properties; when no suitable extractant with a higher K_{ES} is available, the biggest potential to increase the recovery, is a decrease of the phase ratio. This can be achieved either by the use of a lower sample volume, which also results in lower sensitivity of the method, or with more extractant. This is exemplarily depicted in **Figure 1.3**, where the achievable recovery of three environmental contaminants is calculated for three microextraction techniques utilizing different volumes of PDMS.

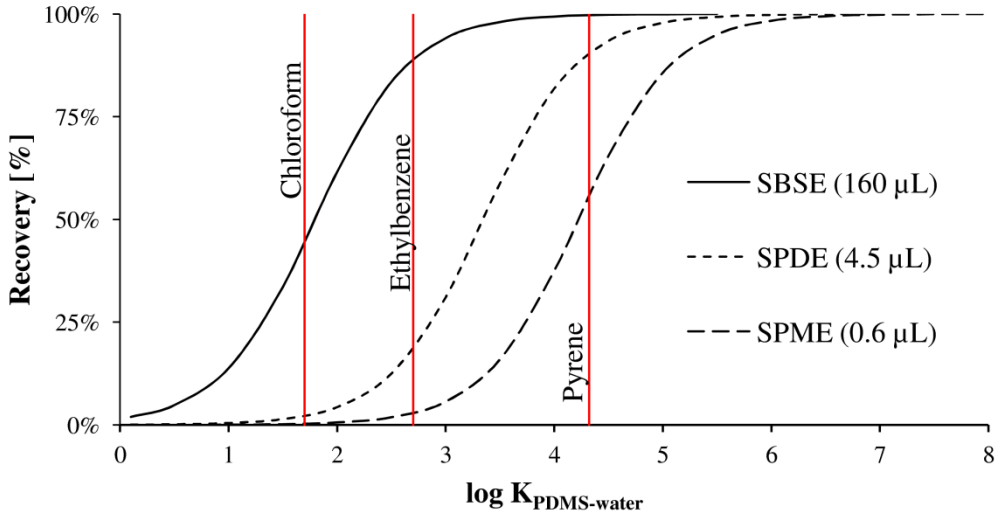

Figure 1.3 Calculated recoveries for the extraction of chloroform, ethylbenzene and pyrene from 10 mL sample volume with three microextraction techniques and their typical PDMS volumes; the log $K_{PDMS\text{-}water}$ of each compound is marked by a red line, where the recoveries can be read at the intercepts with the curves of the extraction techniques

An additional phase transition with a corresponding distribution has to be considered in the 3-phase system of a headspace extraction. This can be achieved by the expansion of **Equation 1.3** with the headspace to sample distribution constant K_{HS} and the headspace volume V_H:

Equation 1.5

$$m_E = \frac{C_0 V_E V_S K_{ES}}{V_E K_{ES} + V_S + V_H K_{HS}}$$

In most analytical applications the condensed sample phase will be an aqueous sample, where K_{HS} is identical to the air-water partitioning constant K_{aw}, which can be found in literature for many compounds.[144, 145] **Equation 1.5** can be further expanded in the same way for any number of phases, if necessary. One problem in headspace analysis is the opposite effect of sample temperature on the distribution equilibria of headspace to sample and extractant to headspace; while K_{aw} rises with increasing temperature, K_{EH} will typically decrease, making it important to find the optimal temperature, where both effects are well balanced.

The kinetics of static sampling is limited by the diffusion of the analytes in the different phases and the phase transition. While diffusion in the gas phase is usually uncritical, shaking or mixing of a liquid phase can be applied to minimize boundary layers between liquid and sorbent or liquid and headspace, to speed up the extraction procedure.

1.2.3.2 Dynamic Sampling

In dynamic sampling, opposed to static sampling, only parts of the sample are in contact with the extractant at any given time and the fraction in contact with the extractant is exchanged perpetually. The dynamic sampling itself can be differentiated in open system and closed system sampling (see **Figure 1.4 b), d) and e)**). The closed system sampling is applied by some microextraction techniques, where parts of the sample are withdrawn with a syringe and in this way pumped over an extractant film (SPDE) or through a sorbent bed (ITEX, NT), before it is re-injected to the sample reservoir and another fraction is withdrawn (**Figure 1.4 d)**). Dynamic sampling in a closed system will result in the same equilibrium conditions as described for static sampling in **Section 1.2.3.1**, although the sampling process is often stopped before equilibrium will be reached.

The "classic" dynamic sampling uses an open system, where a liquid or gaseous sample is pumped through a packed sorbent bed and is discarded afterwards. For liquid and solid samples, there are also methods that use a purge gas to exchange the headspace above the solution (dynamic headspace) or bubble the gas through the sample matrix to strip the analytes to the gas phase before trapping (purge and trap, **Figure 1.4 e)**). The typical

microextraction technique to apply to this open sampling system is the NT, where the sample can also be pulled through the sorbent bed by a syringe, without a following dispensing step (**Figure 1.4 b)**).

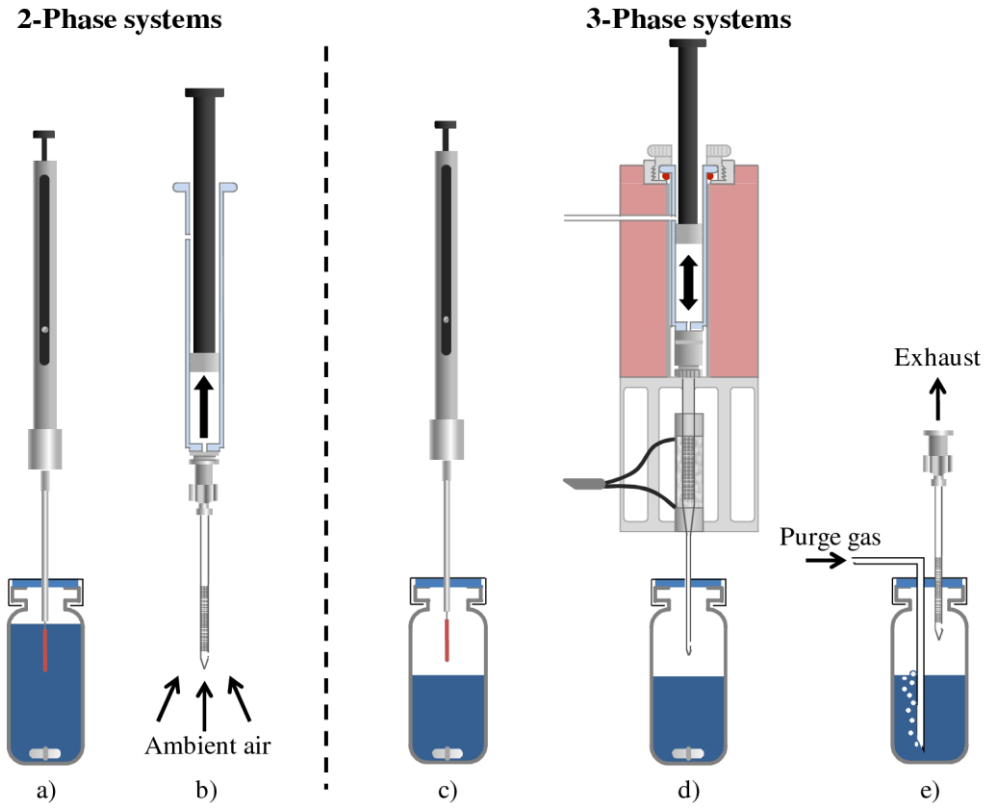

Figure 1.4 Examples for different sampling systems: a) liquid phase sampling with SPME (static sampling), b) ambient air sampling using NT (dynamic sampling, open system), c) HS-SPME (static sampling), d) ITEX (dynamic sampling, closed system) and e) liquid sampling with NT and purge gas (dynamic sampling, open system)

Technically, open system dynamic sampling resembles frontal gas-solid chromatography, where a constant stream of analyte is introduced to the trap, until the extractant is saturated. At the start, when the trap is unloaded, all analytes will be trapped and the concentration in the outflow will be zero. With longer sampling time and more sampled volume, the extractant at the entrance of the trap will get saturated and the concentration of the analyte front will move through the bed like the integral of a Gaussian peak, until it reaches the end of the trap, when a breakthrough will occur and analyte is lost (see **Figure 1.5**, sampling times t_1 to t_3).[146-148] The shape of the front may be different in short traps with low plate numbers, like they are typical for the sorbent beds of microextraction techniques and a correction following *Lövkvist and Jönsson*[147] should be applied. The analyte loss can either be calculated as a percentage of the initial concentration C_0 (differential breakthrough) or as a predetermined amount of analyte mass (integral breakthrough), where the tolerated amount has to be chosen by the user. More information on breakthrough sampling and the calculation of safe sampling

volumes, where no analyte loss occurs, can be found in literature[139, 149], also considering especially needle trap devices[83]. A possible sampling strategy for absorbent type extractants, where no competition and displacement occurs, is to continue sampling until the analytes in the sample stream are in equilibrium with the analytes in the sorbent (equilibrium sampling, see **Figure 1.5**, sampling time t_4).[150]

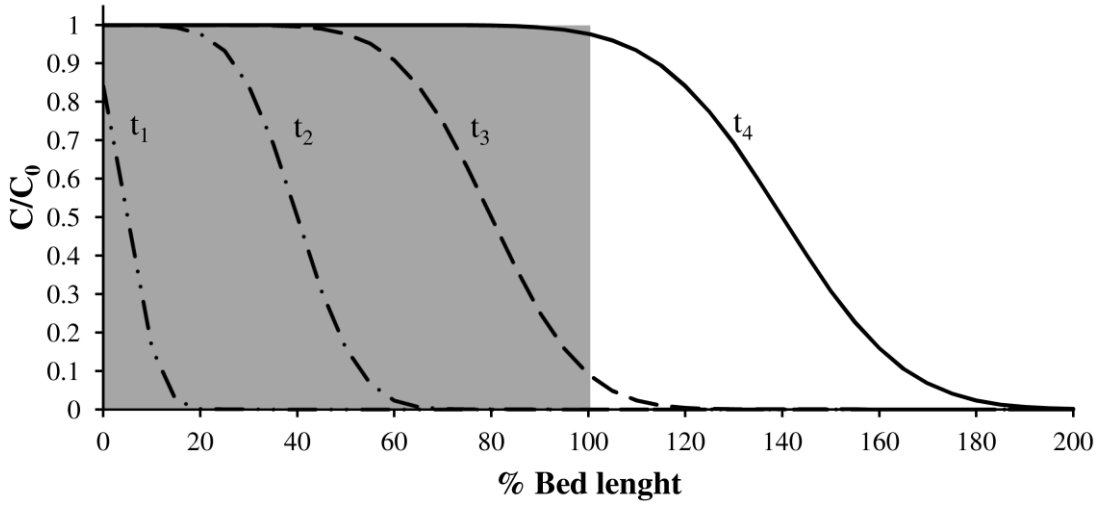

Figure 1.5 Schematic view of the theoretical analyte front in a sorbent bed at four consecutive sampling times t_1 to t_4; 10 % differential breakthrough is reached at t_3, saturation/equilibrium at t_4

1.3 Scope of this Thesis

This thesis focuses on the development and evaluation of new applications for novel, fully automated microextraction techniques. Chapter 2 describes a SPDE method used to build a fingerprinting database of 196 German red wines, predominantly from the vintage of 2006. Furthermore, a quantification and performance evaluation of the method for 22 flavor-relevant aroma compounds and an observation of the long term performance of the extraction needle were conducted.

The following chapters present ITEX methods from different analytical fields. Chapter 3 describes the optimization of extraction parameters for the analysis of regulated environmental contaminants and two unwanted odor compounds from aqueous samples. The applicability is tested with water samples of differing origins and several soft drinks. A method for the analysis of aroma relevant alcohols and esters from beer aroma is presented in Chapter 4. It uses a similar, but enlarged, compound set as the SPDE method discussed in Chapter 2 and the performance of both extraction techniques is compared regarding sensitivity and precision. Finally, 45 beers of different makings (Pilsner, Altbier, Wheat beer, Kölsch, Helles and Schwarzbier), also including alcohol free variants, are analyzed and discriminated by linear discriminant analysis. A summary of the importance of the different optimization steps, together with recommendations for more efficient method development, which is based on experiences made through the time of this thesis, are given in Chapter 5.

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2 Fingerprinting of Red Wine by Headspace Solid Phase Dynamic Extraction of Volatile Constituents

This chapter has been published in modified form in *Laaks, J.; Letzel, T.; Schmidt, T. C.; Jochmann, M. A., Fingerprinting of red wine by headspace solid-phase dynamic extraction of volatile constituents. Analytical and Bioanalytical Chemistry 2012, 403, 2429-2436,* © Springer-Verlag 2012

2.1 Introduction

Wine is considered to be the alcoholic beverage with the greatest variation in flavor[1]. The aroma is a complex mixture of several hundred compounds[1, 2] and over 800 volatiles have been identified in wines[3-6]. The requirements in wine analysis are an extensive and artifact free enrichment, an effective separation by means of gas chromatography and the identification of important and characteristic compounds[7]. Especially S-HS methods without pre-concentration have been used for the analysis of the volatile wine fraction, but low sensitivity, as well as the large concentration range in which different important compounds occur, restrict the application of S-HS methods considerably[8].

Microextraction methods such as SPME fulfill the mentioned demands and have been successfully employed in different aspects of wine analysis such as screening of aroma compounds[2, 3, 5, 9-13], analysis of methoxypyrazines[14, 15], sulphur compounds,[16-19] oak barrel storage related compounds[20] and in wine discrimination[6, 21-23]. SPME has further advantages over more traditional extraction techniques: it is inexpensive, relatively fast, easy to automate with conventional auto samplers, requires low sample volumes, gives high sensitivity and good reproducibility.[24-26] Despite these advantages, SPME itself suffers from certain disadvantages: mechanical damage of the coating due to scraping and needle bending during agitation, limited flexibility in fiber length and coating thickness resulting in low amounts of stationary phase and the limited fiber lifetime.[11, 24, 27-29]

The film thickness is the major leverage to increase the sorption capacity of SPME, but with greater film thicknesses up to 100 µm also the extraction takes longer, diminishing one of the advantages of SPME. To overcome this problem, a thin film has to be spread over a larger area, leading to a larger extraction phase volume, combined with short extraction times, due to faster phase transfer[30]. This was achieved by placing a short length of capillary GC column into a steel needle or by directly coating the inner wall of the needle. Typically the needle is mounted on a 2.5 mL-headspace syringe. Early implementations have been called INCAT[31, 32]. In the year 2000, Chromtech (Idstein, Germany) commercialized the in-needle trapping as

the "magic needle" or SPDE[29, 33]. The sampling can be carried out from the headspace, whereby the analytes are accumulated in the coating by aspirating and dispensing a defined sample volume for several times. Thermal desorption is carried out in the GC injection system, after aspiration of a desorption gas (helium or nitrogen), either from a gas station or directly from the injection system (see **Figure 2.1**).

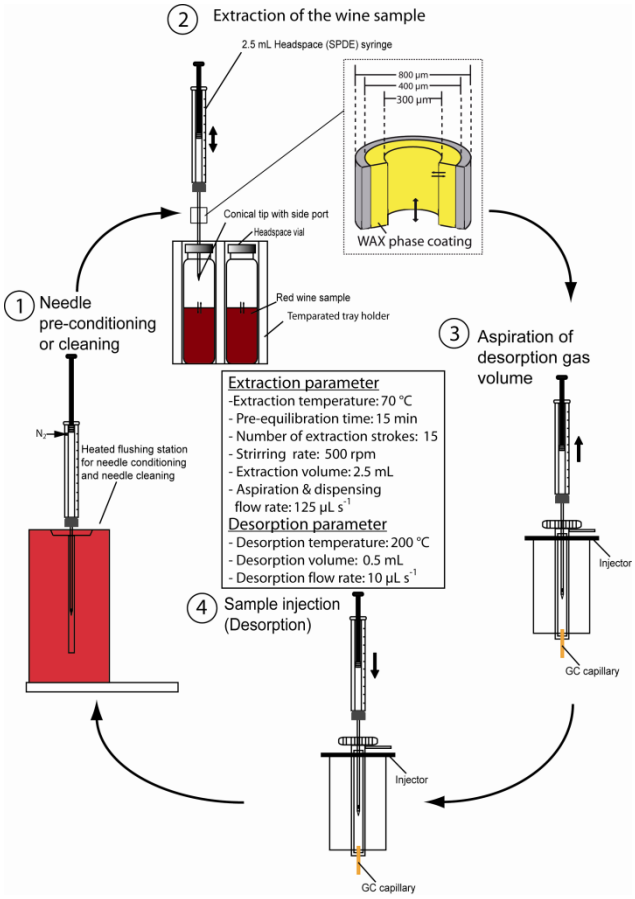

Figure 2.1 Depiction of the SPDE-procedure. 1) Conditioning of the needle before the first use and cleaning after each analysis; 2) headspace extraction of the sample by several extraction strokes; 3) aspiration of desorption gas volume; 4) thermal desorption in the GC injector.

Contrary to equilibrium sampling, under the conditions used here SPDE is a non-equilibrium sampling method and must be carried out with invariable extraction parameters (aspirating and dispensing flow, sampling volume) to obtain reliable results[8]. However, nowadays this can easily be achieved with programmable xyz-autosamplers. The main advantages of SPDE over SPME are the higher sorption capacity, due to the larger amount of stationary phase (about 5-6 times[8, 27, 29, 33-35]); the faster extraction, because of the active pumping, instead of relying only on diffusion[35] and the mechanical stability[33]. SPDE has been applied to a wide scope of target compounds: halogenated pesticides[30], chlorinated hydrocarbons[34], furan[36], BTEX[36-38], drugs of abuse[29, 33, 35] and different food matrices[27, 39]. Other examples, related to

wine analysis, are the quantification of fusel oils[40] and the identification of fermentation problems in wine musts[41].

The aim of this work was the application and evaluation of a headspace SPDE-GC/MS method in the analysis of German red wines. Therefore, 22 aroma active alcohols and esters were chosen as targets for quantification, which would also be used as reference compounds to create a fingerprinting database for authenticity control and similarity searches, based on their retention times, mass spectra and relative peak areas, respectively. Due to the large number of samples analyzed (overall, about 950 analyses were executed), the long term performance of the employed extraction needles was also investigated.

2.2 Experimental

2.2.1 Chemicals

As reference compounds for method validation and calibration standard solutions, 1-butanol (purity ≥99.9%), 2-butanol (≥99.8%), *tert*-butanol (≥99.8%), ethyl acetate (≥99.9%), 2-ethyl-1-hexanol (≥99.5%), ethyl octanoate (≥98%), 1-hexanol (≥99.9%), (*S*)-2-methylbutanol (≥99.5%, sum of enatiomers), 3-methylbutanol (≥99.8%), 2-methyl-1-propanol (≥99.8%), 1-pentanol (≥99.8%), 3-pentanol (≥99.5%), 1-propanol (≥99.9%) and 2-propanol (≥99.9%) were purchased from Fluka (Sigma-Aldrich, Steinheim, Germany). Ethyl decanoate (≥99%), ethyl hexanoate (≥99%), ethyl isovalerate (98%), linalool (≥97%), 3-methyl-1-pentanol (99%), 2-phenethyl acetate (99%) from Aldrich (Sigma-Aldrich); isopentyl acetate (≥99%) from Sigma-Aldrich and diethyl succinate (≥99%) from Merck (Darmstadt, Germany) were used. Ethanol absolute SPECTRANAL® (≥99.8%) from Riedel-deHaën (Sigma-Aldrich) was used for the preparation of stock solutions. Tri-distilled deionised water as solvent for standard solutions and sample dilution was attained from a lab unit (Westdeutsche Quarzschmelze, Geesthacht, Germany).

2.2.2 Instrumentation

All measurements were carried out with a Thermo Trace GC Ultra (S+H Analytik, Mönchengladbach, Germany) equipped with a CTC Combi PAL autosampler (AxelSemrau, Sprockhövel, Germany), holding a TrayCooler for 20-mL headspace vials, a SyrHS2.5mL heated syringe holder and a Single Magnet Mixer (SMM) (Chromtech, Idstein, Germany). 10 mL of each sample solution were transferred into a 20-mL amber headspace vial (BGB Analytik AG, Boeckten, Switzerland), with an 8x3 mm PTFE laminated magnetic stir bar (VWR International GmbH, Darmstadt, Germany). The vials were closed by magnetic screw

caps with rubber/PTFE septa (BGB Analytik AG) and stored in the TrayCooler at 25 °C, because longer storage (about one day) at the extraction temperature of 70 °C revealed to alter the composition of the wine samples. Sample extraction and injection were executed by the autosampler programmed with custom made macros. The HS-SPDE was carried out using a 56 mm long hollow needle with a 50 µm carbowax-coating (Chromtech) mounted on a 2.5-mL headspace syringe (Hamilton, Bonaduz, Switzerland). The syringe temperature was kept at 35 °C. Prior to analysis, the needle was pre-conditioned and flushed with nitrogen 4.0 (Air Liquide, Oberhausen, Germany) for 30 min in an Atas GL Optic 3 injector (Axel Semrau, Sprockhövel, Germany) at 200 °C. For the extraction, the samples were transferred to the SMM, which was set to 70 °C and stirred at 500 rpm for 15 min, to heat up and equilibrate. 15 extraction strokes of 2.5 mL were conducted with an aspiration and dispensing flow of 125 µL s^{-1}, respectively. Sample penetration for the extraction was 20 mm. Desorption was carried out in the Split/Splitless injector (S/SL) with a 5 mm inner diameter (*i.d.*) glass liner (Thermo Scientific; Idstein, Germany) at 200 °C. 500 µL helium 5.0 (Air Liquide) were aspirated as desorption gas from the S/SL with an injector penetration of 20 mm. The desorption flow was set to 10 µL s^{-1} with an injector penetration of 45 mm. The S/SL injector was used in splitless mode (1 minute splitless time) with constant septum purge; helium was used as carrier gas with a constant flow of 1.5 mL min^{-1}. After desorption, the needle was transferred to the Optic 3 and was flushed with nitrogen for 10 min at 200 °C, to prevent carryover. The whole procedure for each sample (including equilibration, extraction, injection and needle flushing) takes about 35 minutes and can be performed in parallel to the GC analysis, thus making the GC program the limiting factor. Separation of compounds was performed on a Stabilwax DA fused-silica capillary column (cross bonded carbowax-PEG (polyethylene glycol)) with 60 m length, 0.32 mm *i.d.* and 1 µm film thickness (Restek GmbH, Bad Homburg, Germany). Initial GC oven temperature was 40 °C for 1 min, to trap the analytes during desorption time and prevent peak broadening. After the desorption phase, the GC was heated with 7 °C min^{-1} to 110 °C, 3 °C min^{-1} to 130 °C and 7 °C min^{-1} to 160 °C with a hold time of 38 min. A Thermo DSQ II single quadrupole mass spectrometer (S+H Analytik) was coupled to the GC for sample detection. The MS transfer line and ion source temperatures were set to 220 °C. Electron ionization mode (EI) with an ionization energy of 70 eV was used in scan mode (m/z = 31-129) with a scan rate of 500 amu s^{-1}. Automatic tuning of the instrument was carried out regularly. Instrument automation, data acquisition and data processing were performed by the Xcalibur 1.4 data system (S+H Analytik).

2.2.3 Samples and Sample Preparation

196 samples of German red wines were supplied for quantification from the Analytical Research Group at the Chair of Biopolymer Chemistry of the Technische Universität München. The samples were delivered in 60-mL amber screw cap bottles, which were flushed with nitrogen and sealed with laboratory film, to prevent oxidation of sample compounds during transportation and storage. Because of the good sensitivity of the method, the wine samples were diluted by a factor of ten to decrease matrix, co-solvent and competition effects[3, 9, 12-14, 40]; no other sample preparation, except the addition of stir bars, was needed.

The required calibration ranges for all compounds were determined by the analysis of five randomly selected wine samples. The ethanolic stock solution, containing all target compounds in the previously determined concentration ratio, was prepared monthly and stored in vials without headspace in the dark at 4 °C. Aqueous calibration standards were prepared from the stock solutions every day. A six point calibration was performed with every set of samples, to monitor the long term stability of the SPDE needles.

2.3 Results and Discussion

2.3.1 Quantification

The Method detection limits (*MDL*s) were determined based on a guideline of the U.S. Environmental Protection agency and are calculated from the standard deviation of a sevenfold measurement at a signal to noise ratio of three to five, resulting in a 99% confidence that the analyte concentration is greater than zero[42]. *MDL*s are in a range from 0.02 µg L^{-1} for ethyl octanoate to 9.3 µg L^{-1} for 1-propanol. As the *MDL* are far below the required concentration range for the wine analysis, the samples were diluted by a factor of ten.

A chromatogram of a typical wine sample is shown in **Figure 2.2**. The begin of the chromatogram with the major compounds is depicted in the larger part, while the zoomed view gives insight to the high number of trace compounds, which are responsible for the richness of wine aroma. The amounts of the 22 quantified compounds ranged from about 1 µg L^{-1} of linalool up to 380 mg L^{-1} of 2-methyl-1-propanol, the ranges for each compound with the corresponding median are listed in **Table 2.1**. The major compounds besides ethanol are mainly fusel alcohols like 2-methyl-1-propanol, 2-/3-methylbutanol and 1-propanol, the main esters are ethyl acetate and diethyl succinate.

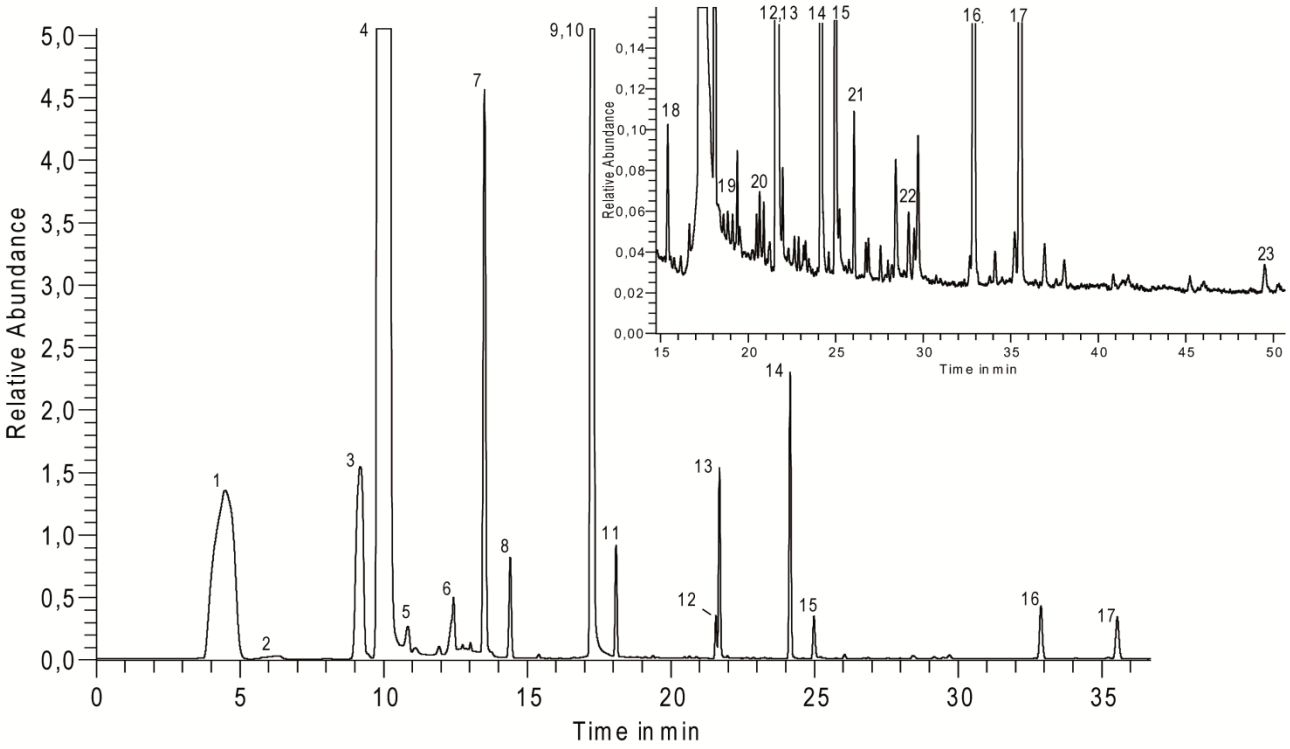

Figure 2.2 Exemplary wine chromatogram. main view showing 1) desorption gas, 2) Acetaldehyde, 3) Ethyl acetate, 4) Ethanol, 5) Ethyl isovalerate, 6) 1-propanol, 7) 2-methyl-1-propanol, 8) Isopentyl acetate, 9) 2-methylbutanol, 10) 3-methylbutanol, 11) Ethyl hexanoate, 12) 1-hexanol, 13) Ethyl lactate, 14) Ethyl octanoate, 15) Acetic acid, 16) Ethyl decanoate, 17) Diethyl succinate. The zoomed view shows lower concentration compounds 18) 1-butanol, 19) 1-pentanol, 20) 3-methyl-1-pentanol, 21) 2-ethyl-1-hexanol, 22) Linalool, 23) 2-phenethyl acetate.

2.3.2 Uncertainty budget of the method

The method error was calculated by propagation of uncertainty in a "top-down" approach[43], by summing up of the uncertainties of the used materials and instruments:

Equation 2.1

$$\boldsymbol{u(c_m) = \sqrt{u(c_{cal})^2 + u(c_s)^2 + RSD_x^2}}$$

where $u(c_m)$ is the total measurement uncertainty, $u(c_{cal})$ the uncertainty of the calibration, $u(c_s)$ the sample uncertainty and RSD_x the relative standard deviation of the extraction and analysis from standard solutions. The relative standard deviations of the samples (RSD_s) for each compound were calculated as the average of the relative standard deviations (RSD) of the 196 triplicate analyses. The results of the compound specific measurement uncertainty and RSD_s are summed up in **Table 2.1**.

The total measurement uncertainty ranges from 2.5% to 7.9%. While $u(c_{cal})$ is about 1% and $u(c_s)$ is only 0.4%, the major proportion of $u(c_m)$ is made up by RSD_x, which is higher for the compounds with retention times between 9 and 15 minutes. The elevated uncertainties in this range mainly originate from co-elution of low concentrated compounds with more abundant

ones and the chromatographic background composed of degradation products of PEG from the column or needle coating material, which will be discusses later.

The values of RSD_s are, in most cases, in good agreement with the calculated measurement uncertainties, indicating little additional matrix effects on the analytical method. However, some compounds exhibit a high RSD_s, because in several samples, *RSD*s of over 100% were observed, which seems to be caused by interferences between the target compounds and substances present in certain wine samples. In the case of 1-butanol, for example, some wines showed *RSD*s of up to 182%, while in most of the remaining samples the deviation for this compound keeps below 10%. Therefore, a one-sided Grubbs' test with a significance level $\alpha = 0.01$ was carried out to remove possible outliers. In other cases, like with ethyl isovalerate, ethyl decanoate, 2-methyl-1-propanol, 1-butanol, 2-ethyl-1-hexanol and 2-phenethyl acetate, wines from certain regions had interferences in the quantification, which could not be removed as outliers, because about 25 samples each have been affected, resulting in average RSD_s of 20% or more.

2.3.3 Fingerprinting Analysis

The fingerprinting database was built using the software MSChromsearch (Axel Semrau), which allows a manual peak by peak comparison of two chromatograms (**Figure 2.3**). Each peak receives a matching score by retention time (R_t), retention index (R_i), relative peak area and height and by comparing their mass spectra with each other (in case of the chosen target compounds also with a previously stored reference spectrum), indicating the similarity of both peaks. Based on the same scoring system, libraries of reference chromatograms can be compiled for automated chromatogram similarity searches, likewise to common mass spectra databases. In this way, unknown samples can be matched to previously acquired chromatograms for identification.

Table 2.1 Investigated compounds with their retention times (Rt), target ions and corresponding method detection limit (*MDL*); concentration ranges found in wine samples with median, measurement uncertainty u(cm) and average relative standard deviation of all analysed samples *RSD*s.

Compound	R_t	Target ions (m/z)	*MDL* (µg L^{-1})	Range (mg L^{-1})	Median (mg L^{-1})	$u(c_m)$ (%)	RSD_s (n=196) (%)
Ethyl acetate	9.15	61, 70	2.8	30 - 168	71	4.3	3.8
tert-butanol	9.17	59, 41	8.5	0.3 - 1.5	0.7	7.0	3.8
2-propanol	9.88	39, 59	8.2	18 - 48	36	7.8	18
2-butanol	12.0	73, 45	4.6	0.2 - 1.4	0.4	6.8	3.4
1-propanol	12.6	31, 59	9.3	18 - 134	42	6.1	13
Ethyl isovalerate	13.1	115, 88	7.1	0.01 - 0.1	0.02	4.5	12
2-methyl-1-propanol	13.5	43, 74	11	16 - 380	68	6.1	16
3-pentanol	14.1	59, 31	3.2	n.d. - 0. 9	0.03	7.9	10
Isopentyl acetate	14.6	43, 70	5.7	0.09 - 2.9	0.4	5.9	3.8
1-butanol	15.4	31, 56	6.2	n.d. - 5.2	2.6	7.5	9.9
2-methylbutanol	17.2	56, 57	0.9	18 - 140	61	4.6	6.6
3-methylbutanol	17.3	55, 42	0.7	56 - 358	161	5.8	6.7
Ethyl hexanoate	18.2	88, 99	0.08	0.1 - 0.9	0.3	5.2	5.4
1-pentanol	18.6	42, 55	0.3	0.03 - 0.1	0.07	6.1	8.4
3-methyl-1-pentanol	20.9	56, 69	0.6	0.04 - 0.8	0.4	4.7	5.8
1-hexanol	21.5	56, 43	0.2	0.3 - 5.6	1.7	4.1	4.8
Ethyl octanoate	24.2	88, 101	0.02	0.09 - 1.2	0.3	7.0	8.3
2-ethyl-1-hexanol	25.8	57, 41	0.1	n.d. - 0.08	0.002	4.5	25
Linalool	28	71, 121	0.4	0.001 - 0.03	0.005	5.0	7.2
Ethyl decanoate	33	88, 101	0.3	n.d. - 0.2	0.05	5.3	12
Diethyl succinate	35.5	101, 129	0.7	0.8 - 41	6.9	3.2	4.2
2-phenethyl acetate	49.5	43, 104	0.3	n.d. - 0.3	0.03	2.5	12

n.d.: not detectable (lower than method detection limit)

While a reliable correlation of given wine samples to the library was no problem, as long as a sample of the same provenance and vintage was measured before, the detection of further similarities based on the varietal or region of origin proved difficult. Only in few cases, larger groups of wines with the same varietal and region of origin could be formed, because the differences in the wine making process of each individual wine maker outweigh the influence of varietal, soil condition or climate on the volatile composition of wine. A more extensive differentiation should be possible by the combination of the volatile data with measurements of non-volatile compounds.

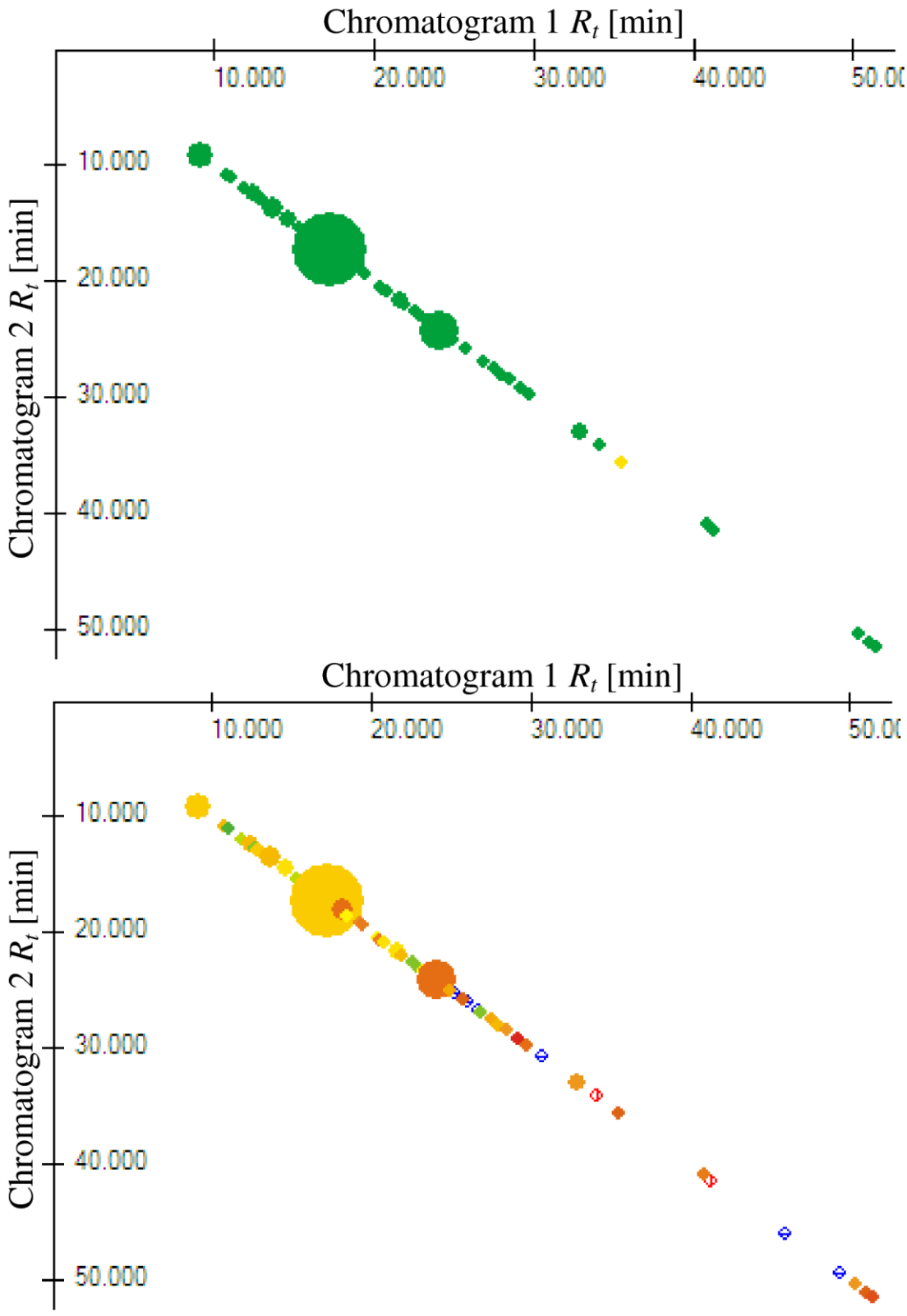

Figure 2.3 Chromatogram match view, showing a peak by peak comparison of a sample and a library chromatogram; the size of the dots indicates the peak area and the color the matching score from red (low score) to green (high score); examples of a good match in the upper figure and a bad match in the lower one.

2.3.4 SPDE Needle Lifetime and Performance

A previous study revealed a long lifetime of a carbowax-coated SPDE syringe needle[40]. Therefore, an in depth study of the SPDE needle lifetime and performance for high through put sampling was carried out during the wine fingerprinting campaign. The long term stability of the SPDE needles was observed using the calibration data, acquired with each sample batch. As any reference standard involved in the sample extraction and injection process also would have been affected by the changes in the needle extraction capacity, the sensitivity of the MS was monitored to make sure any observed loss of extraction capacity only results from deterioration of the SPDE needle. The peak areas of the reference substance of the MS (perfluorotributylamine (PFTBA)), acquired with the auto-tune function on m/z = 69 were used to calculate a relative sensitivity S_r:

Equation 2.2

$$S_r = \frac{A_t}{A_{max}}$$

where A_t is the peak area of the corresponding auto-tune and A_{max} the maximum value of all tunings. During the first 250 measurements, the sensitivity of the instrument dropped to about 50 % of the maximum and stayed at this level till the end of the campaign with over 900 samples. The calculated relative sensitivity was then applied to the calibration data, to allow comparison of the extraction performance over the whole time of the study:

Equation 2.3

$$A_{cor} = \frac{A_{cal}}{S_r}$$

where A_{cor} is the corrected peak area and A_{cal} the primary acquired peak area.

Ethyl acetate, as one of the most abundant compounds, was chosen to discuss the performance of the SPDE needle over time, in detail. The first SPDE needle was also used for the preliminary experiments to determine the required concentration ranges for the target compounds and extraction conditions for the wine samples, resulting in about 200 performed extractions. No data for performance comparison is available for this phase of work, because of the varying extraction conditions. The slopes of the calibration functions for the determination of ethyl acetate, fitted to the uncorrected and corrected calibration data, over time are depicted in **Figure 2.4**. A decrease of the slopes can be observed with an increasing number of performed analyses, indicating a loss of extraction capacity after about 250 analyses. However, after the calibration data was corrected for changing MS sensitivity, a decline of calibration slopes cannot be observed until over 350 analyses. After 542 measurements, the extraction capacity dropped to about 50% of the initial value and the

SPDE needle was replaced by a new one. The same trend as observed for ethyl acetate applies for the other quantified compounds. The second SPDE needle was introduced after 542 samples and was used for the remaining samples. The slopes of the calibration functions are also given in **Figure 2.4**, no decrease of capacity can be observed during the use of the second needle. In total, 399 analyses were performed with this needle without loss of extraction capacity. The initial performance of both needles is quite similar and suggests low variance between different needles.

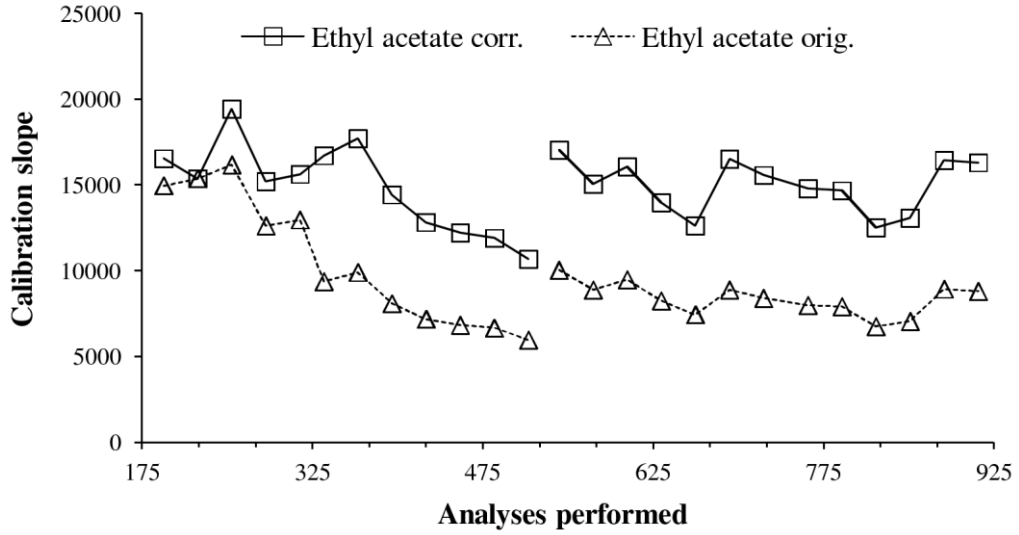

Figure 2.4 Slopes of fittet calibartion functions for the determination of ethyl acetate, before and after correction for MS sensitivity loss.

Contrary to the deterioration of needle extraction capacity, no significant change of retention of the column could be observed over time. Suggesting the earlier mentioned appearance of PEG degradation products in the chromatograms results mainly from decomposition of the extraction phase in the needle, during thermal desorption and conditioning. *Lattimer* observed pyrolysis of PEG in inert atmospheres at temperature levels of 150 °C[44], the main reactions are homolytic cleavage of C-C or C-O bonds followed by hydrogen abstraction reactions[44, 45]. A random scission of the main chain occurs already at temperatures of 80 °C in air[46]. Furthermore, PEG can form stable trihydrates with water[47], which changes the polarity of the extraction phase.

2.4 Conclusions

The headspace SPDE-GC/MS method proved to be appropriate for the analysis of volatile wine constituents. A number of substances could be extracted and quantified down to the low µg L^{-1} range from the diluted samples, with total measurement uncertainties below 10%.

A continuous decrease of the extraction capacity of the SPDE needles over the working period could not be observed; instead constant results were obtained for about 400 extractions before a decline started. This is a notable advantage over solid phase microextraction, where the peak heights in wine analysis were reported to drop to about 25 % after only 30 extraction cycles.[11]

The fingerprinting library was reliable in identification of wine samples and could be used for quality control, but not for the assignment of common characteristics like region of origin or varietal, because of the limited information provided by volatile data only.

2.5 Acknowledgements

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3 In-tube Extraction of Volatile Organic Compounds from Aqueous Samples

This chapter has been published in modified form in *Laaks, J.; Jochmann, M. A.; Schilling, B.; Schmidt, T. C., In-tube extraction of volatile organic compounds from aqueous samples: An economical alternative to purge and trap enrichment. Analytical Chemistry 2010, 82, 7641-7648* © American Chemical Society 2010

3.1 Introduction

The analysis of substances in water which are environmentally hazardous or harmful to health is a major part of the routine work performed in analytical laboratories. It requires sensitive and robust methods, delivering results in a reasonable amount of time with preferably a high degree of automation. Established and standardized methods include liquid-liquid extraction, static headspace without pre-concentration and, particularly common in the US, purge and trap systems (P&T). These methods have certain disadvantages, for example the limited sensitivity of S-HS[1], the amount of manual work and solvent consumption for LLE or the investment cost and susceptibility to contamination of P&T systems.

These drawbacks could be overcome by the use of solventless microextraction techniques, offering sample preparation and pre-concentration in one step, which can be performed fully automated by xyz-autosamplers. Although SPME, featuring a sorbent coated fused-silica fiber in a stainless steel needle, was presented almost 20 years ago[2] and commercialized in 1993, only few standardized procedures have been developed so far (e.g. DIN 38407-34:2006[3], EPA Method 8272[4]). SPME suffers from certain disadvantages: fragility of the fused-silica fiber, mechanical damage of the coating due to scraping and needle bending during agitation, limited flexibility in fiber length and coating thickness, resulting in low amounts of stationary phase (about 0.6 µL on a 100 µm PDMS fiber[5]), ghost peaks from septum particles or fiber glue, a possible memory effect from incomplete sample desorption and the limited lifetime[6-9]. Several in-needle extraction devices were proposed to overcome these drawbacks, by placing a short length of capillary GC column into a steel needle or by directly coating the inner wall of the needle. Early implementations have been called INCAT[10, 11], until it was commercialized as the "magic needle" or SPDE by Chromtech (Idstein, Germany) in 2000[9, 12]. The main advantages are the larger amount of stationary phase (about 4.5 µL in a 50 µm x 56 mm PDMS coating[5]), the mechanical stability[12] and the faster extraction, because of the dynamic process with active pumping of the sample, instead of mere diffusion[13]. SPDE has been successfully utilized in the analysis of pesticides[14],

chlorinated hydrocarbons[15], furan[16] or BTEX compounds[16-18] and proven its stability (up to 400 extractions were achieved with a polyethylene glycol phase)[19]. However, the length of the coating in the needle (56 mm) can cause problems during thermodesorption, if the injector exhibits a temperature profile and the amount of extraction phase is still quite small. An increase in phase material can be achieved with packed sorbents in larger diameter needles, like the tubular cylindrical microconcentrator (TCMC) developed by *Berezkin et al.*[20], the NT presented by *Wang et al.*[21], the in-needle extraction device introduced by Saito et al.[22] and ITEX device, which was commercialized in 2006 by CTC Analytics AG (Zwingen, Switzerland)[23]. ITEX features a sorbent volume of 160 µL and is the only fully automatable device with needle packing, so far. However, the ITEX system required a special autosampler head, which hampered the quick exchange of analytical methods. In 2009, this drawback has been overcome with the release of a new ITEX 2 system, which can be mounted on any PAL-type autosampler without modification.

It features a sorbent packed needle surrounded by an external heater, mounted on a gas-tight syringe with side-port. Enrichment is carried out by aspirating and dispensing the syringe several times, pumping the sample headspace through the sorbent bed. The analytes are thermodesorbed into the GC injector with desorption gas, which can be either a portion of the sample headspace or aspirated carrier gas from the injector. After the syringe is withdrawn from the injector, the plunger is moved above the side port and the heated trap is flushed with inert gas several times to avoid carryover. A schematic depiction of the steps in the ITEX procedure is given in **Figure 3.1**. The extraction yield is influenced by various parameters taking effect in different steps of the analytical process. As with other headspace sampling techniques, the air-water partitioning of the analytes in the vial can be influenced by the variation of the phase ratio, the temperature, the ionic strength and, depending on compound structure, the pH-value of the sample. The first step in the development of an ITEX procedure is the choice of an appropriate sorbent material for the target compounds. Optimization parameters during the extraction are the number of aspirating and dispensing cycles (extraction strokes) and the volume and flow of each stroke, respectively. The desorption temperature of the trap, the desorption gas volume and the desorption flow have to be considered during sample injection.

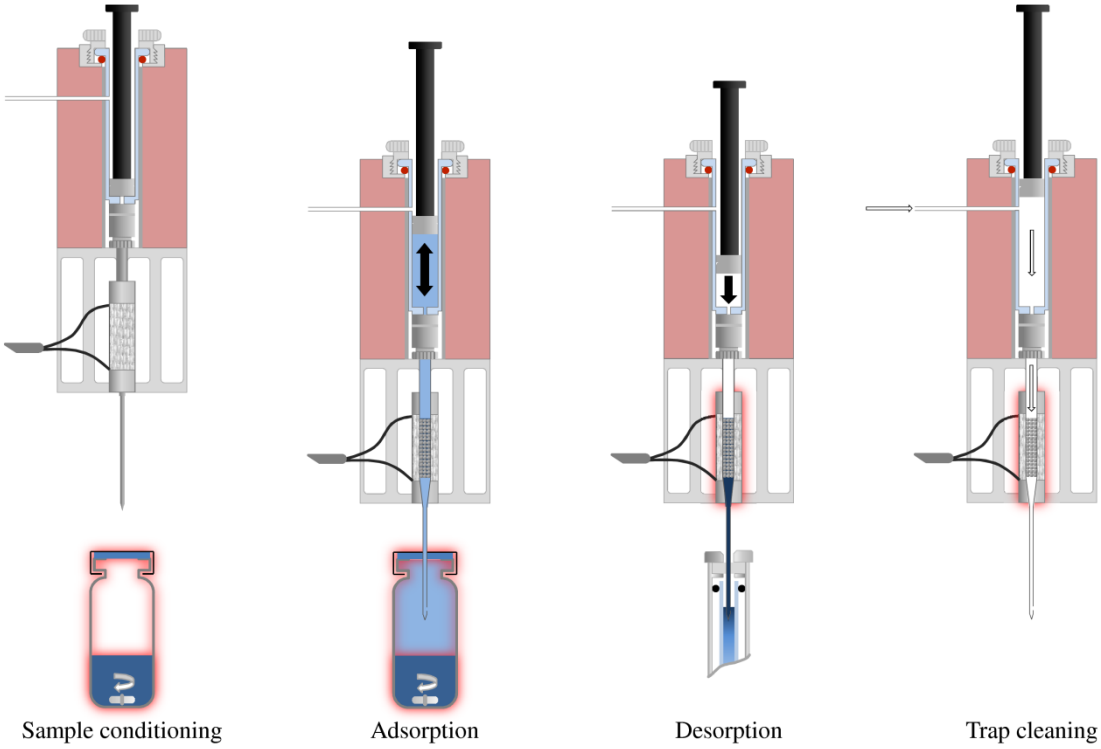

Figure 3.1 Schematic steps of the ITEX procedure: Sample conditioning through heating and stirring/shaking, adsorption by dynamic headspace extraction, thermodesorption from the heated trap, trap cleaning by a stream of nitrogen

The aim of this work was to develop and evaluate a simple ITEX 2 method for the analysis of water contaminants as an alternative to the typically utilized P&T system, following EPA method 524.3[24]. The target compounds include chlorinated hydrocarbons, trihalomethanes (THM), BTEX compounds, fuel oxygenates (ethyl *tert*-butyl ether (ETBE), methyl *tert*-butyl ether (MTBE) and *tert*-amyl methyl ether (TAME)). We also included more polar compounds not typically analyzed by P&T methods such as 1,4-dioxane and the two bacterial or cyanobacterial metabolites 2-methylisoborneol (MIB) and geosmin that cause earthy-musty odors in low concentrations[25]. The latter need to be controlled since their presence leads to the most frequent customer complaints. Guideline or limit values for the target compounds are listed in **Table 3.1**.

Table 3.1 Target compounds with boiling points, air-water partitioning coefficients and their maximum concentrations according to the *"Guidelines for Drinking-water Quality"*[26] of the World Health Organization (WHO), the *"National Primary Drinking Water Regulations"* of the United States Environmental Protection Agency (EPA)[27] and the *"Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption"*[28] of the European Union (EU)

Compound	CAS-Nr.	Boiling point* (°C)	K_{aw}(20°C)	WHO (µg L^{-1})	EPA (µg L^{-1})	EU (µg L^{-1})
Vinyl chloride	75-01-4	-13	0.891[29]	0.3	2	0.5
Dichloromethane	75-09-2	40	0.0903[29]	20	5	-
MTBE	1634-04-4	55	0.0169[29]	-	-	-
ETBE	637-92-3	73	0.0377[30]	-	-	-
Chloroform	67-66-3	61	0.126[29]	300	80[a]	100[a]
Benzene	71-43-2	80	0.191[29]	10	5	1
TAME	994-05-8	86	0.03[30]	-	-	-
1,2-Dichloroethane	107-06-2	83	0.0419[29]	30	5	3
Trichloroethylene	79-01-6	87	0.533[29]	20	5	10[c]
Bromodichloromethane	75-25-2	87	0.076[29]	60	80[a]	100[a]
1,4-Dioxane	123-91-1	102	0.0002[31]	50	-	-
Toluene	108-88-3	110	0.209[29]	700	1000	-
Tetrachloroethylene	127-18-4	121	0.533[29]	40	5	10[c]
Dibromochloromethane	124-48-1	120	0.035[29]	100	80[a]	100[a]
Ethylbenzene	100-41-4	136	0.239[29]	300	700	-
p-Xylene	106-42-3	138	0.248[29]	500	10[b]	-
o-Xylene	95-47-6	145	0.16[29]	500	10[b]	-
Bromoform	75-25-2	150	0.0175[29]	100	80[a]	100[a]
2-Methylisoborneol	2371-42-8	197	0.0027[32]	-	-	-
Geosmin	16423-19-1	270	0.0024[32]	-	-	-

[*] Supplier data
[a] Sum of all trihalomethanes
[b] Sum of all xylenes
[c] Sum of trichloroethylene and tetrachloroethylene

3.2 Experimental

3.2.1 Reagents

Analytical grade methanol (KMF Laborchemie, Lohmar, Germany) was used for the preparation of stock and working solutions. Standard solutions were prepared with reagent water from a PURELAB Ultra Analytic water purification system (ELGA LabWater, Celle, Germany).

Dichloromethane (> 99.5 %), chloroform (> 99.9 %), bromodichloromethane (98+ %), dibromochloromethane (98 %), bromoform (99+ %), trichloroethylene (> 99 %), tetrachloroethylene (> 99 %), ethyl *tert*-butyl ether (ETBE) (99 %), *tert*-amyl methyl ether (TAME) (97 %), toluene (> 99.5 %) and methyl *tert*-butyl ether-d_3 (MTBE-d_3) (99+ atom-%) were purchased from Aldrich (Sigma-Aldrich, Steinheim, Germany); methyl *tert*-butyl ether

(MTBE) (99+ %), 1,4-dioxane (99+ %), benzene (> 99.9 %), ethylbenzene (> 99.5 %), *p*-xylene (> 99.5 %) and *o*-xylene (> 99.5 %) from Fluka (Sigma-Aldrich); 1,2-dichloroethane (99.8 %), geosmin (2 g L^{-1} in methanol) and 2-methylisoborneol (MIB) (10 g L^{-1} in methanol) from Sigma (Sigma-Aldrich) and vinyl chloride (2 g L^{-1} in methanol), toluene-d_8 (99.9 atom-%, 2 g L^{-1} in methanol) and 1,2-dichlorobenzene-d_4 (99.9 atom-%, 2 g L^{-1} in methanol) from Supelco (Sigma-Aldrich).

3.2.2 Standard and Sample Preparation

A methanolic stock solution with a concentration of 100 µL L^{-1} was prepared from the pure substances, using Hamilton syringes (Hamilton, Bonaduz, Switzerland) and volumetric flasks, using the stock solution and the reagents delivered in methanolic solutions (vinyl chloride, geosmin and MIB), methanolic standard solutions with a concentration range from 100 ng L^{-1} up to 2 mg L^{-1} were prepared and stored in vials without headspace in the refrigerator at 4 °C. An internal standard mixture, containing 2 mg L^{-1} of MTBE-d_3, toluene-d_8 and 1,2-dichlorobenzene-d_4, was also prepared. MTBE-d_3 was used as internal standard for vinyl chloride, dichloromethane, MTBE, ETBE, chloroform, TAME, 1,2-dichloroethane, trichloroethylene and bromodichloromethane; Toluene-d_8 was used for benzene, 1,4-dioxane, toluene, tetrachloroethylene,dibromochloromethane, ethylbenzene, p-xylene and o-xylene; 1,2-dichlorobenzene was used for bromoform, MIB and geosmin.

The aqueous calibration solutions were prepared with 10 mL reagent water, where each 10 µL of the methanolic standard solutions and 5 µL of the internal standard mix were added, resulting in calibration levels between 0.1 ng L^{-1} and 2 µg L^{-1} for the target compounds and 1 µg L^{-1} of the internal standards. The only sample preparation step was the addition of 5 µL internal standard mix to each 10 mL of sample. The calibration solutions and samples were filled into amber 20 mL-headspace screw cap vials with rubber-PTFE septa (BGB Analytik AG, Boeckten, Switzerland), containing an 8x3 mm PTFE coated magnetic stir bar (VWR International GmbH, Darmstadt, Germany).

3.2.3 GC/MS Instruments and Parameters

All analyses were performed using a Trace GC Ultra (S+H Analytik, Mönchengladbach, Germany) coupled to a DSQ II single quadrupole mass spectrometer (S+H Analytik). The GC was equipped with a split/splitless injector (S/SL), an Atas GL Optic 3 programmed temperature vaporization injector with cryofocussing unit (Axel Semrau, Sprockhövel, Germany) and a Combi PAL autosampler (Axel Semrau). An uncoated, deactivated fused

silica tubing with 0.53 mm *i.d.* (BGB Analytik AG, Boeckten, Switzerland) was used in the cryofocussing unit, compound separation was performed on a Restek Rtx-VMS column (medium polar, proprietary modified phase) with 60 m length, 0.32 mm *i.d.* and 1.8 µm film thickness (Restek GmbH, Bad Homburg, Germany).

For the ITEX measurements, the injector temperature of the Optic 3 was set to 280 °C, the cryotrap was set to -165 °C with a hold/transfer time of 60 s and a transfer column flow of 1.0 mL min^{-1} He 5.0 (Air Liquide, Oberhausen, Germany) in splitless mode. After the transfer time, the column flow is raised to a constant flow of 1.5 mL min^{-1}, the split is opened at 20 mL s^{-1} and the cryotrap is heated to 250 °C with a heating rate of 30 °C sec^{-1}. The column oven start temperature was set to 40 °C for 1 min, then heating up to 130 °C with 4 °C min^{-1} and to 200 °C with 10 °C min^{-1} and a hold time of 10 minutes. The MS transfer line was set to 250 °C, ion source temp was 220 °C. The MS was set to electron ionization (EI) with an ionization energy of 70 eV in scan mode (m/z = 49-180, 6.5 scans s^{-1}). Instrument control, data acquisition and evaluation were performed by the Xcalibur 1.4 data system (S+H Analytik).

3.2.4 ITEX Method

The ITEX 2 option for the Combi PAL was received by CTC Analytics (Zwingen, Switzerland), it consists of a heated syringe holder, a 1.3 mL Hamilton syringe with side port (Hamilaton, Bonaduz, Switzerland) and a trap heater. The Combi Pal was equipped with a Tray Cooler2 (CTC Analytics) and a Single Magnet Mixer (SMM) (Chromtech, Idstein, Germany). The ITEX extraction procedure was controlled by manufacturer supplied macros, customized in the PAL Cycle Composer (CTC Analytics). ITEX 2 traps with different sorbent packings, common in gas analytics, were supplied by BGB Analytik AG: Carbosieve S III, Carbopack C, Carboxen 1000, Tenax GR and Tenax TA. A custom trap with two parts Tenax GR and one part Carbosieve S III was acquired after the evaluation of the packing materials with the target compounds. The properties of the packing materials are summarized in **Table 3.2**.

Parameters considered for the optimization of the ITEX method were: (i) the extraction flow, at which the sample headspace is passed through the sorbent packing; (ii) the total extraction volume passed over the trap, depending on the number of extraction strokes performed and the volume used for every stroke; (iii) the sample temperature during the extraction; (iv) the desorption temperature of the trap; (v) the desorption gas volume and (vi) the desorption gas

flow. Method optimization was carried out using standard solutions with a concentration of 1 µg L^{-1} in triplicate analysis. Only the optimized parameters are given here, the optimization steps will be discussed in the results part.

The samples were stored in the Tray Cooler at 25 °C, after the transfer to the SMM, the sample was heated and stirred at 500 rpm for 5 min to reach the extraction temperature of 60 °C. 60 extraction strokes with an aspirating and dispensing volume of 1 mL were performed with an aspirating and dispensing flow of 100 µL s^{-1}, respectively. The trap temperature was 40 °C and the syringe temperature was set to 60 °C to avoid condensation of water. After the extraction, the sample vial was moved back to the tray and 500 µL of helium were aspirated as desorption gas from the cold S/SL, to minimize the amount of water transferred to the column and to avoid desorption and condensation of sample components in the syringe. Desorption was performed in the Optic 3 after the ITEX trap was heated to 300 °C with a desorption flow of 10 µL s^{-1}. The autosampler returned to the home position for trap cleaning. To avoid transport of possible residuals from the trap into the syringe, the trap was allowed to cool down to 70 °C, before the plunger of the syringe was moved above the side port. A flow of nitrogen with 5-6 mL min^{-1} was applied and the trap was flushed for 5 minutes at a temperature of 350 °C. Afterwards, the plunger was moved down and the temperature was set to 40 °C, to prepare the trap for the next extraction. The whole procedure (including injection, trap cleaning and extraction of the following sample) was optimized to be completed within the runtime of the GC oven program with cooling (about 45 minutes) to avoid unnecessary downtime.

Table 3.2 Properties of sorbent materials (manufacturer data)

	Carbopack C	Carbosieve S III	Carboxen 1000	Tenax TA	Tenax GR
Sorbent type	Graphitized carbon black	Carbon molecular sieve	Carbon molecular sieve	Porous organic polymer	70 % Tenax 30 % graph. carb.
Mesh size	80/100	80/100	60/80	80/100	80/100
Surface Area	10 m²/g	975 m²/g	1200 m²/g	35 m²/g	24 m²/g
Temperature Limit	500 °C	400 °C	225 °C	350 °C	350 °C
Water affinity	relatively low	moderate	moderate	low	low
Application	low to medium boilers (C_{12}-C_{20})	volatile organics (C_2-C_5)	permanent gases, volatiles (C_2-C_5)	volatiles and semi volatiles (C_7-C_{26})	volatiles, flavors, fragrances

3.3 Results and Discussion

A chromatogram of a 1 µg L^{-1} standard solution is depicted in **Figure 3.2**. An elevated background due to trapped water can be seen at retention times between 4.5 and 7 minutes, the only target compound in this area is vinyl chloride. However, when an extracted ion chromatogram of the base peak at m/z = 62 is created, no negative effects on peak shape and height are visible and no problems in quantification down to the low ng L^{-1} range could be observed.

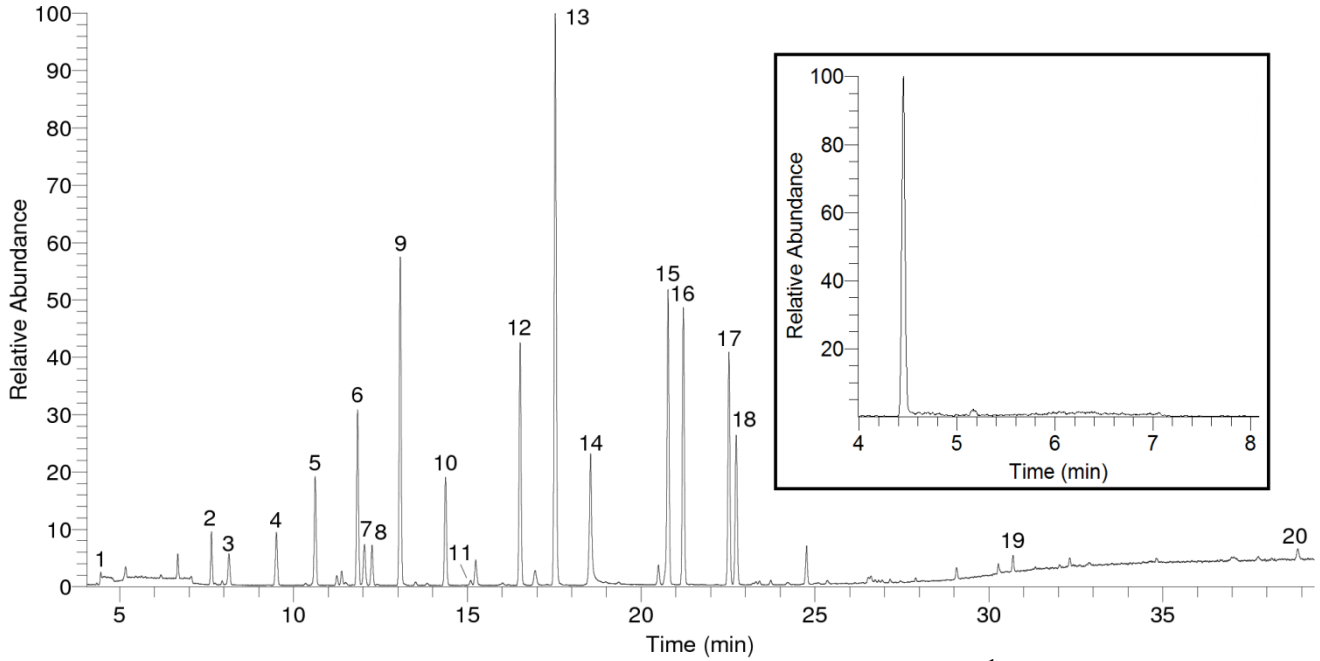

Figure 3.2 GC/MS chromatogram of standard analytes, in full scan mode (m/z 49-180) at 1µg L^{-1}; the first minutes of an extracted ion chromatogram of m/z 62 is shown in the box. Target compounds: 1) vinyl chloride, 2) dichloromethane, 3) MTBE, 4) ETBE, 5) chloroform, 6) benzene, 7) TAME, 8) 1,2-dichloroethane, 9) trichloroethylene, 10) bromodichloromethane, 11) 1,4-dioxane, 12) toluene, 13) tetrachloroethylene, 14) dibromochloromethane, 15) ethylbenzene, 16) p-xylene, 17) o-xylene, 18) bromoform, 19) MIB, 20) geosmin

3.3.1 Sorbent materials

Prior to the ITEX 2 method optimization, the five available packing materials were evaluated for the best yield of target analytes. Therefore, a sample containing 1 µg L^{-1} of each compound was analyzed with the default parameters (20 extraction strokes of 1 mL at 100 µL s^{-1} flow) and desorbed at temperatures of 200 and 300 °C. The obtained peak areas are listed in **Table 3.3**. The higher desorption temperature generally gives better yields, due to the endothermic desorption process and was kept for all measurements. As expected, the Carbopack C trap was more effective with the higher boiling substances and the Carboxen 1000 trap with low boilers. The Carbosieve S III trap was most effective with low boilers, but also with the high boiling MIB and geosmin. Tenax TA and the graphitized Tenax GR

display the best enrichment performance over the whole analyte range, but with lower yield in the low and high boiling substances.

As the characteristics of Tenax GR and Carbosieve S III complemented each other, a mixed bed trap consisting of 2/3 Tenax GR and 1/3 Carbosieve S III was prepared and evaluated under the same conditions. The Tenax GR was placed in the bottom of the trap and the Carbosieve S III on the top, in this way the higher boiling analytes are held by and desorbed from the weaker sorbent and do not reach the stronger molecular sieve as is common practice in air analysis.[33, 34] The overall performance of the mixed bed trap was slightly inferior compared to the plain Tenax GR trap, but because the results for 1,4-dioxane, MIB and geosmin were higher than with the other traps and their peak areas, together with that of vinyl chloride, were the smallest of all compounds, the mixed bed trap was used for the following experiments.

Table 3.3 Obtained peak areas of the target compounds with different sorbent materials and desorption temperatures (average of three measurements); the bars in each row indicate the relative peak area per compound

	Carbopack C		Carbosieve S III		Carboxen 1000		Tenax TA		Tenax GR		T. GR/C. S III 2:1	
Desorption temp.	200 °C	300 °C	200 °C	300 °C	200 °C	300 °C	200 °C	300 °C	200 °C	300 °C	200 °C	300 °C
Vinyl chloride	2.2E+04	2.3E+04	1.9E+05	4.3E+05	2.3E+05	6.0E+05	3.0E+05	3.0E+05	3.5E+05	3.7E+05	2.1E+05	2.9E+05
Dichloromethane	3.5E+05	3.8E+05	5.8E+05	1.5E+06	7.6E+05	2.1E+06	1.6E+06	2.9E+06	2.3E+06	2.0E+06	1.9E+06	1.7E+06
MTBE	8.3E+05	9.3E+05	8.0E+05	1.6E+06	2.5E+05	8.2E+05	2.5E+06	3.2E+06	2.5E+06	2.8E+06	2.3E+06	1.7E+06
MTBE-d3	1.4E+06	1.5E+06	1.4E+06	3.0E+06	4.4E+05	1.5E+06	4.5E+06	5.6E+06	4.7E+06	5.0E+06	4.1E+06	3.0E+06
ETBE	2.0E+06	2.5E+06	1.0E+06	2.2E+06	2.2E+05	8.1E+05	4.2E+06	5.1E+06	4.2E+06	4.8E+06	4.4E+06	3.4E+06
Chloroform	2.3E+06	2.4E+06	3.9E+05	1.3E+06	8.8E+05	2.8E+06	5.4E+06	7.0E+06	5.8E+06	7.0E+06	5.8E+06	4.7E+06
Benzene	8.1E+06	1.2E+07	4.7E+05	1.5E+06	1.0E+06	8.5E+06	1.3E+07	1.8E+07	1.3E+07	1.9E+07	1.3E+07	1.5E+07
TAME	1.7E+06	2.2E+06	6.8E+05	1.6E+06	1.5E+05	5.7E+05	3.1E+06	4.2E+06	3.1E+06	3.9E+06	3.3E+06	2.7E+06
1,2-Dichloroethane	6.0E+05	6.8E+05	7.3E+04	2.7E+05	1.9E+05	6.6E+05	1.4E+06	1.8E+06	1.5E+06	1.9E+06	1.4E+06	1.2E+06
Trichloroethylene	6.4E+06	7.5E+06	1.9E+05	8.0E+05	6.2E+05	2.5E+06	1.0E+07	1.2E+07	1.0E+07	1.3E+07	9.6E+06	8.4E+06
Bromodichloromethane	4.3E+06	4.9E+06	2.3E+05	8.2E+05	6.2E+05	2.2E+06	7.1E+06	1.0E+07	7.4E+06	1.1E+07	7.9E+06	7.2E+06
1,4-Dioxane	1.9E+04	2.7E+04	4.7E+03	1.1E+04	7.3E+03	3.3E+04	2.5E+04	4.3E+04	2.4E+04	4.2E+04	3.4E+04	4.7E+04
Toluene-d8	7.2E+06	1.2E+07	2.5E+04	1.6E+05	1.9E+05	8.5E+05	1.2E+07	1.5E+07	1.2E+07	1.8E+07	1.2E+07	1.1E+07
Toluene	8.8E+06	1.7E+07	4.3E+04	2.7E+05	3.2E+05	3.9E+06	1.4E+07	2.1E+07	1.4E+07	2.3E+07	1.6E+07	2.3E+07
Tetrachloroethylene	1.4E+07	2.1E+07	7.6E+04	4.0E+05	3.8E+05	1.8E+06	1.8E+07	2.6E+07	2.0E+07	3.2E+07	2.2E+07	2.3E+07
Dibromochloromethane	1.1E+06	1.4E+06	2.0E+04	6.7E+04	4.2E+04	2.6E+05	1.2E+06	2.0E+06	1.3E+06	2.1E+06	1.4E+06	1.5E+06
Ethylbenzene	7.1E+06	1.7E+07	1.6E+04	5.6E+04	2.0E+05	4.5E+05	1.3E+07	2.2E+07	1.2E+07	2.5E+07	1.4E+07	1.8E+07
p-Xylene	3.9E+06	1.4E+07	1.4E+04	4.4E+04	1.6E+05	3.1E+05	1.1E+07	1.9E+07	9.7E+06	2.1E+07	1.1E+07	1.7E+07
o-Xylene	3.6E+06	1.3E+07	1.9E+04	4.4E+04	1.4E+05	2.9E+05	8.8E+06	1.8E+07	8.3E+06	1.9E+07	1.0E+07	1.5E+07
Bromoform	6.5E+06	8.7E+06	5.5E+04	2.4E+05	1.9E+05	8.0E+05	5.3E+06	1.1E+07	5.2E+06	1.2E+07	6.4E+06	9.5E+06
1,2-Dichlorobenzene-d4	3.3E+06	1.2E+07	5.1E+04	9.0E+04	2.2E+05	3.0E+05	5.4E+06	1.3E+07	5.4E+06	1.5E+07	7.5E+06	1.3E+07
2-Methylisoborneol	2.1E+05	5.9E+05	1.9E+05	3.6E+05	2.3E+04	3.3E+04	4.2E+05	6.8E+05	3.6E+05	4.8E+05	4.9E+05	6.8E+05
Geosmin	1.4E+05	3.0E+05	2.5E+05	4.8E+05	1.3E+05	1.5E+05	3.3E+05	3.9E+05	3.7E+05	4.4E+05	4.5E+05	5.8E+05

3.3.2 Extraction Parameter Optimization

Some parameters of the extraction process are linked and have to be optimized together, especially considering the total extraction time. These parameters are the aspirating and dispensing flow, the aspirated sample volume and the number of extraction strokes. Higher

extraction flows usually lead to a lower extraction yield; with MIB and geosmin being the only exceptions of the investigated compounds, displaying increasing peak areas with increasing flows. The cause of this behavior is still under investigation. With a difference of about 30 % between 10 µL s^{-1} and 125 µL s^{-1}, the effect of the extraction flow is not as important as the number of extraction strokes performed, where an increase in peak areas by a factor of five can be observed between 10 and 100 strokes of 1 mL aspiration volume. Considering the run-time of the GC program, the yield of 10 extraction strokes at 10 µL s^{-1} was compared to that of 60 strokes at 100 µL s^{-1}. The higher number of extraction strokes, and consequently sampled headspace volume, resulted in considerably higher extraction yields, up to a factor of 11 for MIB. This could be expected, as van Durme et al.[35] reported increasing extraction yield with increased aspirated sample volume for a modified SPDE technique.

Extraction temperature was checked in increments of 10 °C from 40 °C to 70 °C, as *Jochmann et al.*[23] found the optimal extraction temperature to be 50 °C with the first generation ITEX system, using a Tenax TA trap. Up to 60 °C, the extraction yield of all compounds increased with rising temperatures, because of the increasing partitioning coefficient between headspace and sample; when the extraction temperature was raised to 70 °C, stagnation or slight decrease of extraction efficiency was observed for the volatile compounds like vinyl chloride, dichloromethane, ETBE or trichloroethylene, while the extraction of less volatile compounds like MIB and geosmin still improved. This behavior can also be observed with the first generation ITEX[23], SPDE[5, 15, 18] and SPME[36, 37], because the partitioning into the sorbent decreases with higher temperatures. Although the decrease in peak areas from 60 °C to 70 °C for the volatile compounds was only marginal, an extraction temperature of 60 °C was chosen.

Optimized desorption parameters are the desorption temperature, the desorption gas volume and the desorption flow. As the influence of the desorption temperature on the analyte yield has already been covered in the extraction phase evaluation, only the latter two will be discussed here. Desorption gas volumes of 100, 500 and 1000 µL were tested. In general, the effect on the desorption efficiency was small, with only MIB showing a significant increase from 100 to 500 µL; an increase to 1000 µL slightly lowered the yield of many compounds and augmented the error of the measurement, thus 500 µL were set as desorption gas volume. Contrary to the extraction flow, variations of the desorption flow result in significantly increased analyte yields. Lowering the flow from 100 µL s^{-1} to 50 µL s^{-1} and finally to

10 µL s^{-1} increased the obtained peak area for some compounds up to a factor of five, only the yield of the highly volatile vinyl chloride was not influenced by the desorption flow.

Graphical presentations of four optimization parameters are given with the examples of vinyl chloride as most volatile and geosmin as least volatile compound in **Figure 3.3**.

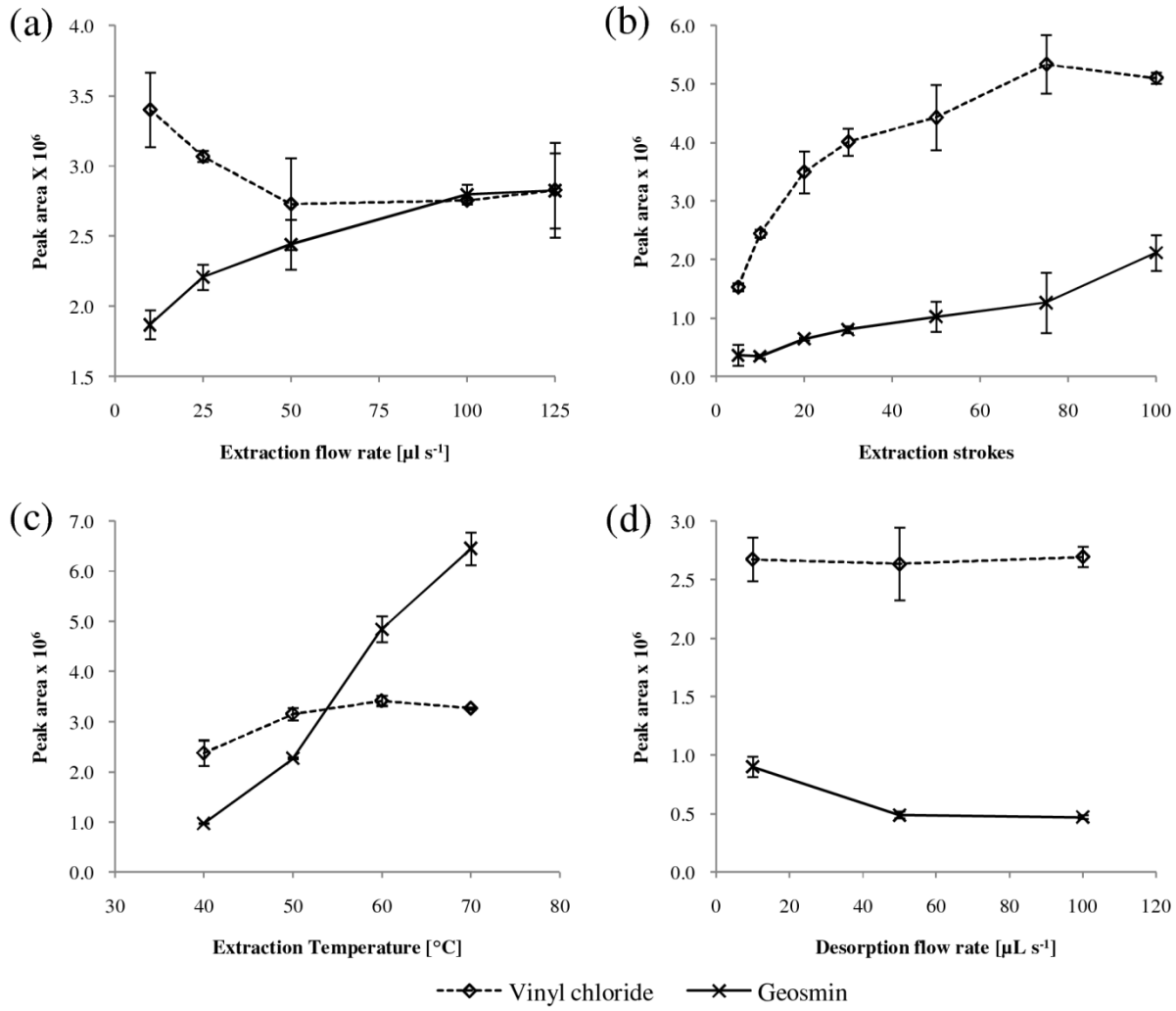

Figure 3.3 Influence of selected extraction parameters on the obtained peak areas: (a) extraction flow; (b) number of extraction strokes; (c) extraction temperature; (d) desorption gas flow

3.3.3 Method Detection Limits, Linear Range, Precision, Recovery and Extraction Efficiency

The method detection limits (*MDL*s) were calculated following a procedure of the U.S. Environmental Protection Agency.[38] The standard deviation of seven replicates (S_c) was calculated for each compound, at a concentration level featuring a signal to noise ratio (S/N) of approximately five to one, or where the slope of the calibration curve exhibited a break and became non-linear nearing low analyte concentrations, due to chromatographic

background or not totally blank free water. The *MDL*s were calculated according to **Equation 3.1**:

Equation 3.1 $$\boldsymbol{MDL = t_{(N-1,1-\alpha=0.99)} \times S_c}$$

where *t* is the student's *t*-value for the one-tailed test with a confidence level of 99% and six degrees of freedom. Sixteen-point calibration curves of seven replicates were measured in a concentration range from 0.1 ng L^{-1} up to 2 µg L^{-1} to obtain the required data for all compounds.

The attained *MDL*s are mostly in a narrow range: halogenated compounds range from 0.7 ng L^{-1} to 10.6 ng L^{-1}, the trihalomethanes in particular from 0.8 ng L^{-1} to 5.2 ng L^{-1}, BTEX compounds from 1.2 ng L^{-1} to 5.3 ng L^{-1} and the fuel oxygenates from 0.8 ng L^{-1} to 3.7 ng L^{-1}; only 1,4-dioxane, MIB and geosmin show higher *MDL*s of 69.6 ng L^{-1}, 32.8 ng L^{-1} and 57.2 ng L^{-1}, respectively, which may result from their low air-water partitioning coefficients and higher chromatographic background noise in the case of MIB and geosmin. The *MDL*s are comparable to those achieved with purge and trap systems found in the literature, except for MIB and geosmin (**Table 3.4**).

The linear range was specified from the concentration level, where the *MDL* calculation has been performed (S/N ≈ 3-5 or changing/discontinuous slope) up to the highest tested concentration level, if no changes in the slope appeared. Resulting linear ranges are between a concentration factor of 20 for 1,4-dioxane and about 2000 for bromodichloromethane, with correlation coefficients ≥ 0.998 for all compounds. A higher dynamic range can be assumed, as all compounds exhibit linear characteristics up to the highest investigated concentration levels and measurements of real samples, containing BTEX concentrations up to 40 µg L^{-1}, gave similar results undiluted and diluted to the calibrated concentration range. Also, *Jochmann et al.* observed a dynamic range over six orders of magnitude for the first generation ITEX.[23]

The precision was determined as the average of the relative standard deviations (*RSD*, n = 7) of all concentration levels in the linear range. The standard deviations were below 10% for all analytes; p-xylene and 1,4-dioxane showing the highest deviation with 8.9%, while the other compounds mostly possess standard deviations in the range of 5-6%. This is also similar to purge and trap results.

Recovery was calculated after tenfold measurements of tap water spiked with 100, 500 and 1500 ng L^{-1} of the target compounds, respectively. It is given as the proportion of the measured concentration minus the initial concentration of the tap water and the spiked amount. The recovery rates are basically between 88% and 103%, only 1,4-dioxane with just 59% recovery stands out. This might result from matrix effects or the overall low sensitivity of the method for this compound. The variations of recovery between the three spiking levels are also small, with *RSD*s well below 5%, except for MTBE, 1,4-dioxane, toluene and geosmin.

The extraction efficiency was calculated by multiple extractions from the same sample vial, according to the depletion method presented by *Zimmermann et al.*.[39] An exponential decrease of peak areas could be observed for all compounds, indicating that the same fraction of the sample is removed with each consecutive extraction. The extracted fractions ranged from 7% to 55% and increased with the air-water partitioning coefficient (see **Table 3.1**) of the compounds. When the extracted fraction was plotted against the air-water partitioning coefficient, a logarithmic trend could be observed. However, the correlation is not quantitative, because of the compound specific partitioning to the sorbent material.

Table 3.4 Quality parameters obtained with the ITEX 2-GC-MS system, compared to purge and trap systems

		ITEX 2						Purge & Trap		
Compound	**Target ions (m/z)**	***MDL*** **(µg L^{-1})**	**Linear range (µg L^{-1})**	**R^2**	**Precision (%)**	**Recovery (%)**	**Extraction yield (%)**	***MDL*** **(µg L^{-1})**	***RSD*** **(%)**	**Ref.**
Vinyl chloride	62	0.008	0.02-2.0	0.999	5.3	103 ± 3.8	36	0.008		[40]
Dichloromethane	49, 84	0.01	0.03-2.7	0.999	5.5	97 ± 2.2	21			
MTBE	73	0.004	0.01-1.5	0.999	6.1	88 ± 10.1	28	0.001	4.7	[41]
ETBE	59, 87	0.001	0.004-1.5	0.998	6.6	94 ± 1.8	34	0.009	5.1	[41]
Chloroform	83	0.004	0.007-2.9	0.999	5.4	99 ± 3.7	35	0.008		[40]
Benzene	78, 77	0.001	0.002-1.8	0.999	5.4	89 ± 2.2	24	0.002	5	[41]
TAME	73, 55	0.001	0.004-1.5	0.999	5.8	95 ± 3.4	29	0.01	3	[42]
1,2-Dichloroethane	62, 98	0.002	0.006-2.5	0.999	5.3	97 ± 1.1	22			
Trichloroethylene	95, 130	0.001	0.007-2.9	0.999	5.2	95 ± 1.3	49	0.003	5.3	[40]
Bromodichloromethane	83, 85	0.001	0.002-4.0	0.999	6.1	97± 1.6	31	0.007	5.2	[40]
1,4-Dioxane	88, 58	0.07	0.1-2.1	0.998	8.9	59 ± 13.5	10			
Toluene	92, 91	0.005	0.009-1.7	0.998	7.3	96 ± 7.7	46	0.001	4.5	[41]
Tetrachloroethylene	166, 168	0.001	0.003-3.2	0.999	5.7	97 ± 0.9	55	0.004	7.3	[40]
Dibromochloromethane	129, 127	0.005	0.02-4.9	0.999	5.8	98 ± 2.8	27	0.001	4.1	[40]
Ethylbenzene	91, 106	0.002	0.009-1.7	0.999	8.7	93 ± 3.2	50	0.001	5.5	[41]
p-Xylene	91, 106	0.004	0.009-1.7	0.999	8.9	117 ± 2.3	49	0.001	4.9	[41]
o-Xylene	91, 106	0.005	0.02-1.7	0.999	6.9	90 ± 2.6	45	0.002	4.7	[41]
Bromoform	173, 175	0.002	0.006-5.8	0.999	5.7	94 ± 4.2	19			
2-Methylisoborneol	95, 107	0.03	0.1-2.0	0.999	5.5	94 ± 2.1	7	0.001	5.6	[25]
Geosmin	112, 55	0.06	0.1-2.0	0.999	5.1	88 ± 9.1	10	0.002	6.1	[25]

3.3.4 Application to Real Samples

The method was tested on different aqueous samples: (i) tap water, which was also used for the recovery calculations, (ii) water from a pond near the university campus, (iii) water from a reservoir for drinking water production, withdrawn in 30 m depth and (iv) four kinds of soda in plastic bottles, acquired in the university cafeteria.

The results are summarized in **Table 3.5**. The guideline or limit values for drinking water were exceeded neither in the tap water and sodas, nor in the reservoir- or pond water. Except for chloroform and toluene in soda a and the xylenes in soda d, all concentrations were well below 1 µg L^{-1}, but traces of many compounds can be found in all samples. Although the sampling took place in late autumn, outside the growing period of blue-green algae, MIB could be detected in the water of the pond, which is heavily eutrophicated.

Table 3.5 Results for different types of aqueous real samples (n=3)

	Tap water		Pond water		Reservoir water		Soda a		Soda b		Soda c		Soda d	
	(µg L^{-1})	*RSD*	(ng L^{-1})	*RSD*	(µg L^{-1})	*RSD*	(µg L^{-1})	*RSD*	(µg L^{-1})	*RSD*	(µg L^{-1})	*RSD*	(µg L^{-1})	*RSD*
Vinyl chloride	<0.008				<0.008		<0.008		<0.008		<0.008		<0.008	
Dichloromethane	0.02	1%	0.02	9%	<0.01		<0.01		<0.01		0.09	5%	<0.01	
MTBE	0.01	14%	0.02	12%	0.02	10%	<0.004		<0.004		0.03	16%	<0.004	
ETBE	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Chloroform	0.20	10%	0.20	6%	0.55	4%	0.98	23%	<0.004		<0.004		0.09	63%
Benzene	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
TAME	<0.001		<0.001		0.01	31%	<0.001		<0.001		<0.001		<0.001	
1,2-Dichloroethane	0.04	7%	0.05	9%	0.10	13%	<0.002		<0.002		<0.002		<0.002	
Trichloroethylene	0.08	10%	0.08	6%	0.17	13%	0.33	19%	<0.001		<0.001		0.04	44%
Bromodichloromethane	0.01	12%	0.01	11%	0.02	13%	0.19	17%	0.08	15%	0.14	2%	0.06	13%
1,4-Dioxane	<0.07		<0.07		<0.07		<0.07		<0.07		<0.07		<0.07	
Toluene	0.25	1%	0.43	3%	0.61	2%	1.85	15%	0.25	5%	0.33	7%	0.40	3%
Tetrachloroethylene	0.07	1%	0.07	2%	0.13	5%	0.10	1%	0.03	6%	0.02	10%	0.01	15%
Dibromochloromethane	<0.005		0.07	3%	<0.005		0.54	6%	0.53	9%	0.63	3%	0.11	3%
Ethylbenzene	0.01	8%	0.06	11%	0.07	12%	0.07	8%	<0.002		<0.002		0.05	5%
m/p-Xylene	0.06	12%	0.05	14%	0.12	9%	0.16	5%	0.07	20%	0.07	11%	0.79	5%
o-Xylene	0.03	4%	0.04	7%	0.07	11%	0.14	4%	0.06	7%	0.04	5%	0.06	3%
Bromoform	0.09	1%	0.09	1%	0.10	1%	0.16	4%	0.16	1%	0.16	3%	0.10	2%
MIB	<0.03		0.08	11%	<0.03		<0.03		<0.03		<0.03		<0.03	
Geosmin	<0.06		<0.06		<0.06		<0.06		<0.06		<0.06		<0.06	

3.4 Conclusions

The results show that the ITEX 2 option can be used as sensitive and robust method in trace analysis of water samples, delivering *MDL*s well below any regulatory limit values. The sensitivity is similar to that of P&T systems, with less instrumental effort and susceptibility to contamination, because of the easy exchange of the needle including the sorbent. The heating unit makes the desorption step independent from the injector temperature profile, which can cause troubles especially for SPDE, where the coating is spread over the length of the needle. Many sorbents well known from gas analysis and P&T are available as trap materials and mixed bed traps tailored for broad analyte ranges are also possible.

3.5 References

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4 In-tube Extraction-GC/MS analysis of volatile beer aroma compounds

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4.1 Introduction

The aroma of beer is the main quality feature for brewers, as well as for the consumers and it is important that an established, well known brand character is consistent over time, to satisfy the expectations of customers.[1] However, quality ratings by expert panels may be based on different criteria than ratings performed by consumers[2] and a stale beer has been found to be more acceptable for drinkers, when it was presented unbranded and they had no expectations how the beer should taste, compared to a branded presentation.[3]

A wide variety of compounds is responsible for the beer aroma. Depending on their contribution to the overall beer aroma, the flavor-active compounds can be classified in four groups (i) primary flavor constituents, (ii) secondary flavor constituents, (iii) tertiary flavor constituents and (iv) background flavor constituents. Examples for primary flavor constituents are ethanol, carbon dioxide or hop bitter substances, which cause a significant change of aroma, if either one of them is removed. The major element of beer aroma is contributed by the entity of the secondary flavor constituents but the absence of a single compound of this group would only lead to a minor change in flavor. Tertiary flavor constituents cannot be perceived individually and all together they add only complementary aromas to the beer, while background flavor constituents may require many similarly flavored compounds to result in a perceptible effect.[4]

The most important alcohols in beer, next to ethanol, are the fusel alcohols 1-propanol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol and phenethylalcohol, which belong to the secondary flavor constituents and can also be oxidized to the corresponding aldehydes during beer aging.[5] *Trans*-2-nonenal was first identified to cause cardboard flavor in beer, with a flavor threshold of 0.1 µg L^{-1} but other linear C_4-C_{10} alkanals, alkenals and alkedienals have been found to have similar flavor properties, too. Another group of aldehydes is formed by the Strecker degradation of amino acids; but only two of the so-called Strecker aldehydes, methional and phenylacetaldehyde, are considered to be relevant for stale flavor formation,

while the others could be used as suitable markers for beer oxidation.[6] Benzaldehyde however, which also belongs to the Strecker aldehydes and gives an almond aroma, can be formed during the malting process, also.[7] Acetaldehyde is considered as an off-flavor, which gives beer an unfinished "green apple" aroma. Other secondary flavor constituents with fruity flavors are volatile esters like ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate, also called "apple esters", 3-methylbutyl acetate or "banana ester" and ethyl acetate as a light, solvent-like flavor.[4] They are produced during fermentation and can be degraded by yeast induced ester hydrolysis during storage, while other esters, like ethyl 3-methylbutanoate or diethyl succinate, are formed during beer aging.[6] A general decrease of fruity flavors and an increase of caramel and burnt sugar aromas can be observed during beer storage.[8]

The main ingredients of beer are water, malt, hop and yeast; although other ingredients like fruits and spices may be added for specialty beers. The yeast is responsible for the synthesis of the main flavor constituents and can be separated in two general classes, top- or warm-fermenting yeast, which ferments at temperatures of 15-25 °C, while bottom- or cold-fermenting yeast ferments at temperatures of 6-14 °C and can also use melibiose, while top-fermenting yeast cannot.[9] The fermentation at higher temperatures is faster and results in higher concentrations of higher alcohols and esters with fruity aromas.[10] Traditionally, top-fermenting yeast is also known as ale yeast and bottom-fermenting yeast as lager yeast, although the brewing procedure of some top-fermented beers also includes cold storage for conditioning.

The analysis of alcoholic beverages usually requires an extraction procedure, to separate the aroma compounds, which come from many chemical classes with varying polarity and volatility, from the matrix. Classical methods are liquid-liquid extraction, vacuum or steam distillation, supercritical fluid extraction, ultrasound extraction and solid phase extraction.[11-14] In this way, more than 620 constituents have been reported in different types of beers by 1996, but many of them do not contribute to the beer aroma and the flavor impact of volatiles is now more in the focus of research.[15] This can be achieved by methods, which reflect the release of aroma compounds from the matrix in a similar way as in a sensory analysis, i.e. static or dynamic headspace techniques.[12] They include purge and trap, desorption tubes, sorbent packed micro columns and, particularly in recent years, microextraction techniques like SPME.[1, 7, 8, 13-21]

ITEX is a relatively new, fully automated microextraction technique for xyz-autosamplers which uses a sorbent filled tube with a fixed steel needle, which is attached to a gas-tight syringe and surrounded by an electrical heater for thermal desorption.[22, 23] The analytes are enriched on the sorbent material by repeated pumping of the sample headspace through the sorbent bed by aspirating and dispensing of the syringe. Analyte injection to the GC is performed after the trap is heated to the set desorption temperature, facilitated by a desorption gas, which can either be a portion of the sample headspace or carrier gas aspirated from the inlet system. Afterwards, the trap is heated and flushed with nitrogen through the syringe side-port hole, to avoid carryover. So far, ITEX has been used for the analysis of volatile environmental pollutants[22, 23], alcohol degradation products from blood and urine[24], aliphatic hydrocarbons from petroleum source rock[25] and in the food area for the analysis of *Torreya grandis* Aril extracts[26], wine and beer[20, 27] and sea-buckthorn[28]. The aim of this work was to develop and evaluate an ITEX method for the analysis of major aroma compounds from alcoholic beverages. Therefore, a wide variety of custom prepared ITEX traps, featuring different kinds of commercial sorbent materials, has been prepared and evaluated, including (to my best knowledge) the first use of an absorbent ITEX trap filled with PDMS. Finally, the found method was applied in the analysis of 46 samples of six beer varieties, established in Germany.

4.2 Experimental

4.2.1 Chemicals, Samples

Pure substances for standard preparation were purchased from different suppliers: acetaldehyde, 1-butanol, 2-butanol, *tert*-butanol, ethyl acetate, 2-ethylhexanol, ethyl octanoate, 1-hexanol, 2-methylbutanol, 3-methylbutanol, 2-methylpropanol, 1-pentanol, 3-pentanol, 1-propanol and 2-propanol from Fluka (Sigma-Aldrich, Steinheim, Germany); ethyl butanoate, ethyl decanoate, ethyl hexanoate, ethyl 3-methylbutanoate, geraniol, linalool, 3-methylpentanol, 2-phenethyl acetate and *trans*-2-nonenal from Aldrich (Sigma-Aldrich); benzaldehyde, 3-methylbutyl acetate and (R)-(+)-limonene from Sigma-Aldrich and diethyl succinate from Merck (Darmstadt, Germany). CAS registry numbers, analytical grades, logarithmic air-water ($log\ K_{aw}$) and octanol-water partitioning constants ($log\ K_{ow}$) of all compounds can be found in **Table 4.1**. Stock solutions were prepared with ethanol (99.8%) from Riedel de Haën (Seelze, Germany). Analytical grade water from a PURELAB Ultra Analytic water purification system (ELGA LabWater, Celle, Germany) was used to prepare standard solutions and to dilute samples.

4.2.2 Standard- and Sample Preparation

Two stock solutions were made; the first (*Stock 1*) was used for extraction phase evaluation and method optimization and contained 500 µL L^{-1} of each analyte, except of the aldehydes and limonene, which have been added to the compound set, later. The second stock solution (*Stock 2*) was prepared to adjust for the differences in sensitivity between analytes and was composed to cover the whole linear range of all analytes for a more efficient calibration procedure. The composition of the second stock solution is given in **Table 4.1**. The highest concentrated calibration solution was prepared with 200 µL of *Stock 2* filled up to 200 mL with water; the lower concentration levels were prepared by a serial dilution with a dilution factor of two, resulting in a 14-point calibration spanning a concentration factor of 8192.

46 Beers from six German varieties Altbier (A, n=4), Helles (H, n=6), Kölsch (K, n=6), Pilsener Bier (P, n=25), Schwarzbier (S, n=1) and Weizenbier (wheat beer) (W, n=4) were analyzed. Three varieties are produced with top-fermenting yeast, they were Kölsch, which is a light lager, Weizenbier, where a major proportion of barley is replaced by malted wheat and Altbier, a dark lager which gets its colour from roasted malt. The other three varieties are produced with bottom-fermenting yeast and are Helles, a light lager, Schwarzbier, a dark lager and Pilsener Bier, which is a pale lager with hoppy aroma. Two Pilsener and one Weizen were alcohol-free beers (marked with \alc). The beer samples have been cooled on ice to minimize the loss of volatile aroma compounds to the gas phase, before they were diluted by a factor of ten. Because precise measurement of the liquid volume of beer is difficult due to foam formation, 10 g of beer have been weighted in a 100-mL volumetric flask and were filled up with water. The gas content of the diluted solution was then low enough to be handled by a volumetric pipette.

10 mL of each standard or sample solution were transferred into a 20-mL amber headspace vial (BGB Analytik AG, Boeckten, Switzerland), with an 8×3 mm PTFE laminated magnetic stir bar (VWR International GmbH, Darmstadt, Germany). The vials were closed by magnetic screw caps with rubber/PTFE septa (BGB Analytik AG)

Table 4.1 Compound information and composition of stock solutions; *log K_{aw}* and *log K_{ow}* taken from the experimental data database of the EPI Suite™ v4.11 of the U.S. Environmental Protection Agency[29], if not stated otherwise

Compound	CAS-nr.	Purity (%)	log K_{aw} (25°C)	log K_{ow}	*Stock 2* concentration mmol L^{-1}	(g L^{-1})
Alcohols						
1-Propanol	71-23-8	≥ 99.9	-3.52	0.25	40.1	2.41
2-Propanol	67-63-0	≥ 99.9	-3.48	0.05	39.2	2.36
2-Methylpropanol	78-83-1	≥ 99.8	-3.40	0.76	43.3	3.21
1-Butanol	71-36-3	≥ 99.9	-3.44	0.88	43.7	3.24
2-Butanol	78-92-2	≥ 99.8	-3.43	0.61	43.5	3.23
tert-Butanol	75-65-0	≥ 99.8	-3.43	0.35	31.6	2.34
2-Methylbutanol	137-32-6	≥ 99.5	-3.24	1.29	37.2	3.28
3-Methylbutanol	123-51-3	≥ 99.8	-3.24	1.16	36.7	3.24
1-Pentanol	71-41-0	≥ 99	-3.27	1.51	37.0	3.26
3-Pentanol	584-02-1	≥ 99.5	-3.09	1.21	37.0	3.26
3-Methylpentanol	589-35-5	99	-3.14*	1.75*	32.3	3.30
1-Hexanol	111-27-3	98	-3.16	2.03	31.8	3.25
2-Ethylhexanol	104-76-7	≥ 99.5	-2.97	2.73*	38.4	5.00
Aldehydes						
Acetaldehyde	75-07-0	≥ 99.5	-2.56	-0.34	35.6	1.57
Benzaldehyde	100-52-7	≥ 99	-2.96	1.48	39.6	4.20
trans-2-Nonenal	18829-56-6	97	-2.02*	3.06*	36.2	5.08
Esters						
Ethyl acetate	141-78-6	≥ 99.9	-2.26	0.73	40.7	3.59
Ethyl butanoate	105-54-4	99	-1.79	1.85*	37.8	4.40
Ethyl 3-methylbutanoate	108-64-5	98	-1.54	2.26*	33.2	4.32
3-Methylbutyl acetate	123-92-2	> 99	-1.62	2.25	33.6	4.38
Ethyl hexanoate	123-66-0	99	-1.53*	2.83*	30.3	4.37
Ethyl octanoate	106-32-1	≥ 98	-1.28*	3.81*	35.2	6.07
Ethyl decanoate	110-38-3	99	-1.04*	4.79*	34.4	6.90
Diethyl succinate	123-25-1	99	-4.67	1.20	35.8	6.24
2-Phenethyl acetate	103-45-7	99	-3.11*	2.30*	37.7	6.20
Terpenes						
Geraniol	106-24-1	98	-3.33	3.56	34.6	5.33
Limonene	5989-27-5	97	0.12	4.57	37.0	5.04
Linalool	78-70-6	≥97	-3.06	2.97	33.8	5.22

* Values estimated by EPI Suite™ v4.11 programs HenryWin v3.2 and KOWWIN v1.68

4.2.3 Sorbent Materials

Nine sorbent materials were tested for their extraction efficiency for the target analytes, they were Carbopack C (CC), Carboxen 1000 (C1000), Carbosieve S III (CSIII), Tenax TA (TTA) (Poly-(2,6-diphenyl-)-p-phenyloxide), Tenax GR (TGR) (Tenax TA with graphitized carbon), HayeSep D (HSD) (divinylbenzene (DVB)), multi-walled carbon nanotubes (MWCNTs) (Baytubes C 150 HP, Bayer Material Science, Leverkusen, Germany), polydimethylsiloxane (PDMS) and Carbowax 20M (polyethylene glycol (PEG) with a molecular weight of 20000); more information can be found in **Table 4.2**. PDMS and PDMS blue (PDMSb) particles were prepared from white silicone and IceBlue septa (Restek Corporation, Bellefonte, PA, USA) which have been frozen with liquid Nitrogen and were ground and sieved subsequently. Carbowax 20M could only be applied as a fraction of 10% in a mixed bed trap with PDMS

because it melts at desorption temperature and might drip out of the trap or block it, after cooling down again. A two-sorbent bed, prepared of ⅔ Tenax GR and ⅓ Carbosieve S III, was used based on previous experiences,[23] resulting in a total of eleven different ITEX traps tested.

Table 4.2 Properties of sorbent materials used (manufacturer data, if not stated otherwise; n.a. is not available)

Sorbent	Sorbent type	Specific surface area	Temperature limit	Water affinity	Typical applications
Carbopack C	Graphitized carbon black	10 $m^2 g^{-1}$	500 °C	Relatively low	Low to medium boilers (C_{12}-C_{20})
Carboxen 1000	Carbon molecular sieve	1200 $m^2 g^{-1}$	225 °C	Moderate	Permanent gases, volatiles (C_2-C_5)
Carbosieve S III	Carbon molecular sieve	975 $m^2 g^{-1}$	400 °C	Moderate	Volatile organics (C_2-C_5)
Tenax GR	70% porous organic polymer/ 30% graphitized carbon	24 $m^2 g^{-1}$	350 °C	Low	Volatiles, flavors
Tenax TA	Porous organic polymer	35 $m^2 g^{-1}$	350 °C	Low	Volatiles and semi-volatiles (C_7-C_{26})
HayeSep D	Porous organic polymer	795 $m^2 g^{-1}$	290 °C	Low	Volatiles (C_1-C_6)
MWCNT	Multi wall carbon nanotubes	[a)] 211 $m^2 g^{-1}$	n.a.	n.a.	n.a.
PDMS & PDMS blue	Silicone rubber	Absorbent	250 °C	Low	Unpolar volatiles, semi-volatiles
Carbowax 20M	Polyethylene glycol	Absorbent	225 °C	High	Polar semi-volatiles

[a)] BET measurement

4.2.4 Sample Extraction and Injection

Sample extraction and injection were executed by a CTC Combi PAL autosampler (Axel Semrau, Sprockhövel, Germany), holding a thermostated TrayCooler2 for 20-mL headspace vials, a heat able Single Magnet Mixer (SMM) (Chromtech, Idstein, Germany) and the ITEX II option kit (CTC Analytics AG, Zwingen, Switzerland), consisting of a heated syringe holder for a 1.3-mL gas-tight Hamilton syringe with side port (Hamilton, Bonaduz, Switzerland) and the ITEX trap heater. The extraction was performed by custom made macros. Only the optimized ITEX procedure, used for sample analysis, is presented here, more details regarding method development are given in the results and discussion section.

The samples were incubated and stirred at 70 °C for 20 minutes in the SMM to equilibrate the sample headspace prior to extraction. During the incubation time, the ITEX trap was heated to 250 °C, while being flushed with 5 mL min^{-1} of nitrogen for 10 minutes, for preconditioning. After the trap was cooled down to 30 °C and the incubation time was over,

65 extraction strokes of 1 mL were performed with a flow of 100 µL s^{-1}. Then 1 mL of helium was aspirated and dispensed at an unconnected ATAS GL Optic 3 injection port (Axel Semrau), to remove residual carbon dioxide of the sample from the void volume of the trap. Following the transfer of the trap to the split/splitless injector (S/SL), 500 µL of helium were aspirated as desorption gas and after the trap was heated to 250 °C, injected with a desorption flow of 50 µL s^{-1}. The whole procedure for each sample (including incubation/equilibration, extraction, injection and needle flushing) takes about 42 minutes and can be performed in parallel to the GC analysis, thus making the GC temperature program the total analysis time-determining step.

4.2.5 GC/MS Measurements

All measurements were made using a Thermo Trace GC Ultra, coupled to a Thermo DSQ II single quadrupole mass spectrometer (S+H Analytik, Mönchengladbach, Germany). The GC was equipped with an Optic 3 programmable temperature vaporizer with a nitrogen cooled cold trap for on-column focusing and a S/SL injector, where the column was connected. Injection was performed into the S/SL set to 200 °C in splitless mode, the column flow was set to 1.5 mL min^{-1} constant flow for 31 minutes and was then raised to 2 mL min^{-1} until the end of the temperature program, to accelerate the elution of low volatile compounds. After injection, the analytes were transferred to an uncoated 0.53 mm inner diameter (*i.d.*) fused silica capillary (BGB Analytik AG) and cryo-focused in the cold trap at -150 °C. After a hold time of two minutes, the trap was heated with 50 °C s^{-1} to 250 °C and the analytes were transferred to the chromatographic column. Separation of compounds was performed on a Stabilwax-DA fused-silica capillary column (cross bonded carbowax (PEG)) with 60 m length, 0.32 mm *i.d.* and 1 µm film thickness (Restek GmbH, Bad Homburg, Germany). The GC oven temperature program started at 35 °C for 5 min and then heated with 5 °C min^{-1} to 110 °C, held for 2 min and heated further with 10 °C min^{-1} to 200 °C with a hold time of 10 min. The MS transfer line was set to 220 °C, the ion source temperature was 200 °C; Electron ionization (EI), with an ionization energy of 70 eV, was used in scan mode (m/z = 29-200), with a scan rate of 500 amu s^{-1}. Instrument automation, data acquisition and data processing were performed using the Xcalibur 1.4 data system (S+H Analytik).

4.3 Results and Discussion

4.3.1 Method Optimization

The ITEX procedure comprises of three main steps: sample extraction, sample injection and trap conditioning for the next analysis; several parameters can be optimized in each of these steps. The extraction parameters, next to the choice of the most suitable extraction material, are the temperatures of the trap and the sample solution and the number, volume and flow of the extraction strokes. The parameters for injection are the desorption gas volume and flow and the desorption temperature; trap conditioning includes conditioning temperature and time.

Two of the extraction parameters will not be discussed here. The first is the choice of the extraction material, which will be covered in the next section, because it was performed after the optimization of the parameters and the second is the trap temperature, which should normally be set to the lowest possible value, due to the exothermic nature of the sorption process. Sample temperatures were tested from 40 °C to 80 °C in intervals of 10 °C. The majority of analytes shows increasing extraction efficiency in the whole tested range, but about a third of the analytes begin to lose sensitivity, when the temperature is raised from 70 °C to 80 °C. Here it is an advantage of ITEX that the sorption material is located outside of the heated sample vial, because a similar loss of sensitivity has been observed for the analysis of terpenes using HS-SPME, already at temperatures above 40 °C.[30] The extraction flow and the number of extraction strokes have to be optimized together, because their combination defines the efficiency and duration of the extraction procedure. An extraction flow of 10 μLs^{-1} gives the highest peak areas for all compounds, which drop to 33-94% of the area at 50 μLs^{-1} and 28-88% at 100 μLs^{-1}, with averages of 63% and 52%, respectively. On the other hand, when 10 extraction strokes are defined as 100%, the extracted amount increases to 119-1337% for 100 extraction strokes, with an average of 626%. The average peak areas for 25, 50 and 75 extraction strokes were 228%, 299% and 399%, respectively. No analyte reached equilibrium within the tested 100 extraction strokes. As an increase in the number of extraction strokes by a factor of ten results in about six times larger peak areas, while the same increase of the extraction flow only halves the peak areas, a high number of extraction strokes with a high flow gives a better extraction efficiency in the same extraction time. Consequently, 65 extraction strokes with an extraction flow of 100 μLs^{-1} were chosen, which was the maximum to be performed in parallel to the GC-oven runtime.

Desorption volumes of 100 µL, 500 µL and 1000 µL were tested. An increase of the resulting peak areas for most compounds can be observed from 100 µL to 500 µL, while a further increase is only visible for low volatile compounds, like geraniol, ethyl decanoate or diethyl succinate, when the desorption volume is raised from 500 µL to 1000 µL. Because the gain in sensitivity was not significant and limited to few compounds, a desorption volume of 500 µL was chosen. The desorption flow showed a similar behavior, with a decline of peak areas from a flow of 10 $\mu L\ s^{-1}$ to 50 $\mu L\ s^{-1}$, while only the peak areas of the low volatile compounds further decrease at a flow of 100 $\mu L\ s^{-1}$. As the reproducibility got better with higher desorption flows, 50 μL^{-1} were used. The optimization results also agree with the ones found for environmental contaminants in an earlier work.[23]

4.3.2 Choice of Extraction Phase Material

The comparison of the extraction materials was performed with a 5 $\mu g L^{-1}$ standard solution and the optimized method parameters given before, but with adapted desorption temperatures for each trap. Based on previous experiences, which indicated that the best desorption efficiency is reached at high desorption temperatures[23], the desorption temperature was set at or near the maximum temperature for each sorbent (see **Table 4.2**). The desorption temperatures were set to 300 °C for Carbopack C, Carbosieve S III, Tenax GR, Tenax TA and the MWCNTs, to 250 °C for HayeSep D and PDMS and to 200 °C for the trap containing Carbowax 20M and C1000.

A summary of the total extracted amounts with each trap is given in **Figure 4.1**. The best overall extraction yield was achieved with HayeSep D, Tenax GR, Tenax TA and Carbopack C; the PDMS based absorbent traps were in a medium range and the traps using molecular sieves and carbon nanotubes had the lowest yield. Information on the extracted amount per compound can be found in **Table 4.3**. HSD was most effective for the trapping of long chain esters and terpenes, but less suitable for the more polar alcohols, where TTA was the best sorbent. TGR had a lower efficiency for alcohols than TTA and was best suited for short chain esters. CC as an apolar material also had the highest yields for esters and long chain alcohols, but, complementary to HSD, very low efficiency for long chain esters and terpenes, which may be caused by irreversible adsorption or the degradation of analytes; similar reasons might be responsible for the low extraction yields of the traps containing carbon molecular sieves. It has been found, that the surface of carbon based adsorbents can be activated during the conditioning, even with a stream of inert gas and then cause analyte loss by transformation reactions, especially for alcohols and carbonyl compounds.[31] The same

may also apply for the MWCNTs, where more research is undergoing at the moment. Because the sensitivity for esters was generally better than for alcohols and the differences in the total extracted amount were quite small, the carbon based adsorbents were excluded and TTA was initially chosen as sorbent material.

However, when the linear range was determined during method evaluation, it proved to be quite limited for TTA (see **Table 4.4**) which lead to an inferior repeatability for beer samples, than for standard solutions, because of competition and displacement effects of ethanol. Moreover, the compound set was extended with aldehydes, including benzaldehyde, which can be formed as degradation product of Tenax TA during thermal desorption.[32] When the carbon and Tenax based sorbents were ruled out because of their reactivity with alcohols and carbonyl compounds or interfering degradation product formation, only HSD and the PDMS based traps remained. However, HSD as adsorbent material would suffer from the same competition effects as TTA while the equilibrium conditions of absorbent materials do not vary until loadings of several percent are present.[33] This rendered a re-evaluation of sorbent materials for the enhanced compound set unnecessary and consequently, the trap containing PDMS blue was used, which had the best performance of the remaining sorbents and promised to possess a wider linear range, being an absorbent material.

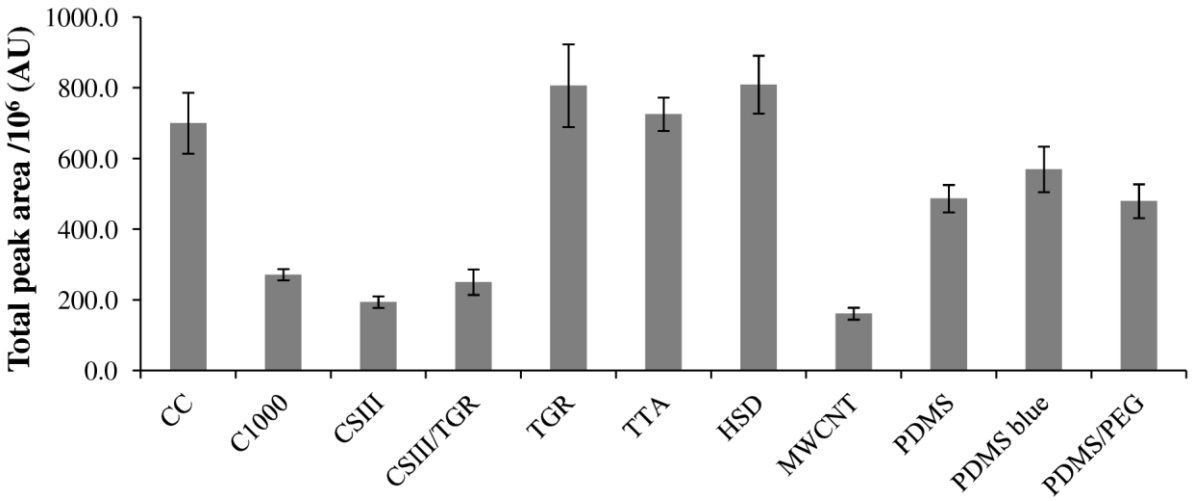

Figure 4.1 Summarized peak areas with average repeatability of all analytes

Table 4.3 Comparison of extraction trap efficiencies by the average peak areas of a triplicate analysis of a 5 µgL^{-1} standard solution and the average of the relative standard deviations of all compounds

	CC	C1000	CSIII	C.SIII/ TGR	TGR	TTA	HSD	MW CNT	PDMS	PDMS blue	PDMS / PEG
	Peak area /1,000,000										
Alcohols											
1-Propanol	5.2	7.6	7.8	2.7	7.6	6.4	2.0	0.1	3.7	3.4	2.7
2-Propanol	0.3	0.6	0.8	n.d.	0.5	0.4	0.4	n.d.	0.3	0.3	0.2
2-Methylpropanol	0.6	1.4	1.2	1.2	1.2	5.1	1.5	1.6	1.0	0.9	0.8
1-Butanol	2.8	0.7	3.0	1.0	4.9	4.8	4.4	0.1	1.9	2.4	1.8
2-Butanol	10.8	18.5	16.1	2.5	22.2	14.5	14.3	0.07	28.7	41.0	36.2
tert-Butanol	14.4	31.5	11.4	4.8	27.6	5.5	24.7	0.7	22.1	12.2	11.9
2-Methylbutanol	5.7	5.4	3.7	2.5	9.1	7.4	6.6	0.3	3.1	4.1	3.3
3-Methylbutanol	5.6	7.0	2.3	2.1	5.8	4.6	3.9	1.2	1.9	2.5	2.0
1-Pentanol	4.3	2.6	1.9	1.5	3.4	6.0	4.4	0.2	1.9	2.5	2.1
3-Pentanol	4.0	3.7	6.3	1.1	7.9	30.4	4.9	0.2	6.4	6.5	7.6
3-Methylpentanol	6.3	2.6	1.6	1.6	8.0	8.3	5.9	0.4	3.1	3.9	3.0
1-Hexanol	9.0	3.0	1.9	2.3	10.2	12.9	7.9	1.2	4.4	5.0	3.9
2-Ethylhexanol	24.0	4.0	3.1	4.4	24.1	40.5	39.2	6.7	24.9	14.4	11.8
Esters											
Ethyl acetate	10.4	26.1	20.9	15.9	21.9	18.7	20.2	4.5	10.1	6.5	6.5
Ethyl butanoate	144.1	69.3	37.3	43.9	129.8	109.3	145.2	7.2	56.1	39.7	47.6
Ethyl 3-methylbutanoate	34.8	9.7	4.6	12.5	94.0	36.7	38.3	4.1	26.8	37.7	16.6
3-Methylbutyl acetate	113.4	7.5	10.4	18.0	61.3	1.4	33.9	47.2	49.6	99.3	57.2
Ethyl hexanoate	111.5	16.1	7.5	23.7	134.9	118.1	92.8	11.2	40.6	71.8	52.2
Ethyl octanoate	73.7	7.1	5.9	28.5	85.1	154.5	124.8	35.3	115.6	73.8	55.0
Ethyl decanoate	114.5	40.8	41.8	73.5	129.5	114.3	181.7	32.8	58.5	126.8	141.4
Diethyl succinate	0.07	0.7	0.3	0.3	1.4	0.7	1.7	0.05	0.8	0.5	0.7
2-Phenethyl acetate	1.2	2.1	1.2	4.4	10.8	6.6	23.5	0.7	9.8	4.5	5.8
Terpenes											
Geraniol	0.3	2.2	2.1	0.8	3.2	2.2	6.5	0.4	2.2	3.4	3.6
Linalool	3.3	1.4	1.0	1.2	1.8	16.5	20.6	5.1	13.2	6.3	5.6
Sum	700.3	271.6	194.1	250.4	806.2	725.8	809.3	161.3	486.7	569.4	479.5
Average *RSD* (%)	12.3	5.8	8.4	14.4	14.5	6.5	10.1	10.4	8.0	11.3	10.0

4.3.3 Method Performance Parameters

Method performance parameters were evaluated for the initially used TTA trap and the finally used PDMSb trap. The method detection limit (*MDL*), precision and linear range were determined for both traps, the recovery and extracted analyte fraction only for the latter one. The results are summarized in **Table 4.4**.

The *MDL* of each analyte was determined by a procedure of the U.S. Environmental Protection Agency, as the minimum concentration of an analyte that can be reported greater than zero with a confidence of 99%. It is based on the standard deviation of a sevenfold analysis with an analyte concentration resulting in a signal to noise ratio of about three to five.[34] The precision was calculated as the average of all calibration points within the linear range and a range factor (*RF*) was defined by dividing the upper limit of the linear range by the lower limit, for an easier comparison of linear ranges between sorbent materials. The

*MDL*s which were achieved with the TTA trap ranged from 0.01 µg L^{-1} to 17.5 µg L^{-1} with a median of 0.2 µg L^{-1}; except for benzaldehyde, where no quantification was possible because of artifact formation by TTA during desorption. The average *MDL* for the alcohols is 1 µg L^{-1}, while it is 0.17 µg L^{-1} for the esters without diethyl succinate (2.1 µg L^{-1} when included), which is mainly influenced by the much higher air-water partitioning coefficient (K_{aw}, see **Table 4.1**) of most of the esters, than by the selectivity of TTA towards the analytes. K_{aw} of diethyl succinate is three orders of magnitude lower, than of the other esters and the lowest of all analytes. Precision with TTA was consistent in a narrow band between 6.2% and 10.0%, but the linear range was quite limited and *RF* was spanning from 25 to 540, with an average of 136 and a median of 88, giving less than two orders of magnitude for most analytes. The precision achieved for beer samples was worse than for standard solutions (>20% *RSD*), due to the low *RF*, the wide range of analyte concentrations in the samples and competition effects, even when the beer has been diluted by a factor of 50. *Zapata et al.* experienced similar problems with mass saturation of TTA and solved it by using a stronger sorbent (Bond Elut ENV) and very small sample amounts (10 µL, diluted by a factor of 5) to achieve quantitative analyte extraction.[20] A different approach has been chosen here, where an absorbent material was used, which does not suffer from competition and saturation effects.[35]

The *MDL*s for the PDMSb trap were mostly higher than for TTA, ranging from 0.3 µg L^{-1} to 13 µg L^{-1}, with a median of 1.6 µg L^{-1}. They were lower for diethyl succinate, which had by far the highest *MDL* with TTA, 2-phenethyl acetate and geraniol; furthermore, the quantification of benzaldehyde was possible with PDMSb, because no artifacts were formed by the sorbent. The largest difference was observed with 3-methylbutyl acetate, where the *MDL* was 410 time higher with PDMSb, but apart from this, the difference was about a factor of ten and the sensitivity was still good enough, to dilute the samples by a factor of ten, before the analysis. The same trend for the sensitivities of alcohols and esters that has been observed with TTA also applied for the extraction with PDMSb. The precision was less consistent, ranging from 1.2% for 3-pentanol to 19.9% for *trans*-2-nonenal, but overall it was better, with less than 5% for the majority of analytes. The linear range improved, as it was expected using a sorbent based on analyte partitioning, rather than adsorption. *RF* was between 32 and 4485, with an average of 666 and a median of 391, being about 5 times larger. The limit of the linear range was not reached within the applied concentration range for 1-propanol, 2-propanol, 2-methylpropanol, 2-butanol, 2-methylbutanol, 3-methylbutanol and 3-pentanol, which could make the difference even bigger.

When the *MDL*s are compared to those of the SPDE (solid phase dynamic extraction) method for a similar compound set in Chapter 2 (**Table 2.1**), which was previously used to analyze wine samples[36], the ITEX method is more or less sensitive, depending on the applied sorbent material. Compared to the results with TTA, the *MDL*s of the SPDE method are lower for diethyl succinate and 2-phenethylacetate, equal for ethyl hexanoate and 3-pentanol, and between 1.3 to 570 times higher for the other compounds, with a median of 3.6. With the PDMSb phase, the *MDL*s of the ITEX method are lower by a factor of four for seven of 22 analytes, equal for 2-phenethyl acetate and higher by an average factor of five for the other 14 compounds, analyzed in both methods. The lower *MDL* using the TTA phase should be caused by stronger sorption of the analytes and the larger sorbent volume of ITEX, compared to the 4.5 µL PEG phase which was used in SPDE. On the other hand, PEG is more polar than PDMS, making the SPDE method more sensitive for nine of 13 of the analyzed alcohols. As no data on the linear range of the SPDE method is available, it is difficult to estimate if the smaller sorbent volume has negative effects on the extraction capacity. *Liu et al.* presented a method for similar analytes from beer samples, using sol-gel derived SPME fibers; The limits of detection are comparable to those of the ITEX method with TTA sorbent and lower than ITEX with PDMSb, but the linear range of the SPME method is smaller.[37] The detection limits of the earlier mentioned ITEX method for quantitative extraction by *Zapata et al.* are between 3 and 117 times larger than for the method presented here; however, they were still low enough for the analysis in natural abundance in beer and wine. A comparison of linear ranges is not feasible, because it has only been determined in the expected analyte range.[20]

The recovery was calculated by the analysis of pure and spiked beer samples, which were measured undiluted, diluted by a factor of ten and diluted by a factor of 100; the spike was made using 1 µL, 5 µL and 10 µL of the standard solution *Stock 2* in 100 mL sample, respectively. In this way, the recovery for all analytes could be calculated from at least one analysis within the linear range; when two or all measurements were within the linear range, an average was calculated. The recoveries were between 91.8% for diethyl succinate and 131.7% for 3-pentanol, with an average of 111%. Considering the difficult handling of the undiluted samples, due to foam formation, that had to be transferred to the sample vials after spiking, it seems acceptable that most analytes show recoveries of 100% ±15%. The recovery for 2-propanol could not be determined because the peak co-eluted with ethanol and in this period, the detector was turned off to avoid detector saturation, when analyzing beer samples.

Table 4.4 Method performance parameters of the evaluated ITEX-traps. Method detection limit (*MDL*), precision (as relative standard deviation (*RSD*)), linear range (absolute and range factor (RF), +: upper limit not reached within applied concentration range), recovery from spiked samples and extracted analyte fraction (F_e)

	Tenax TA				PDMS blue					
	MDL	*RSD*	Linear range		*MDL*	*RSD*	Linear range		Recovery	F_e
	(µg L^{-1})	(%)	(µg L^{-1})	*RF*	(µg L-1)	(%)	(µg L^{-1})	*RF*	(%)	(%)
Alcohols										
1-Propanol	2.1	6.2	3.2 – 80	25	7.0	1.7	37.7 – 2410+	64+	97.2	30.3
2-Propanol	3.6	9.9	3.6 – 236	66	11.3	7.8	36.8 – 2343+	64+	-	8.2
2-Methylpropanol	1.6	9.7	3.2 – 241	75	13.0	8.8	50.1 – 3208+	64+	105.8	12.6
1-Butanol	0.3	8.4	0.4 – 41	103	5.0	1.8	12.7 – 1620	128	121.4	2.5
2-Butanol	1.1	8.8	1.6 – 242	151	3.1	2.1	6.3 – 3225+	512+	120.9	5.3
tert-Butanol	0.3	8.9	0.6 – 47	78	2.2	4.5	2.3 – 146	64	104.5	5.6
2-Methylbutanol	0.1	7.7	0.3 – 10	33	1.6	2.1	1.6 – 3276+	2048+	109.3	5.8
3-Methylbutanol	0.1	7.4	0.3 – 24	80	3.0	4.2	6.3 – 3236+	512+	110.4	9.3
1-Pentanol	0.2	7.1	0.2 – 81	405	1.4	7.6	6.4 – 204	32	100.0	7.2
3-Pentanol	3.1	8.6	3.3 – 815	247	4.3	1.2	6.4 – 3260+	510+	131.7	6.2
3-Methylpentanol	0.3	7.0	0.3 – 33	110	1.5	7.1	1.6 – 1648	1030	111.0	6.6
1-Hexanol	0.08	6.9	0.2 – 16.3	82	1.1	3.5	1.6 – 1627	1017	117.3	6.4
2-Ethylhexanol	0.08	7.3	0.08 – 33	413	1.8	15.3	2.4 – 1250	521	102.4	5.8
Aldehydes										
Acetaldehyde	3.1	8.3	3.1 – 157	51	5.1	2.3	6.1 – 784	128	113.3	15.9
Benzaldehyde	-	-	-	-	7.2	9.3	4.1 – 1050	256	106.0	5.6
trans-2-Nonenal	0.2	7.8	0.2 – 25.4	127	0.5	19.9	0.6 – 1269	256	112.3	11.9
Esters										
Ethyl acetate	0.1	8.5	0.1 – 54	540	0.6	1.8	0.4 – 1794	4485	106.8	9.8
Ethyl butanoate	0.02	7.3	0.04 – 3.5	88	0.5	2.3	0.5 – 1099	2198	108.4	24.9
Ethyl 3-methylbutanoate	0.06	7.3	0.4 – 26	65	0.5	2.2	0.5 – 68	136	115.4	38.9
3-Methylbutyl acetate	0.01	8.1	0.04 – 1.1	28	4.1	1.9	4.3 – 1095	255	121.3	34.7
Ethyl hexanoate	0.08	9.3	0.2 – 18	90	0.4	3.2	0.5 – 546	1092	120.7	35.8
Ethyl octanoate	0.01	8.6	0.02 – 1.7	85	0.5	4.4	0.7 – 379	541	123.3	37.3
Ethyl decanoate	0.1	10.0	0.2 – 26	130	0.7	6.4	0.8 – 108	135	125.2	47.3
Diethyl succinate	17.5	7.6	21 – 1040	50	2.1	6.3	6.1 – 3120	511	91.8	3.6
2-Phenethyl acetate	1.0	8.9	1.0 – 155	155	0.3	2.9	0.8 – 194	243	96.1	3.8
Terpenes										
Geraniol	5.8	9.3	5.8 – 667	115	0.8	4.0	2.6 – 1334	513	103.7	5.7
Limonene	0.07	9.5	0.08 – 4.2	53	0.7	11.6	1.2 – 1260	1050	122.6	77.9
Linalool	0.06	7.5	0.2 – 44	220	0.9	8.4	0.9 – 163	272	109.5	2.3

The extracted fraction (F_e) of analyte, which is removed from the sample vial in each analysis, was calculated following the depletion method proposed by *Zimmermann et al.*, by consecutive analyses from the same sample vial.[38] To that end three samples have been extracted for five times each and the logarithmic peak areas of the analytes were plotted over the number of extractions to obtain a linear graph, from which F_e could easily be calculated by the slope. F_e ranged from 2.3% for linalool to 77.9% for limonene. Here it was apparent that the octanol-water partitioning coefficient (K_{ow}, **Table 4.1**), which can be used to estimate the sorption of analytes to PDMS,[35, 39] has lower influence on the extraction efficiency than K_{aw}. Limonene has the highest F_e and the highest values for K_{aw} and K_{ow}, but ethyl hexanoate and linalool have a similar log K_{ow} at 2.83 and 2.97 with extracted fractions of 34.7% and 2.3%, so that the difference must originate in K_{aw}. Also, 1-pentanol and geraniol have similar

log K_{aw} at -3.27 and -3.33, respectively but while log K_{ow} of geraniol is larger by a factor of two, F_e of 1-pentanol is two times larger (7.2%) than for geraniol (3.56%). When F_e is plotted over log K_{aw} (**Figure 4.2 a**)) and log K_{ow} (**Figure 4.2 b**)), a roughly linear trend can be observed for K_{aw}, while no trend is visible for K_{ow}. However, this should be considered merely as a rule of thumb for ITEX-users to estimate the probable extraction efficiency, because the system is not in equilibrium and the air-water and octanol-water partitioning coefficients that can be found in literature can show quite large variations for some compounds.

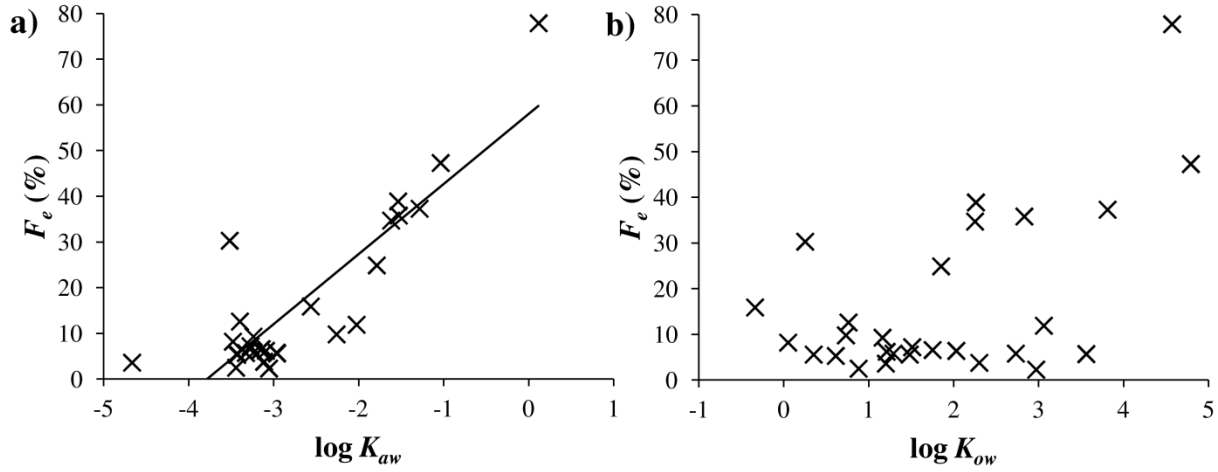

Figure 4.2 Graphical depiction of a) F_e over log K_{aw}, b) F_e over log K_{ow}

4.3.4 Quantitative Analysis

An overview on the detected concentration ranges of all analytes and the number of samples, in which they were detected, is given in **Table 4.5**. A complete list of the beer samples and analytes is given in **Table S 4.1** et seqq. in the supporting information of this chapter. 2-Propanol could not be detected in the actual beer samples, because it co-eluted with the ethanol peak, for which the detector was turned off, to avoid saturation. If the peak would have been detected, quantification could have been performed by the mass selective detector, but, as previous work showed, only with a high *RSD* of about 20%.[36] Three analytes were not detected in any beer sample, 3-pentanol, 3-methylpentanol and benzaldehyde were always below the method detection limits. *Trans*-2-nonenal was only detected in two samples (H06 and P04) in a concentration of about 6 µg L^{-1}, making acetaldehyde the only one of the investigated aldehydes, which was found in considerable amounts, with concentrations between 2.6 mg L^{-1} and 13.2 mg L^{-1}.

The sample concentration exceeded the determined linear range for four analytes, acetaldehyde ethyl acetate, 2-methylpropanol and 3-methylbutanol. In the case of ethyl

acetate, five of 46 samples had higher concentrations, four were about 20% higher and one was 78% higher. Eleven samples contained acetaldehyde above the linear range of the method, but only four exceeded it by more than 20%. 21 samples contained 2-methylpropanol above the upper limit of the determined linear range, which was also the upper limit of the used calibration solutions, with the highest value being almost 3 times higher than the linear range. 33 of 46 samples had concentrations of 3-methylbutanol above the highest concentrated calibration solution, similar as 2-methylpropanol. The problems with 2-methylpropanol and 3-methylbutanol have not been evident during the method development, because the concentration in the beer, which was used for test measurements, was within the linear ranges of each analyte. For future applications it needs to be checked if the problem can be solved by an increased calibration range, a stronger dilution of the samples or a combination of both.

Eight analytes were detected in all 46 beer samples; they were 1-propanol, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol, ethyl acetate, ethyl hexanoate, ethyl octanoate and acetaldehyde. Ethyl butanoate, 3-methylbutyl acetate and 2-phenethyl acetate were detected in 45 samples and all three were missing in sample P25\alc, an alcohol-free Pilsener beer. Ethyl decanoate was not found in two alcohol-free beers, P25\alc and W01\alc and diethyl succinate was absent in four samples (P05, P24\alc, P25\alc and W01\alc), three of them were again alcohol-free variants. Most of these compounds belong to the secondary flavor constituents and play an important role in the overall beer aroma. Acetaldehyde as an off-flavor has an individual flavor threshold of 10 mg L^{-1} in beer[4], which was only reached in three samples: H06, P23 and W03 from the groups of Helles, Pilsener beer and wheat beer. H06 is a beer which is brought to higher alcohol content by the addition of glucose syrup, P23 is a rather cheap beer which might probably have insufficient storage time and W03 is a special dark wheat beer.

The sum of the higher alcohols is dominated by the four fusel alcohols 1-propanol, 2-methylpropanol, 2-methylbutanol and 3-methylbutanol, which contribute more than 99% of the measured higher alcohol content. The top-fermenting beers had, other than expected, a similar average concentration of higher alcohols ($\varnothing$ = 97.0 mg L^{-1}) as the bottom-fermenting beers ($\varnothing$ = 97.5 mg L^{-1}) and no significant difference (Welch's *t*-test, on-tailed, $\alpha = 0.05$, *p*-value = 0.476) could be observed. The variations between the different varieties in each beer class were quite big and the variations within of each variety of beer could also be considerably. The sum of alcohols in the Pilsener beers for example was ranging from

51.4 mg L^{-1} in P06 to 168.4 mg L^{-1} in P15, which is a beer with increased ethanol content. No significant difference (p-value = 0.343) was observed for the terpenes, also. A changed outcome was achieved for acetaldehyde and the esters, where significant differences between bottom- and top-fermenting beers could be observed. With an average acetaldehyde concentration of 4.9 mg L^{-1} in the top-fermenting beers and 6.9 mg L^{-1} in the bottom-fermenting beers, the difference was significant (p-value = 0.003). This can result from the higher fermenting temperatures of the top-fermenting beers, where initially more acetaldehyde is formed, but it is also faster reduced, resulting in a lower concentration in the finished beer.[40] The same applied for the esters, where the average concentrations were 12.3 mg L^{-1} for the bottom-fermenting beers and 17.8 mg L^{-1} for the top-fermenting, resulting in a p-value of 0.025. This result was expected, as top-fermenting yeast is known to produce more esters due to the higher fermentation temperature.[9, 10] The total ester content was dominated by ethyl acetate, which had a concentration about ten times as high as that of the second highest concentrated ester, 3-methylbutyl acetate, except for the alcohol free wheat beer, where the difference was only about 2.5 times. The alcohol free beers lost, apart from the ethanol, also most of its secondary aroma constituents like the higher alcohols and esters, while the reduction in acetaldehyde is not as prominent. A direct comparison is only possible for the wheat beer, as the brand of the tested alcohol-free Pilsener beers does not offer an alcohol containing beer.

Table 4.5 Lowest and highest detected analyte concentrations, with *RSD*, median and the number of samples, in which the analytes have been detected and average (⌀) concentrations of all analytes in the different beer varieties with the average sum of each of the four analyte classes

	Lowest (µg L^{-1})	Highest (µg L^{-1})	*RSD* (%)	Median (µg L^{-1})	Samples (n)	⌀ (A)	⌀ (H)	⌀ (K)	⌀ (P)	⌀ (Pale) (µg L^{-1})	⌀ (S)	⌀ (W)	⌀ (Wale)
Alcohols													
1-Propanol	761	23400	1.7	7400	46	8530	7330	10100	9630	848	5020	10500	1660
2-Propanol	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Methylpropanol	5190	84300*	7.7	31000	46	22700	32900	34500	38200	7540	17100	46400	5190
1-Butanol	36	676	2.2	124	16	86	113	630	323	n.d.	36	78	191
2-Butanol	73	73	1.6	-	1	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	73	n.d
tert-Butanol	25	46	3.5	28	6	31	25	n.d.	25	n.d.	n.d.	44	n.d.
2-Methylbutanol	1010	21700	2.1	14000	46	9820	12100	14300	14800	1090	8610	11300	1640
3-Methylbutanol	1800	57900*	4.2	38400*	46	37700	39200	40600	37900	2460	29300	46900	4030
1-Pentanol	14	102	7.1	24	32	24	19	28	23	93	n.d.	n.d.	n.d.
3-Pentanol	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-Methylpentanol	n.d.	n.d.	n.d.	n.d.	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-Hexanol	12	55	3.3	30	44	27	23	39	33	12	16	20	23
2-Ethylhexanol	22	43	12.9	27	10	n.d.	38	32	23	n.d.	n.d.	n.d.	n.d.
∑ (alcohols)						78900	91600	99900	99000	12000	60100	115000	12800
Aldehydes													
Acetaldehyde	2640	13200*	2.3	5470	46	3220	7290	4960	6870	3190	4140	6830	3480
Benzaldehyde	n.d.	n.d.	n.d.	n.d.	0	n.d	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trans-2-Nonenal	6	6	52.2	6	2	n.d	6	n.d.	6	n.d.	n.d.	n.d.	n.d.
∑ (aldehydes)						3220	7290	4960	6880	3190	4140	6830	3480
Esters													
Ethyl acetate	706	32500*	1.8	10300	46	9680	9470	15000	10400	753	6560	21300	1010
Ethyl butanoate	2	159	2.1	65	45	65	61	82	64	2	47	89	6
Ethyl 3-methylbutanoate	5	6	2.8	5	5	5	n.d.	n.d.	5	n.d.	n.d.	n.d.	n.d.
3-Methylbutyl acetate	47	3540	1.8	884	45	770	807	1230	854	47	599	2240	394
Ethyl hexanoate	9	501	3.2	208	46	203	210	196	221	10	191	313	26
Ethyl octanoate	7	850	4.4	295	46	448	316	449	297	9	253	375	20
Ethyl decanoate	9	166	6.4	65	44	108	69	72	60	10	71	81	n.d.
Diethyl succinate	41	1000	6.3	135	42	113	156	291	247	n.d.	91	107	n.d.
2-Phenethyl acetate	18	1690	2.9	451	45	197	432	871	540	18	195	522	31
∑ (esters)						11600	11500	18200	12600	809	8000	25100	1488
Terpenes													
Geraniol	39	241	3.7	88	13	53	170	n.d.	88	n.d.	72	241	n.d.
Limonene	7	20	45.7	11	6	n.d.	20	11	10	11	n.d.	n.d.	n.d.
Linalool	9	32	5.4	14	19	10	17	19	13	28	n.d.	n.d.	10
∑ (terpenes)						63	207	30	111	39	72	241	10

* Upper limit of determined linear range exceeded
n.d. not detected or below *MDL*

4.3.5 Alcohol-free Beer

A regular wheat beer and the corresponding alcohol-free variant of the same brand were analyzed The overlaid total ion chromatograms of the two beers are presented in **Figure 4.3**, where it can clearly be seen that the peaks of most compounds are much smaller in the alcohol free version. A detailed view on the analytes found in these two samples, together with the fraction which was lost in the making of the alcohol-free beer, is given in **Table 4.6**. The average loss was 21% for acetaldehyde, 79% for the alcohols and up to 88% for the esters. The levels dropped below the method detection limit for ethyl decanoate, diethyl succinate and *tert*-butanol; where, with the corresponding *MDL*s set as maximum possible remaining concentrations, the losses were at least 87%, 66% and 49%, respectively. Exceptions were 1-butanol and linalool, where the levels in the alcohol-free beer were higher than in the regular beer. However, the value for the regular beer of 1-butanol is below the lower limit of the linear range, which starts at 127 $\mu g\ L^{-1}$, due to sample dilution. Linalool is just above the *MDL*, in this way the differences might, as well as for 1-hexanol, originate in measurement uncertainty.

There are several ways to make alcohol-free beer: vacuum rectification, thin film evaporation, dialysis, reverse osmosis and arrested fermentation; common to all techniques is that a major part of volatile beer aroma constituents is also removed with the ethanol, but some methods also allow a reintroduction of parts of the aroma compounds to the beer after the dealcoholization. The method used in the production of the tested beers is undisclosed, but when the reduction ratios are compared with literature, it could be assumed to be a reverse osmosis or arrested fermentation technique. However, studies have shown, that the aroma threshold concentrations in alcohol-free beer are lower than in regular beers and in this way even the lower concentrations can result in a satisfying overall aroma.[41]

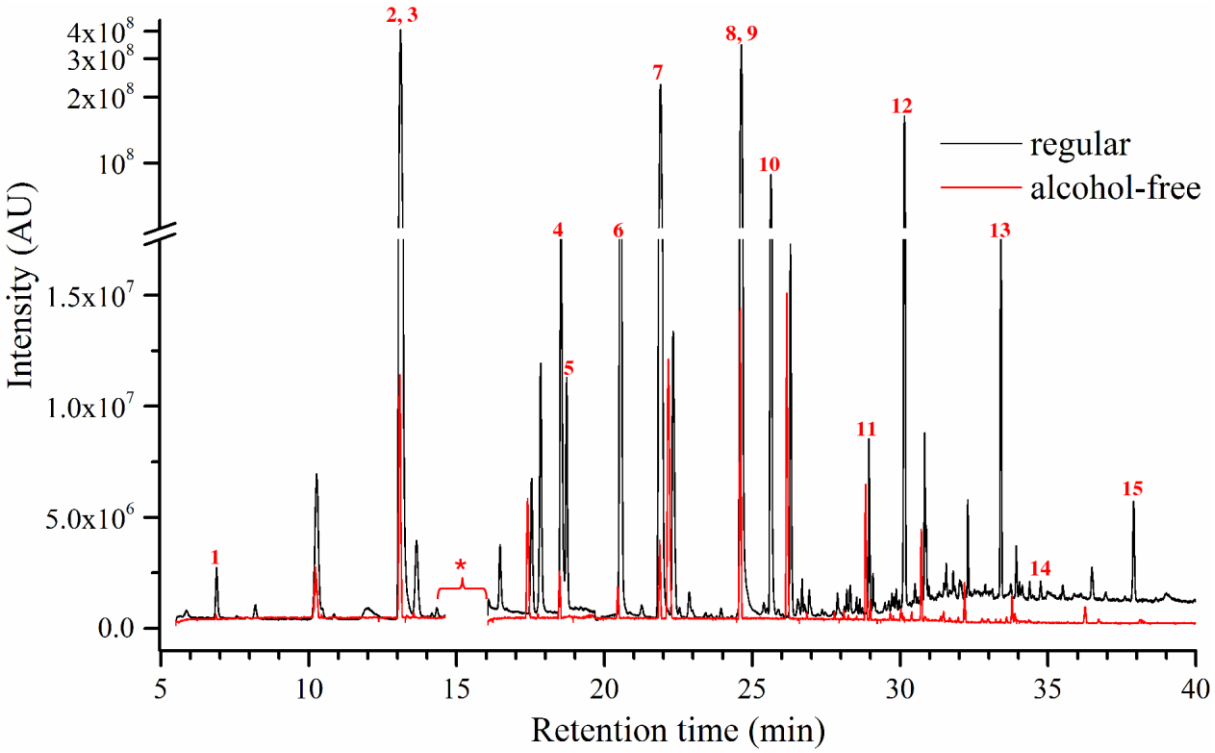

Figure 4.3 Total ion current chromatogram of a regular wheat beer and the alcohol-free beer of the same brand: (1) acetaldehyde, (2) ethyl acetate, (3) *tert*-butanol, (*) detector turned off for ethanol peak, (4) 1-propanol, (5) ethyl butanoate, (6) 2-methylpropanol, (7) 3-methylbutylacetate, (8) 2-methylbutanol, (9) 3-methylbutanol, (10) ethyl hexanoate, (11) 1-hexanol, (12) ethyl octanoate, (13) ethyl decanoate, (14) diethyl succinate, (15) 2-phenethyl acetate

Table 4.6 Comparison of the measured analyte concentrations of W01, a regular wheat beer and W01\alc, the alcohol-free beer of the same brand, with the fraction of analytes lost in the production of the alcohol free version

	Regular (µg L^{-1})	Alcohol-free (µg L^{-1})	Loss (%)		Regular (µg L^{-1})	Alcohol-free (µg L^{-1})	Loss (%)
Alcohols				**Esters**			
1-Propanol	14702	1662	89	Ethyl acetate	22834	1012	96
2-Methylpropanol	35716	5188	85	Ethyl butanoate	97	6	94
1-Butanol	59	191	-	3-Methylbutyl acetate	2079	394	81
2-Butanol	n.d.	11	-	Ethyl hexanoate	501	26	95
tert-Butanol	43	n.d.	≥49	Ethyl octanoate	461	19	96
2-Methylbutanol	8940	1640	82	Ethyl decanoate	55	n.d.	≥87
3-Methylbutanol	35952	4034	89	Diethyl succinate	61	n.d.	≥66
1-Hexanol	21	23	-	2-Phenethyl acetate	506	31	94
Aldehydes				**Terpenes**			
Acetaldehyde	4414	3477	21	Linalool	n.d.	10	-

4.3.6 Variety Discrimination by LDA

Because a significant discrimination of top- and bottom-fermenting beers has been possible by just comparing the concentration of acetaldehyde and the sum of esters, the data was subjected to a multivariate analysis, to perform a discrimination of the varieties within each class, also. A Linear Discriminant Analysis (LDA) was performed with the free software environment *R 3.0.1* (The R Foundation for Statistical Computing, www.r-project.org) using the "lda"-function from the "MASS" package [42], on semi-quantitative data. To that end, the peak areas of the quantified analytes present in almost all samples were combined with the

peak areas of several other compounds that could also be detected in nearly all samples, like 2- and 3-methylbutanal, some formate- and acetate esters and dimethyl sulphide, resulting in 31 components as explanatory variables for the LDA. The number of useful discriminant functions is limited to the minimum of either the number of the groups minus one or the number of variables. Because the beer samples originated from eight different varieties, seven discriminant functions could be found. Their proportions of trace are shown in **Figure 4.4**. This shows that the first two linear discriminants explain about 95% of the between-group variance and should provide good separation; The main loadings on LD1 were given to isoamyl propionate in the positive direction and to 1-hexanol in the negative direction; the main loadings on LD2 were ethyl 3-methylbutanoate in the positive and diethyl succinate in the negative direction.

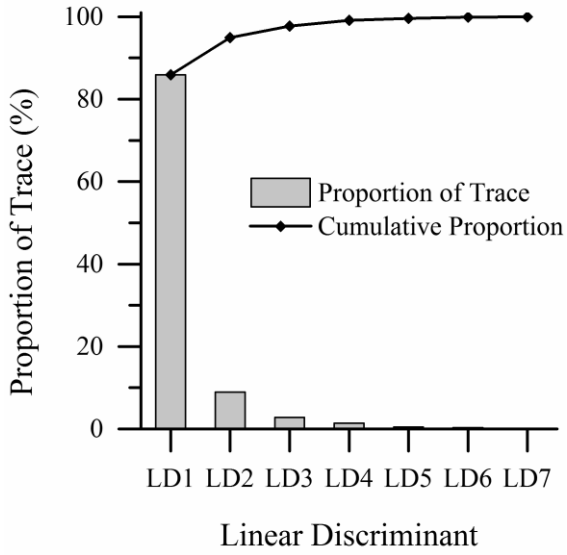

Figure 4.4 Proportion of trace and cumulative proportions of the seven found liniear discriminants

Table 4.7 shows the prediction results using these two first discriminants. The proportion of correct predictions was almost 90% and four of the five false predictions are between Pilsener beer and Helles, which have a very similar brewing process and their main difference is the lower hop content of Helles. Moreover, one of the falsely predicted Pilsener beers is marketed to be especially mild and another one had an increased alcohol content, which might separate them from the other beers of their variety.

Table 4.7 Predicted results of the analyzted samples

Variety	Predicted variety								Correct predictions
	A	H	K	P	P\alc	S	W	W\alc	(%)
A (n=4)	4								100.0
H (n=6)		5		1					83.3
K (n=6)			5				1		83.3
P (n=23)		3		20					87.0
P\alc (n=2)					2				100.0
S (n=1)						1			100.0
W(n=3)							3		100.0
W\alc (n=1)								1	100.0
								Total	89.1

A graphical representation of this situation is given in **Figure 4.5** which shows a scatter diagram based on the first two discriminant functions with boundaries for each beer variety. The distance of the two kinds of alcohol free beers between themselves and to the rest of the beer varieties was so large, that they were not included into the plot. As already discussed using **Table 4.7** a good discrimination is achieved and only the quite similar varieties of Pilsener beer and Helles are partially overlapping. A better discrimination between those two varieties might be achieved by the inclusion of an HPLC analysis of the hop-bitter substances, as Helles has typically lower hop content than Pilsener beer. The production conditions of these two varieties can also be quite different, as the samples originated from breweries all over Germany, but there were also beers from Poland and Turkey and specialty beers which were brewed with higher alcohol content. On the other hand, the variations within the small groups of the top-fermenting beers Alt, Kölsch and wheat beer was relatively small, as they are regional beers; while Alt originates in Düsseldorf and the lower Rhine region, wheat beer is predominantly brewed in southern Germany and Kölsch is exclusively produced in and around the city of Cologne and typically fermented at 14 °C to 16 °C, which is quite low for a top-fermenting beer. A separation between the two general classes of top-fermenting (K, W, A) and bottom-fermenting (H, S, P) is also visible, as the top-fermenting beers are by trend more located in the lower resp. lower left area of the plot, while the bottom-fermenting beers tend to be on the upper resp. upper-right hand area.

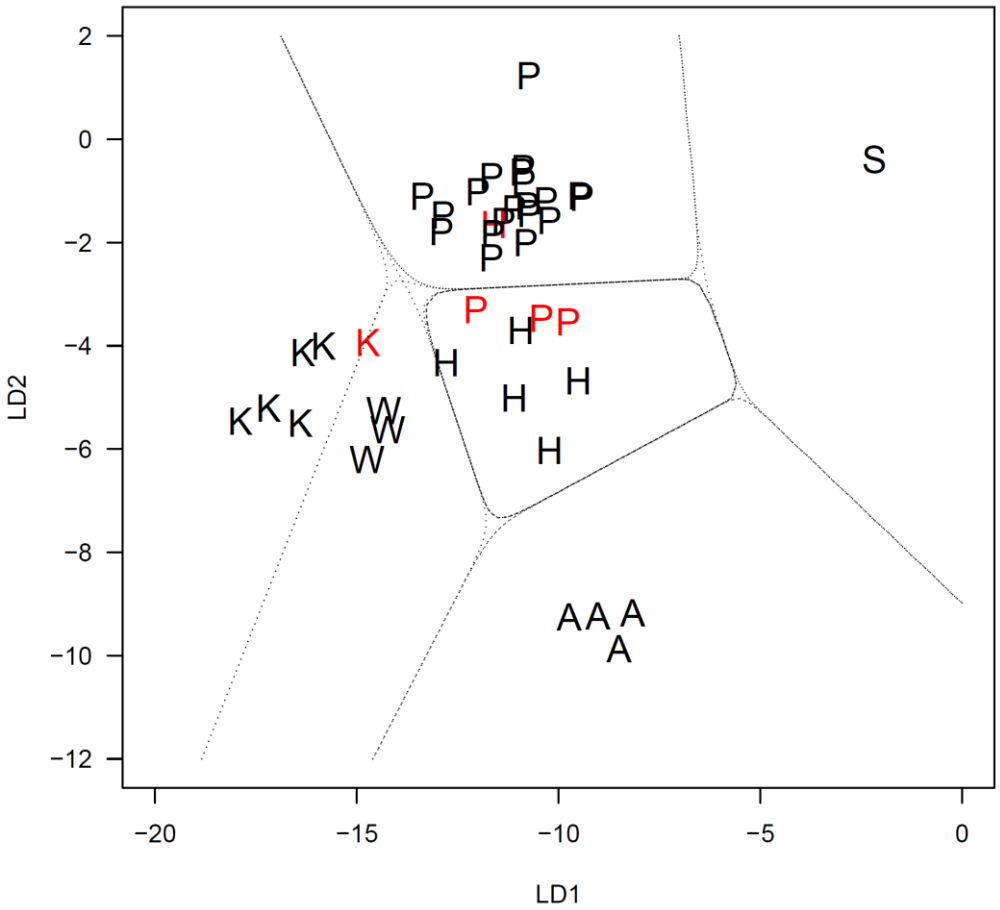

Figure 4.5 Plot of the discriminant functions with boundaries for each beer variety, falsely classified samples shown in red; alcohol free beers not shown (coordinates would be at LD1=80, LD2=0 for the alcohol free Pilsener beers and LD1=-18, LD2=25 for the alcohol free wheat beer)

4.4 Conclusions

The developed method was appropriate to perform a sensible and robust analysis of the beer samples. The performance was mostly comparable to results obtained with other microextraction techniques like SPDE or SPME and sometimes also better. The data, obtained during method evaluation, highlights that it is not always significant to have the most sensitive method, but also a robust method, which also complies with factors like precision and linear range, where it is important to choose the appropriate sorbent material for the analytical task at hand. Still, the presented ITEX method was sensitive enough to allow the dilution of the samples to minimize the influence of the matrix. A satisfying chemometric discrimination of all analyzed beer varieties was possible and the alcohol free beers could clearly be separated from the regular beers, also.

4.5 References

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4.6 Supporting Information

Table S 4.1 Sample numbers with corresponding sample name (H: Helles, P: Pilsener beer, S: Schwarzbier, A: Altbier, K: Kölsch, W: Wheat beer)

Top-fermenting beers		Bottom-fermenting beers	
Number	**Name**	**Number**	**Name**
H01	Hövels	A01	Diebels Alt
H02	Borbecker Helles Dampfbier	A02	Gatz Altbier
H03	Stauder Spezial	A03	Schlösser Alt
H04	Maingold Landbier	A04	Frankenheim Alt
H05	Oettinger Urtyp	K01	Früh Kölsch
H06	Tyskie	K02	Sester Kölsch
P01	Bitburger	K03	Sion Kölsch
P02	König Pilsener	K04	Mühlen Kölsch
P03	Veltins	K05	Reissdorf Kölsch
P04	Brinkhoffs Nr.1	K06	Küppers Kölsch
P05	Stauder	W01	Erdinger Weissbier
P06	Beck's	W01\alc	Erdinger Alkoholfrei
P07	Radeberger	W02	Paulaner
P08	Astra Urtyp	W03	Franziskaner Weissbier Dunkel
P09	Tuborg Pilsener		
P10	Jever		
P11	Moritz Fiege Pils		
P12	Hansa Pils		
P13	Holsten		
P14	Flensburger Pilsener		
P15	Astra Rotlicht		
P16	Krombacher Pils		
P17	Beck's Gold		
P18	Warsteiner		
P19	Efes Pils		
P20	Lech		
P21	DAB Pilsener		
P22	Landfürst		
P23	Paderborner		
P24\alc	Clausthaler Extra Herb		
P25\alc	Clausthaler Classic Alkoholfrei		
S01	Köstritzer Schwarzbier		

Table S 4.2 Measured concentrations of the analyzed compounds in Altbier and Kölsch (empty field: not detected or below *MDL*)

Compound		A01	A02	A03	A04	K01	K02	K03	K04	K05	K06
1-Propanol	**c (µg L^{-1})**	8573	7767	9290	8502	9310	6921	5685	18029	15570	5180
	RSD	0.3%	5.5%	3.5%	0.6%	1.1%	0.9%	1.4%	0.4%	0.7%	4.6%
2-Methylpropanol	**c (µg L^{-1})**	27967	23774	23021	16065	38726	31789	35021	29852	46470	25449
	RSD	2.6%	53.5%	2.6%	6.6%	1.2%	28.2%	5.6%	2.9%	6.8%	19.5%
1-Butanol	**c (µg L^{-1})**		116		55				585	676	
	RSD		2.0%		0.1%				0.9%	1.4%	
2-Butanol	**c (µg L^{-1})**										
	RSD										
***tert*-Butanol**	**c (µg L^{-1})**			31							
	RSD			3.5%							
2-Methylbutanol	**c (µg L^{-1})**	14940	10592	8573	5163	16991	14429	13038	13455	12908	15059
	RSD	2.4%	3.2%	1.3%	0.6%	2.2%	4.3%	1.2%	1.2%	0.5%	10.0%
3-Methylbutanol	**c (µg L^{-1})**	34496	51736	38430	26193	42969	39252	36691	38880	42981	42782
	RSD	2.9%	4.7%	1.9%	1.5%	1.8%	5.8%	1.5%	1.8%	0.7%	11.4%
1-Pentanol	**c (µg L^{-1})**	35			14	20	27	29	31	28	30
	RSD	2.4%			3.6%	18.4%	14.3%	5.6%	6.0%	7.0%	11.6%
1-Hexanol	**c (µg L^{-1})**	51	14	24	19	34	36	40	46	37	42
	RSD	2.1%	9.4%	3.2%	3.1%	1.0%	8.4%	1.7%	2.6%	3.3%	5.4%
2-Ethylhexanol	**c (µg L^{-1})**					23	31	33	43	38	23
	RSD					4.3%	0.0%	38.5%	45.4%	6.0%	3.6%
Acetaldehyde	**c (µg L^{-1})**	2639	2836	3224	4166	4485	4795	4978	5086	5162	5253
	RSD	5.4%	2.1%	1.5%	2.3%	2.0%	6.3%	1.8%	1.4%	2.0%	3.4%
***trans*-2-Nonenal**	**c (µg L^{-1})**										
	RSD										
Ethyl acetate	**c (µg L^{-1})**	10954	7550	14350	5884	21695	8860	10708	16385	20184	12128
	RSD	2.7%	3.7%	2.4%	0.2%	0.8%	3.1%	1.4%	0.0%	0.8%	6.2%
Ethyl butanoate	**c (µg L^{-1})**	72	41	90	55	159	36	43	81	130	44
	RSD	0.2%	2.4%	2.9%	1.0%	0.7%	2.0%	2.1%	0.5%	1.1%	2.9%
Ethyl-3-methylbutanoate	**c (µg L^{-1})**	5									
	RSD	1.4%									
3-Methylbutyl acetate	**c (µg L^{-1})**	618	775	1375	311	1464	884	997	1206	1758	1084
	RSD	1.0%	3.7%	2.0%	1.3%	0.9%	1.4%	1.4%	0.8%	2.0%	1.5%
Ethyl hexanoate	**c (µg L^{-1})**	218	138	219	236	311	94	120	213	321	117
	RSD	2.1%	2.4%	3.0%	0.4%	1.8%	3.5%	3.3%	1.6%	3.5%	6.8%
Ethyl octanoate	**c (µg L^{-1})**	575	295	465	459	850	188	275	405	699	276
	RSD	0.2%	3.3%	2.3%	0.1%	1.1%	12.2%	1.2%	3.3%	5.5%	13.1%
Ethyl decanoate	**c (µg L^{-1})**	154	94	90	94	62	72	68	46	82	99
	RSD	0.9%	2.4%	5.0%	2.0%	6.3%	7.8%	15.7%	5.6%	11.6%	3.4%
Diethyl succinate	**c (µg L^{-1})**	223	47	107	72	52	91	151	1000	207	246
	RSD	4.2%	14.2%	3.3%	4.2%	12.9%	13.1%	2.7%	3.5%	7.4%	2.9%
2-Phenethyl acetate	**c (µg L^{-1})**	238	200	298	52	888	451	581	956	1690	658
	RSD	4.0%	4.0%	1.0%	3.1%	1.6%	4.2%	2.1%	1.9%	1.5%	5.5%
Geraniol	**c (µg L^{-1})**		40		67						
	RSD		1.8%		0.1%						
Limonen	**c (µg L^{-1})**							11			
	RSD							12.5%			
Linalool	**c (µg L^{-1})**	9							27	15	14
	RSD	0.5%							0.3%	9.9%	3.9%

Table S 4.3 Measured concentrations of the analyzed compounds in Wheat beer and Helles (empty field: not detected or below *MDL*)

Compound		W01	W01\alc	W02	W03	H01	H02	H03	H04	H05	H06
1-Propanol	**c (µg L^{-1})**	14702	1662	5595	11281	11049	11369	6234	4272	5608	5425
	RSD	1.5%	3.5%	3.5%	0.5%	2.7%	0.6%	1.6%	1.5%	2.0%	1.8%
2-Methylpropanol	**c (µg L^{-1})**	35716	5188	19155	84343	30339	49418	15502	42453	28280	31619
	RSD	5.4%	5.2%	12.4%	1.3%	6.3%	1.2%	15.2%	5.2%	15.3%	7.1%
1-Butanol	**c (µg L^{-1})**	59	191		97	131	194	58		70	
	RSD	2.7%	8.8%		1.7%	3.2%	1.5%	2.3%		2.2%	
2-Butanol	**c (µg L^{-1})**				73						
	RSD				1.6%						
***tert*-Butanol**	**c (µg L^{-1})**	43			45	25				25	
	RSD	1.5%			5.7%	3.2%				2.4%	
2-Methylbutanol	**c (µg L^{-1})**	8940	1639	10754	14180	10056	13379	7822	14524	12741	14009
	RSD	0.3%	6.1%	1.1%	3.0%	3.3%	1.6%	0.2%	2.5%	1.9%	1.4%
3-Methylbutanol	**c (µg L^{-1})**	35952	4034	46715	57894	49202	49042	30052	31426	43825	31385
	RSD	0.4%	5.0%	0.7%	2.9%	3.9%	0.9%	1.2%	2.4%	1.4%	0.8%
1-Pentanol	**c (µg L^{-1})**								26	14	17
	RSD								12.8%	4.3%	10.7%
1-Hexanol	**c (µg L^{-1})**	21	23	17	23	25	24	13	30	25	24
	RSD	0.9%	9.1%	0.2%	5.9%	1.4%	2.0%	1.2%	4.2%	3.3%	1.7%
2-Ethylhexanol	**c (µg L^{-1})**								38		
	RSD								7.1%		
Acetaldehyde	**c (µg L^{-1})**	4414	3477	5975	10108	3684	4128	5683	8485	8563	13184
	RSD	2.5%	6.2%	1.7%	3.3%	5.0%	2.1%	0.1%	0.3%	3.6%	0.9%
***trans*-2-Nonenal**	**c (µg L^{-1})**										6.3
	RSD										67.6%
Ethyl acetate	**c (µg L^{-1})**	22834	1011	8752	32453	10613	5657	5069	11603	10355	13525
	RSD	1.1%	6.1%	0.9%	5.5%	2.5%	0.6%	0.8%	1.5%	2.5%	0.5%
Ethyl butanoate	**c (µg L^{-1})**	97	6	64	106	61	29	25	94	59	98
	RSD	2.0%	12.8%	1.7%	5.6%	3.1%	1.0%	0.9%	1.1%	1.6%	0.7%
Ethyl-3-methylbutanoate	**c (µg L^{-1})**										
	RSD										
3-Methylbutyl acetate	**c (µg L^{-1})**	2079	394	1100	3538	1011	201	128	1083	1217	1200
	RSD	1.2%	0.7%	1.9%	5.6%	3.4%	1.4%	4.5%	0.6%	2.9%	1.1%
Ethyl hexanoate	**c (µg L^{-1})**	501	26	240	197	158	92	65	328	270	342
	RSD	0.9%	8.4%	0.8%	7.7%	5.1%	1.5%	3.0%	0.9%	2.0%	0.8%
Ethyl octanoate	**c (µg L^{-1})**	461	19	299	365	403	173	127	388	311	494
	RSD	0.6%	12.8%	3.0%	7.5%	1.7%	0.5%	2.7%	2.2%	3.7%	4.7%
Ethyl decanoate	**c (µg L^{-1})**	55		22	166	62	23	14	117	56	140
	RSD	1.1%		5.1%	10.3%	1.3%	1.4%	6.0%	5.4%	7.1%	14.3%
Diethyl succinate	**c (µg L^{-1})**	61		137	123	300	111	46	231	133	113
	RSD	4.2%		1.3%	2.1%	0.4%	3.8%	7.9%	7.6%	1.2%	12.4%
2-Phenethyl acetate	**c (µg L^{-1})**	506	31	250	808	274	70	50	861	352	982
	RSD	1.7%	0.5%	6.4%	4.3%	2.0%	1.2%	0.4%	0.5%	1.2%	2.4%
Geraniol	**c (µg L^{-1})**				241				223	104	183
	RSD				9.5%				2.8%	3.0%	1.5%
Limonen	**c (µg L^{-1})**										20
	RSD										24.8%
Linalool	**c (µg L^{-1})**		10						25		9
	RSD		5.9%						8.5%		22.8%

Table S 4.4 Measured concentrations of the analyzed compounds in Pilsener beers (empty field: not detected or below *MDL*)

Compound		P01	P02	P03	P04	P05	P06	P07	P08	P09	P10
1-Propanol	**c (µg L^{-1})**	7230	11065	15339	9668	5136	7336	6157	16170	16335	7719
	RSD	1.8%	0.7%	0.9%	2.6%	2.2%	0.1%	1.5%	0.5%	1.9%	0.8%
2-Methylpropanol	**c (µg L^{-1})**	19418	26838	54708	64501	14784	16965	30378	59110	62364	39605
	RSD	1.2%	1.9%	2.1%	3.3%	8.4%	11.8%	5.2%	3.0%	6.9%	0.7%
1-Butanol	**c (µg L^{-1})**						60				
	RSD						2.0%				
2-Butanol	**c (µg L^{-1})**										
	RSD										
***tert*-Butanol**	**c (µg L^{-1})**										
	RSD										
2-Methylbutanol	**c (µg L^{-1})**	13972	16213	16096	16486	7188	5311	10419	19715	16703	20355
	RSD	1.2%	1.3%	1.3%	2.3%	2.5%	0.6%	1.2%	2.0%	2.7%	0.4%
3-Methylbutanol	**c (µg L^{-1})**	29882	32500	36594	40891	26886	21684	44447	44155	41017	49073
	RSD	1.1%	1.2%	1.6%	2.3%	2.2%	0.9%	2.0%	3.1%	3.2%	0.5%
1-Pentanol	**c (µg L^{-1})**	27	20	28	21				25	23	20
	RSD	6.6%	4.6%	10.0%	6.3%				6.2%	6.2%	5.2%
1-Hexanol	**c (µg L^{-1})**	40	39	37	37	13	15	20	47	36	33
	RSD	1.6%	1.0%	1.0%	3.5%	1.8%	2.4%	1.9%	5.4%	2.1%	1.6%
2-Ethylhexanol	**c (µg L^{-1})**	24	22							24	
	RSD	0.2%	23.9%							0.0%	
Acetaldehyde	**c (µg L^{-1})**	4278	4492	4908	5059	5356	5373	5561	5816	5882	5908
	RSD	1.4%	2.0%	2.6%	2.4%	2.1%	1.2%	0.9%	2.1%	0.8%	1.8%
***trans*-2-Nonenal**	**c (µg L^{-1})**				6.1						
	RSD				36.8%						
Ethyl acetate	**c (µg L^{-1})**	9887	10328	18204	14535	4634	4008	5906	8714	8373	13501
	RSD	1.1%	0.5%	2.2%	1.7%	0.5%	0.7%	0.4%	2.1%	0.9%	0.9%
Ethyl butanoate	**c (µg L^{-1})**	74	87	72	64	30	31	40	70	66	75
	RSD	1.5%	0.9%	0.7%	1.1%	1.0%	2.0%	1.4%	2.2%	1.3%	0.9%
Ethyl-3-methylbutanoate	**c (µg L^{-1})**									5	5
	RSD									3.5%	0.1%
3-Methylbutyl acetate	**c (µg L^{-1})**	687	702	1421	1201	128	218	803	593	639	963
	RSD	1.0%	1.0%	1.6%	1.5%	3.1%	4.2%	0.4%	0.7%	1.4%	1.4%
Ethyl hexanoate	**c (µg L^{-1})**	251	323	287	178	77	125	158	238	188	208
	RSD	2.1%	1.6%	2.7%	4.0%	3.2%	3.9%	0.8%	6.1%	3.6%	1.2%
Ethyl octanoate	**c (µg L^{-1})**	501	557	345	296	124	218	211	316	226	259
	RSD	1.6%	3.5%	0.7%	5.3%	5.3%	1.9%	1.5%	17.8%	6.3%	0.4%
Ethyl decanoate	**c (µg L^{-1})**	92	165	25	74	9	29	26	80	127	39
	RSD	4.0%	3.5%	3.2%	1.4%	4.7%	2.6%	0.2%	2.9%	5.7%	2.4%
Diethyl succinate	**c (µg L^{-1})**	220	301	351	437		50	41	388	729	165
	RSD	1.8%	1.6%	4.3%	5.8%		10.1%	3.9%	4.0%	5.1%	4.4%
2-Phenethyl acetate	**c (µg L^{-1})**	325	368	1233	1201	37	113	276	336	461	507
	RSD	2.2%	1.5%	2.1%	2.8%	3.1%	4.7%	4.6%	4.3%	4.1%	2.5%
Geraniol	**c (µg L^{-1})**		39								
	RSD		0.0%								
Limonen	**c (µg L^{-1})**				14						
	RSD				118.4%						
Linalool	**c (µg L^{-1})**	11							18	13	11
	RSD	5.7%							1.7%	4.1%	5.1%

Table S 4.5 Measured concentrations of the analyzed compounds in Pilsener beers (empty field: not detected or below *MDL*)

Compound		P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
1-Propanol	**c (µg L^{-1})**	6659	7473	14916	6232	23372	12657	4854	8257	5772	5524
	RSD	2.1%	2.1%	1.1%	1.2%	1.5%	2.3%	0.7%	0.3%	1.7%	1.5%
2-Methylpropanol	**c (µg L^{-1})**	21093	43829	54496	48822	70938	43234	18515	36751	20841	33481
	RSD	5.2%	1.1%	3.6%	7.6%	2.1%	6.1%	3.6%	6.1%	3.5%	7.3%
1-Butanol	**c (µg L^{-1})**				249				654		
	RSD				2.1%				0.0%		
2-Butanol	**c (µg L^{-1})**										
	RSD										
***tert*-Butanol**	**c (µg L^{-1})**				25						
	RSD				3.9%						
2-Methylbutanol	**c (µg L^{-1})**	8349	17483	16122	15892	21680	19119	5497	18035	11539	14675
	RSD	4.2%	2.6%	0.9%	0.9%	0.0%	4.4%	0.3%	3.4%	1.9%	2.2%
3-Methylbutanol	**c (µg L^{-1})**	35431	42135	38324	51450	52338	41340	25740	34904	34164	32867
	RSD	5.9%	2.3%	0.9%	1.7%	0.4%	5.7%	1.2%	4.1%	1.9%	2.1%
1-Pentanol	**c (µg L^{-1})**	16	21	29	14	33	22		29	18	18
	RSD	15.6%	7.3%	3.7%	3.7%	2.8%	9.4%		7.6%	5.8%	8.5%
1-Hexanol	**c (µg L^{-1})**	23	38	44	16	55	35		38	30	23
	RSD	5.2%	2.0%	2.3%	5.5%	0.7%	3.1%		4.0%	8.0%	5.4%
2-Ethylhexanol	**c (µg L^{-1})**										
	RSD										
Acetaldehyde	**c (µg L^{-1})**	6321	6422	6476	6853	6859	7384	8333	8442	8445	9149
	RSD	5.0%	2.5%	2.3%	1.2%	2.3%	4.0%	1.4%	1.7%	1.7%	1.8%
***trans*-2-Nonenal**	**c (µg L^{-1})**										
	RSD										
Ethyl acetate	**c (µg L^{-1})**	8183	12155	9359	10838	9846	13827	8367	11193	9602	14253
	RSD	0.5%	1.6%	1.8%	3.0%	0.4%	2.8%	1.0%	2.5%	0.5%	0.6%
Ethyl butanoate	**c (µg L^{-1})**	55	65	71	57	73	74	35	56	65	95
	RSD	5.1%	0.5%	0.8%	6.6%	2.0%	2.8%	1.0%	0.5%	0.1%	1.8%
Ethyl-3-methylbutanoate	**c (µg L^{-1})**			5		6					
	RSD			5.6%		3.4%					
3-Methylbutyl acetate	**c (µg L^{-1})**	639	983	660	1679	686	1153	758	941	770	1230
	RSD	0.7%	0.4%	0.9%	7.6%	1.0%	3.2%	0.4%	1.7%	0.9%	0.8%
Ethyl hexanoate	**c (µg L^{-1})**	168	240	207	237	247	270	111	276	177	290
	RSD	0.1%	7.4%	1.3%	8.6%	3.2%	5.5%	0.4%	3.0%	2.9%	1.6%
Ethyl octanoate	**c (µg L^{-1})**	240	366	103	352	173	361	147	280	254	389
	RSD	3.5%	1.7%	2.5%	11.8%	2.5%	4.2%	2.3%	6.5%	3.1%	1.4%
Ethyl decanoate	**c (µg L^{-1})**	16	91	16	40	90	49	17	31	45	117
	RSD	9.3%	12.0%	11.6%	2.7%	3.4%	5.5%	4.1%	8.5%	2.3%	6.4%
Diethyl succinate	**c (µg L^{-1})**	358	271	93	70	314	92	56	67	816	179
	RSD	5.7%	9.2%	7.2%	2.4%	3.7%	9.2%	4.4%	18.1%	2.6%	7.2%
2-Phenethyl acetate	**c (µg L^{-1})**	175	730	463	831	485	878	344	558	416	914
	RSD	3.2%	4.7%	2.4%	2.7%	2.9%	6.9%	1.7%	5.1%	0.9%	5.8%
Geraniol	**c (µg L^{-1})**		106		67						80
	RSD		5.9%		3.3%						2.8%
Limonen	**c (µg L^{-1})**		7								
	RSD		65.7%								
Linalool	**c (µg L^{-1})**			18		16	9			14	10
	RSD			2.1%		8.8%	4.8%			2.0%	9.4%

Table S 4.6 Measured concentrations of the analyzed compounds in Pilsener beers and Schwarzbier (empty field: not detected or below *MDL*)

Compound		P21	P22	P23	P24\alc	P25\alc	S01
1-Propanol	**c (µg L^{-1})**	7127	6888	9634	761	935	5018
	RSD	0.8%	6.6%	0.3%	0.7%	3.9%	1.2%
2-Methylpropanol	**c (µg L^{-1})**	35653	33762	29162	6409	8670	17126
	RSD	4.4%	28.4%	4.5%	6.2%	9.3%	6.8%
1-Butanol	**c (µg L^{-1})**			328			36
	RSD			0.0%			4.6%
2-Butanol	**c (µg L^{-1})**						
	RSD						
***tert*-Butanol**	**c (µg L^{-1})**						
	RSD						
2-Methylbutanol	**c (µg L^{-1})**	15575	19362	14376	1011	1168	8611
	RSD	1.4%	2.0%	1.4%	2.3%	2.9%	1.9%
3-Methylbutanol	**c (µg L^{-1})**	42595	41040	32156	1795	3133	29299
	RSD	2.2%	0.1%	2.0%	86.6%	3.1%	2.7%
1-Pentanol	**c (µg L^{-1})**	20	28	21	102	84	
	RSD	0.7%	0.1%	13.7%	2.0%	5.1%	
1-Hexanol	**c (µg L^{-1})**	32	44	30		11	16
	RSD	0.9%	7.9%	1.8%		1.9%	4.6%
2-Ethylhexanol	**c (µg L^{-1})**						
	RSD						
Acetaldehyde	**c (µg L^{-1})**	9301	9725	11674	2759	3618	4143
	RSD	0.6%	3.0%	1.0%	2.0%	2.4%	2.1%
***trans*-2-Nonenal**	**c (µg L^{-1})**						
	RSD						
Ethyl acetate	**c (µg L^{-1})**	10696	13713	8404	706	800	6558
	RSD	0.2%	5.8%	2.1%	1.8%	3.2%	0.5%
Ethyl butanoate	**c (µg L^{-1})**	76	93	50	2		47
	RSD	0.5%	5.6%	1.2%	5.9%		1.3%
Ethyl-3-methylbutanoate	**c (µg L^{-1})**						
	RSD						
3-Methylbutyl acetate	**c (µg L^{-1})**	832	1233	715	47		599
	RSD	1.1%	1.0%	1.8%	3.1%		1.0%
Ethyl hexanoate	**c (µg L^{-1})**	191	467	175	9	11	191
	RSD	1.9%	4.1%	2.3%	5.4%	8.2%	2.4%
Ethyl octanoate	**c (µg L^{-1})**	293	578	252	10	7	253
	RSD	0.9%	15.4%	4.9%	10.1%	5.0%	2.3%
Ethyl decanoate	**c (µg L^{-1})**	89	89	24	10		71
	RSD	12.7%	35.9%	4.7%	16.8%		2.7%
Diethyl succinate	**c (µg L^{-1})**	251	120	59			91
	RSD	3.4%	21.2%	15.9%			4.6%
2-Phenethyl acetate	**c (µg L^{-1})**	565	805	391	18		195
	RSD	1.5%	5.1%	1.1%	2.2%		1.0%
Geraniol	**c (µg L^{-1})**	88	147				72
	RSD	8.4%	1.8%				7.2%
Limonen	**c (µg L^{-1})**			8	11		
	RSD			44.8%	7.8%		
Linalool	**c (µg L^{-1})**		12		32	23	
	RSD		0.1%		2.4%	5.0%	

5 Systematic optimization of in-tube extraction (ITEX) methods

5.1 Introduction

Method development for microextraction techniques can be a very time consuming task, because a multitude of different parameters influences the efficiency of extraction. Even in the simplest system, where only a coated fiber (SPME) is immersed in a liquid sample, the extraction can be influenced by several parameters. They are, for example, (i) the choice of the polymeric coating, (ii) the extraction time, together with (iii) shaking or stirring, (iv) the extraction temperature, (v) the pH for ionizable compounds, (vi) the ionic strength and (vii) the presence of organic solvents or humic substances.[1] Dynamic microextraction techniques, where the sample is actively passed over the sorbent material or through a sorbent bed are more complex and thus have even more parameters to optimize during the steps of the extraction and injection procedure; e.g. the volume and the corresponding flows that are applied during extraction and desorption. [2-4]

ITEX is a fully automated microextraction technique for CTC PAL-series autosamplers and uses a gas-tight syringe to pump the sample headspace repeatedly through an attached tube, filled with a sorbent material for analyte enrichment. The syringe, as well as the sorbent tube, is enclosed by an electric heater, to avoid sample condensation in the syringe and to facilitate thermal desorption to the inlet system of the gas chromatograph, respectively. The syringe also features a side-port hole, which allows the flushing of the syringe and the sorbent tube with a pure, inert gas for trap conditioning to avoid carry-over between analyses. The four stages of the ITEX-procedure (sample conditioning, analyte extraction/sorption, desorption/injection and trap conditioning), together with the main parameters governing the performance of each stage are depicted in **Figure 5.1**.

The aim of this work is to summarize the experiences gained in the ITEX-method development and to present a guideline that allows future user to minimize the number of experiments, which are required to find the appropriate parameters for their analytical task.

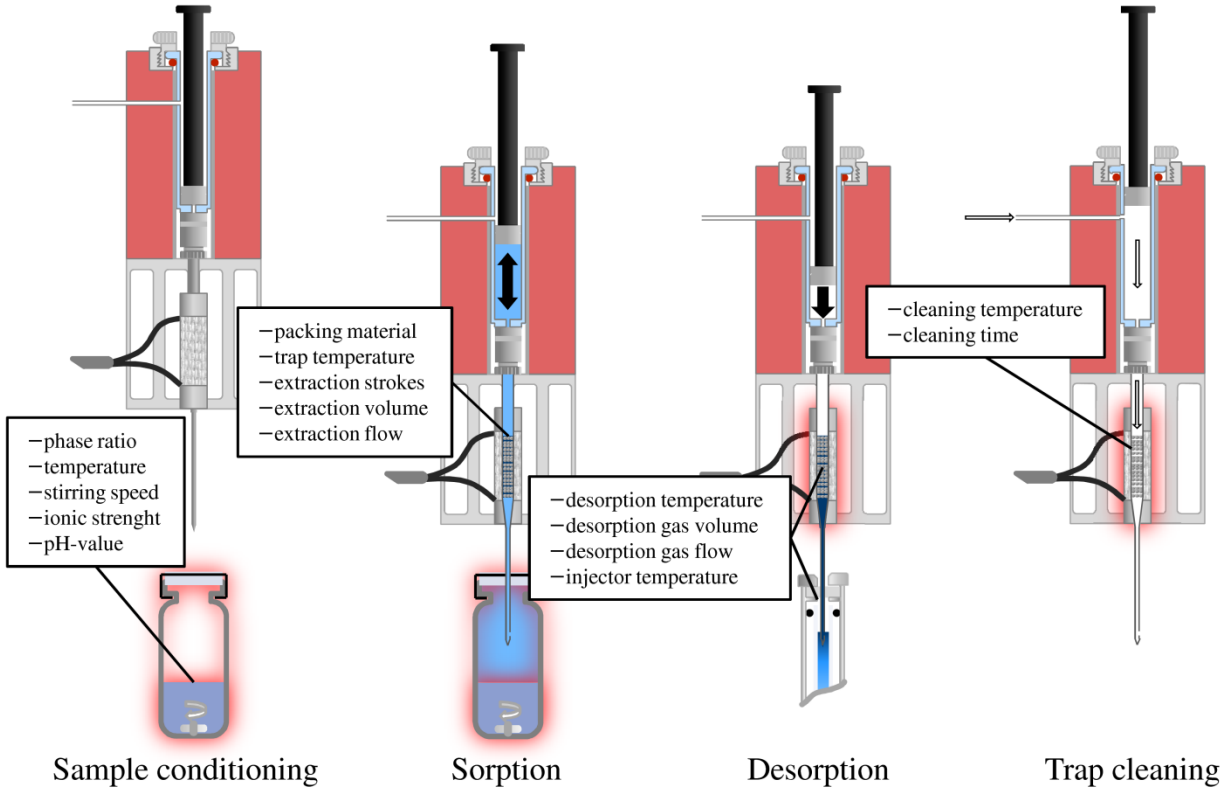

Figure 5.1 Stages of the ITEX-procedure with the corresponding parameters for optimization, adapted from [4]

5.2 Experimental

5.2.1 Target Compounds

The target compounds used in the developed methods can be sorted in two categories, VOCs as water contaminants and aroma compounds in food matrices. The VOCs are comprised of halogenated hydrocarbons, BTEX compounds and gasoline oxygenates (ETBE, MTBE, TAME). The aroma compounds include several alcohols, aldehydes, esters, terpenes and 2,3-butanedione, pyridine, methylpyrazine and 2-furanmethanol. A complete list, together with the sample matrix and the used sorbent material are given in **Table 5.1**.

Table 5.1 Analyzed target compounds with corresponding sample phase and sorbent material

	VOCs[4]	Aroma compounds	
Sample matrix	Water	Beer	Coffee powder
Sorbent material	Tenax GR/Carbosieve SIII	Tenax TA, PDMS	PDMS
Target compounds	Vinyl chloride Dichloromethane Chloroform 1,2-Dichloroethane Trichloroethylene Bromodichloromethane Tetrachloroethylene Dibromochloromethane Bromoform MTBE ETBE TAME Benzene Toluene Ethylbenzene p-Xylene o-Xylene 1,4-Dioxane 2-Methylisoborneol Geosmin	1-Propanol 2-Propanol 2-Methylpropanol 1-Butanol 2-Butanol *tert*-Butanol 2-Methylbutanol 3-Methylbutanol 1-Pentanol 3-Pentanol 3-Methylpentanol 1-Hexanol 2-Ethylhexanol Ethyl acetate Ethyl butanoat Ethyl 3-methylbutanoate 3-Methylbutyl acetate Ethyl hexanoate Ethyl octanoate Ethyl decanoate Diethyl succinate 2-Phenethyl acetate Geraniol Linalool	Acetaldehyde Propanal 2-Methylpropanal 2-Methylbutanal 3-Methylbutanal 2,3- Butanedione Pyridine Methylpyrazine 2-Furanmethanol

5.2.2 Instrumentation

The experiments were performed on two instruments. The first instrument was a Thermo Trace GC Ultra (S+H Analytik, Mönchengladbach, Germany), equipped with a CTC Combi PAL autosampler with ITEX-2 option (Axel Semrau, Sprockhövel, Germany) and a Single Magnet Mixer (SMM) (Chromtech, Idstein, Germany); the autosampler was modified with a small electric fan, for faster cooling of the ITEX-trap. The GC featured a split/splitless injector (S/SL) and an Atas GL Optic 3 programmable temperature vaporizer with a nitrogen cooled cold trap for on column focussing (Axel Semrau). On column focussing was performed on a deactivated, uncoated 0.53 mm inner diameter (*i.d.*) fused silica capillary with a length of about 1 m (BGB Analytik AG, Boeckten, Switzerland). A Rtx-VMS column (medium polar, proprietary modified phase) with 60 m length, 0.32 mm *i.d.* and 1.8 µm film thickness (Restek GmbH, Bad Homburg, Germany) was used for the separation of VOCs and a Stabilwax-DA fused-silica capillary column (cross bonded carbowax (PEG)) with 60 m length, 0.32 mm *i.d.* and 1 µm film thickness (Restek GmbH) was used for the separation of

aroma compounds. The GC was coupled to a Thermo DSQ II single quadrupole mass spectrometer (S+H Analytik, Mönchengladbach, Germany) in EI mode for analyte detection.

The second instrument was a ThermoQuest Trace GC (ThermoQuest GmbH, Egelsbach, Germany) outfitted with a CTC PAL Combi-xt with ITEX-2 option (Axel Semrau) and a SMM. The GC had a split/splitless injector, an Optima 5 MS (5% Diphenyl-95% Dimethylpolysiloxan) chromatographic column with 30 m length, 0.25 mm *i.d.* and 0.25 µm film thickness (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was installed for analyte separation and a Finnigan Polaris Q (ThermoQuest GmbH) external source ion trap mass spectrometer was connected as detector in EI mode.

If not stated otherwise, 10 mL of each standard or sample solution were transferred into a 20-mL amber headspace vial (BGB Analytik AG), containing an 8×3 mm PTFE laminated magnetic stir bar (VWR International GmbH, Darmstadt, Germany), which were closed by magnetic screw caps with rubber/PTFE septa (BGB Analytik AG).

5.2.3 Sorbent Materials

The applied sorbents are mostly standard materials, which are also used in desorption tubes for gas analysis, in purge and trap instruments or as stationary phase in packed GC-columns. Carbopack C (CC), Carboxen 1000 (C1000), Carbosieve S III (CSIII), Tenax TA (TTA) and Tenax GR (TGR) are commercially available as single- and also as multi-sorbent ITEX-traps, while HayeSep D (HSD), multi-walled carbon nanotubes (MWCNTs) (Baytubes C 150 HP, Bayer Material Science, Leverkusen, Germany) PDMS (polydimethylsiloxane) and Carbowax 20M (polyethylene glycol (PEG) with a molecular weight of 20000) are custom prepared taps, which were, to our knowledge, first used here. The properties of the applied sorbent materials are given in **Table 4.2**.

5.3 Results and Discussion

The effects of the essential parameters of the ITEX-procedure will be discussed here with detailed examples; they include the selection of the sorbent material and the extraction and injection parameters, but also ways to shorten the analysis time by modifications of both the ITEX-hardware and the macros of the control software. The initial step of sample conditioning will not be discussed here, because it is basically the same as for other headspace techniques, which can be found in literature (e.g. *Static Headspace-Gas Chromatography: Theory and Practice* [5]).

5.3.1 Sorbent Selection

5.3.1.1 Theoretical Considerations

The first step in ITEX-method development should be the selection of a suitable sorbent for the analytical task. One way to achieve this is to compare the extraction efficiency of all available sorbent materials for all target analytes, like it has been performed here for the sake of completeness. Another option is to save time and limit the number of possible extraction phases, based on the target compounds and sample characteristics. To that end, it is important to check for known unintended interactions between analytes and sorbents. For example: (i) activated carbon possesses several functional groups like hydroxyl-, carbonyl-, and carboxylic functions where polar analytes like alcohols might be adsorbed, irreversible by thermodesorption, through hydrogen bonds [6], (ii) the surface of carbon based adsorbents can be activated during conditioning (even in a stream of inert gas) and then cause analyte loss by transformation reactions, especially for alcohols and carbonyl compounds[7-9] and (iii) Tenax is known to release aldehydes (e.g. benzaldehyde) and ketones during thermodesorption, which can obscure the determination of these compounds [7, 10]; on the other hand, the degradation products of PDMS can easily be identified by mass selective detectors and are usually unproblematic.[11, 12]

Afterwards, the class of sorbent material can be chosen. The sorbent materials suitable for ITEX can be separated in two classes, adsorbents and absorbents. Adsorbents rely on surface interactions of the sorbent material with analyte molecules, while in absorption the analyte molecules are solvated in the extraction phase like in an organic solvent. Absorptive interactions are weaker than adsorption on active surfaces, which makes the trapping of highly volatile analytes difficult, but also allows lower desorption temperatures and shorter desorption times, which minimizes the degradation of unstable analytes.[7, 12] Because the available active sites on the adsorbent surface are limited, problems in quantitative analysis can occur, when the analyte mass is high (either by too high concentration or too large sample amount), due to competition or displacement effects, while the equilibrium conditions of absorbents do not vary until the extracted amount is large enough (a few percent of the sorbent mass) to modify the properties of the sorbent phase.[13] This makes adsorbent materials ideal for trace/ultra-trace analysis of samples with little matrix interferences and for samples where all analytes are in a similar concentration range [4], so that a saturation of the sorbent can be excluded. Absorbents in contrast, are best used when the concentration range of the

analytes is wide or high concentrated matrix components could saturate an adsorbent, for example like ethanol often does in the aroma analysis of alcoholic beverages [14].

5.3.1.2 Exemplary Extraction Efficiencies

In the following, the relative extraction efficiencies of commercially available, but also of custom prepared ITEX-traps, obtained for the analytes listed in **Table 5.1** will be discussed. To that end, for each compound the sorbent with the highest resulting peak area was used as reference to normalize the peak areas of the other traps. The results for the analysis of VOCs are shown in **Figure 5.2**. The overall best extraction yield was achieved with TGR, which was the most efficient for eleven compounds, followed by TTA which had the highest yield for six compounds and very similar results as TGR for most other analytes, while C1000 was the most efficient for vinyl chloride and the mixed TGR and CSIII trap was good for 1,4-dioxane, 2-methylisoborneol and geosmin.

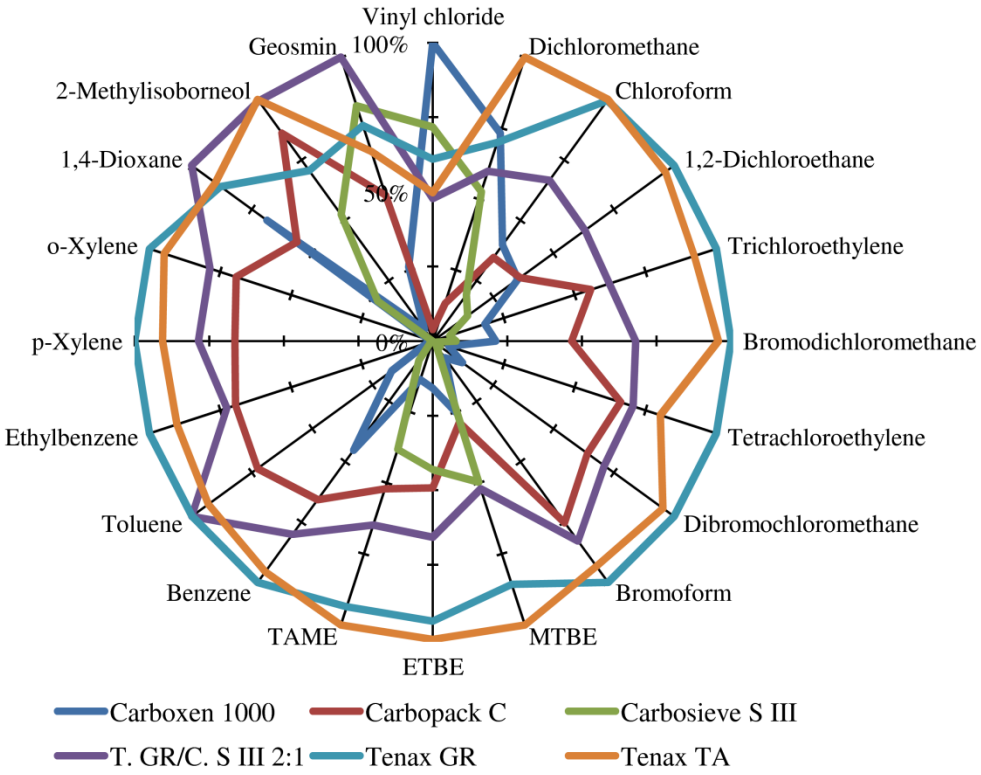

Figure 5.2 Relative extraction yields of six tested standard sorbent traps for the analysis of VOCs, result for each compound was normalized to the most efficient sorbent (data taken from Table 3.3)

The relative extraction yields of aroma compounds from beer analyis have been split in two diagrams, because of the higher number of evaluated sorbents, but both corresponding diagrams were normalized to the same scale. The standard sorbents, also used for the VOC analysis, are shown in **Figure S 5.1** in the supporting information, the custom filled traps, first applied in this project, are shown in **Figure S 5.2**. While the sorbents with the highest

yields were TTA for alcohols and HSD for longer chain esters and terpenes, a lower, but more balanced performance for all compounds could be observed for the PDMS containing traps. The average extraction yield of the C1000 and CSIII traps was quite low, except for few compounds like ethyl acetate and propanol.

The extraction yields of major coffee aroma compounds are shown in **Figure S 5.3** and **Figure S 5.4**. Good results for the extraction of acetaldehyde could be obtained wth CSIII and C1000, while they were not as well suited for the other analytes. The best results could be achieved with TGR, TTA and HSD, while the PDMS and MWCNT traps performed not so well.

5.3.2 ITEX Extraction

5.3.2.1 Sorbent and Sample Temperature

Another parameter that can be set without much experimental effort is the trap temperature; it should be set to the lowest value that can be reached in the laboratory, because analyte sorption to the trap material is typically an exothermic process[15, 16]. On the other hand, the air-water partitioning coefficient increases with higher sample temperatures, which results in a competition between both effects, when the sorbent phase is inserted directly into the sample vial like it is the case with techniques like SPME or SPDE. In this case, it is an advantage of the ITEX device, that the sorbent material is placed in a tube outside the heated sample vial and that the trap temperature can be controlled independently from the conditioning temperature of the sample. However, when the temperature difference between sample vial and trap becomes too large, problems with condensation of water on the sorbent material can arise, depending on the sorbent material. The influence of the sample and sorbent temperature on the extraction efficiency of toluene from water with four different sorbent materials, with increasing water affinity, is shown in **Figure 5.3** and **Figure 5.4** and **Figure S 5.5** and **Figure S 5.6** in the supporting information. For all investigated sorbents, the peak area increases in the direction of rising sample temperatures and decreasing packing temperatures and the highest peak area tends to be reached, when the highest sample and the lowest packing temperatures are applied. However, it is also visible that for the sorbents with higher water affinity, i.e., CSIII or PEG, the structure of the plotted surface shows discontinuous behavior at those points, where the sample temperature is higher than the packing temperature, which is most likely caused by water condensing on the sorbent surface, influencing the precision and accuracy of the measurement.

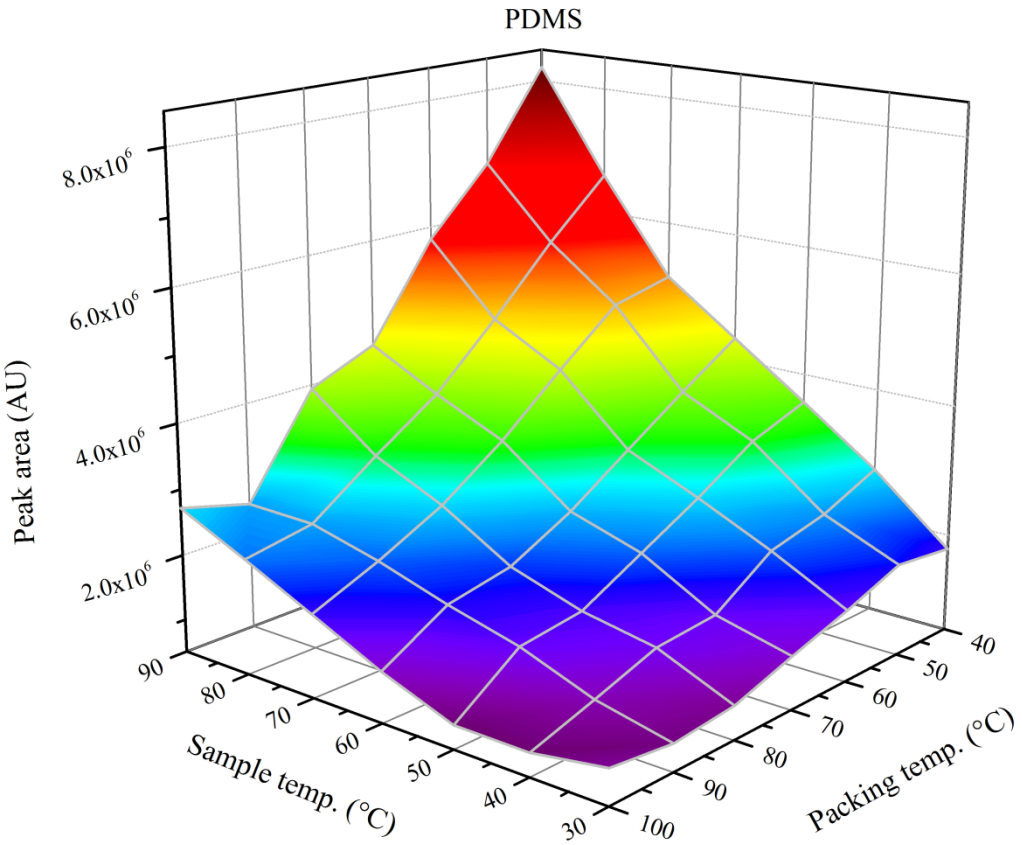

Figure 5.3 Influence of sample and packing temperature on the resulting peak area of toluene for PDMS

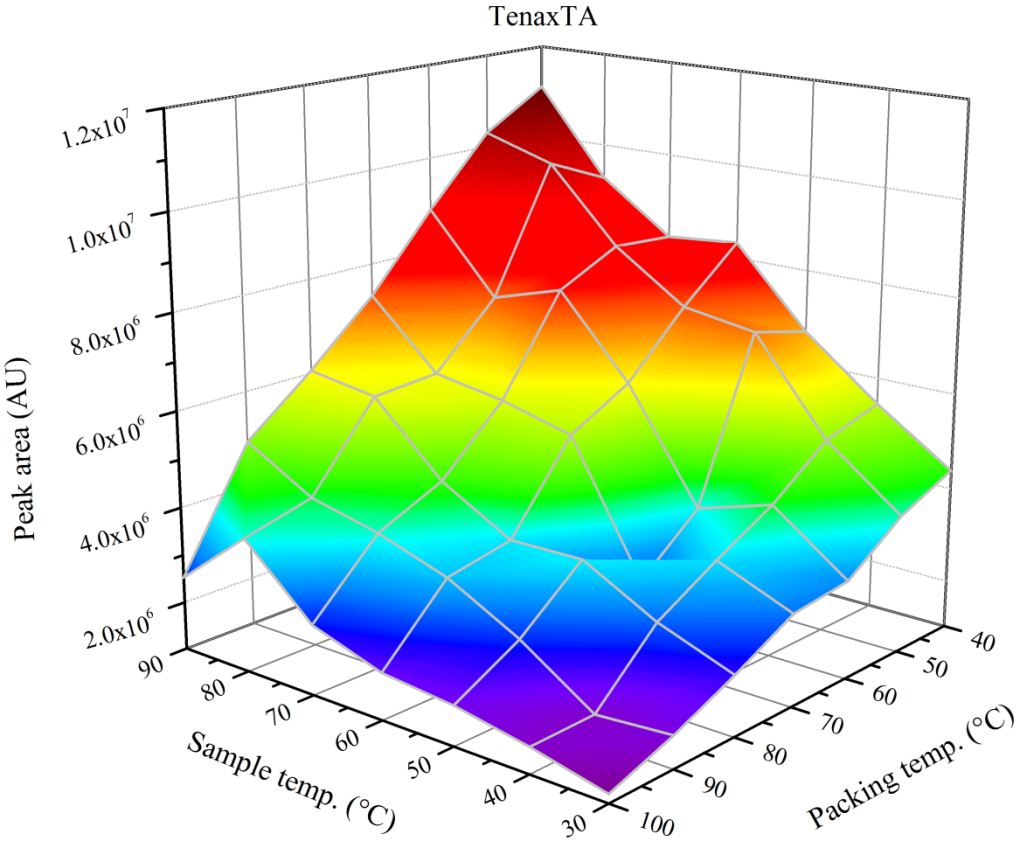

Figure 5.4 Influence of sample and packing temperature on the resulting peak area of toluene for Tenax TA

5.3.2.2 Extraction Flow and Extraction Strokes

While the extraction flow through the trap and the number of performed extraction strokes are the defining factors for the extracted amount and necessary extraction time in analytical applications, they are mostly optimized to the maximum extraction yield, individually.[3, 14] Although, most authors used a method with a high extraction flow and a large number of extraction strokes,[3, 14, 17-19] the measurement of different combinations of extraction strokes and flows has so far only been reported by two authors.[4, 20] Therefore, after discussing both individual parameters, special emphasis will be laid on the interaction of both parameters, to achieve an optimum extraction yield in a predefined extraction time, for instance in parallel to the GC oven runtime.

Figure 5.5 and **Figure 5.6** show the influence of the extraction flow on the extraction yield of six analytes from different compound classes, using a TGR/CSIII trap and a TTA trap. As a general trend, it could be observed that the extraction yield decreased towards higher extraction flows and that the effect was stronger at lower analyte concentrations. The largest influence was observed for ethyl acetate, where the extraction yield at an extraction flow of 10 µL s^{-1} was almost twice as high as at 100 µL s^{-1}, whereas no significant influence on the extraction yield could be observed for geraniol and vinyl chloride. *Jochmann et al.* suggested diffusion into the sorbent pores to be the rate limiting effect at higher extraction flows,[3] as it can also be observed by increasing plate heights in gas chromatography[21]. The lesser retention of analytes would also result in a lower breakthrough volume, however, this can be neglected as ITEX is a closed sampling system. Furthermore, the extraction flow was the parameter with the least influence on extraction yield, when it was compared to the extraction temperature and the number of extraction strokes[20], which allows more flexibility to achieve time efficient analyte enrichment.

The peak areas, obtained from the extraction of the headspace of a toluene solution with 1 mg L^{-1}, using a TTA trap with varying numbers of extraction strokes, are presented in **Figure 5.7**. The development of the peak areas can be separated in two ranges; the increase was linear from the beginning up to ten extraction strokes and then changed to a logarithmic trend until the upper limit of 100 strokes. At the start of the extraction process, the pre-conditioned sorbent material was not loaded and all analytes, which were pumped through the sorbent bed were trapped, therefore the resulting peak areas were proportional to the sampled volume, which is defined by the number of extraction strokes.[22] The loading on the trap increased with the sampled volume until the analytes, which were transported through the

sorbent bed with each extraction stroke, could not be trapped completely, anymore. From this point on, the increase of peak areas changed to the logarithmic trend and in an open sampling system, this would result in analyte loss,[23] but as ITEX is a closed system, the analyte fraction that was not adsorbed would be re-injected to the sample vial. The sampling volume with linear increase depends on the distribution constant between the analyte and the sorbent, the amount of used sorbent and the analyte concentration;[22] while it extended up to 20 extraction strokes for the low volatile geosmin, no linear trend could be observed for the very volatile vinyl chloride; in both cases under the same conditions as for toluene[4]. The following logarithmic trend was also observed in other experiments which were performed up to 200 extraction strokes and which data are not shown here, because they were only performed as single determinations due to the long extraction times. In this way, the response per extraction stroke could easily be predicted by just a few measurements. However, this simple relation is only valid for low analyte masses, because the limited sorption sites of adsorbent materials lead to saturation effects, when samples with higher concentrations or mixtures containing several compounds are analyzed. So, while toluene alone did not reach equilibrium in more than 100 extraction strokes, it only took 40 extraction strokes to reach steady state in a mixture containing 23 compounds. When the extraction is continued beyond this point, analytes with a low affinity to the sorbent material can be displaced by stronger retained analytes. This will show as non-linear behavior in the calibration functions at the higher concentrated mixed standard solutions. Thus, the number of extraction strokes can be used to tune the sensitivity of the method to the expected concentration level of the samples; a high number of extraction strokes should be applied for trace analysis in the ng L^{-1} to µg L^{-1} range, while for higher concentrated samples in the mg L^{-1} range, a lower number might be more suitable.

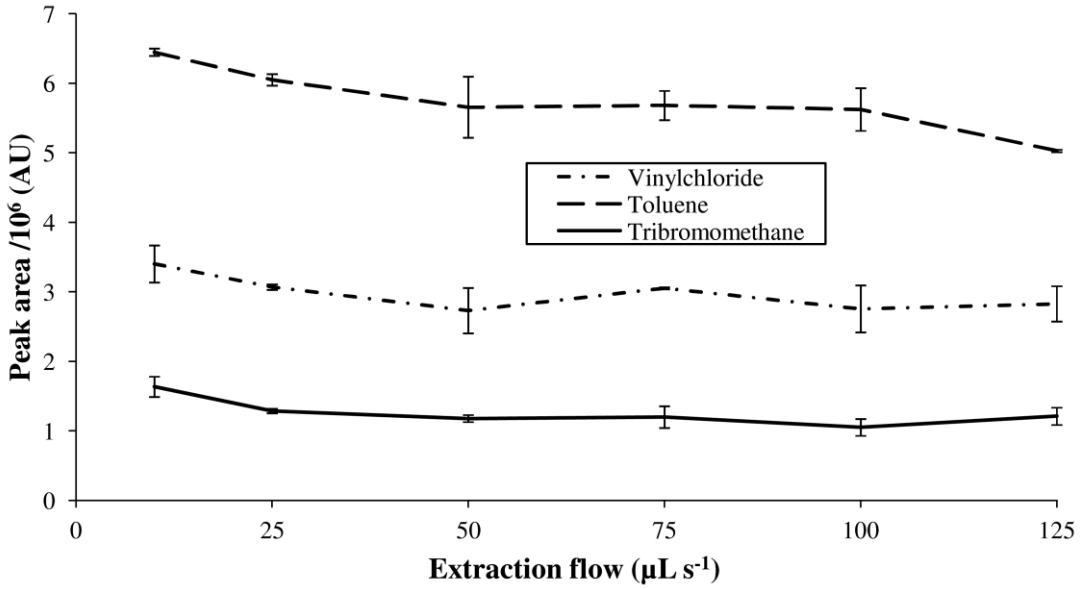

Figure 5.5 Influence of the extraction flow on the obtained peak areas of vinylchloride, toluene and tribromomethane with a TGR/CSIII trap using 10 extraction strokes, from a 23-compound standard mixture with a concentration of 1mg L^{-1} per compound

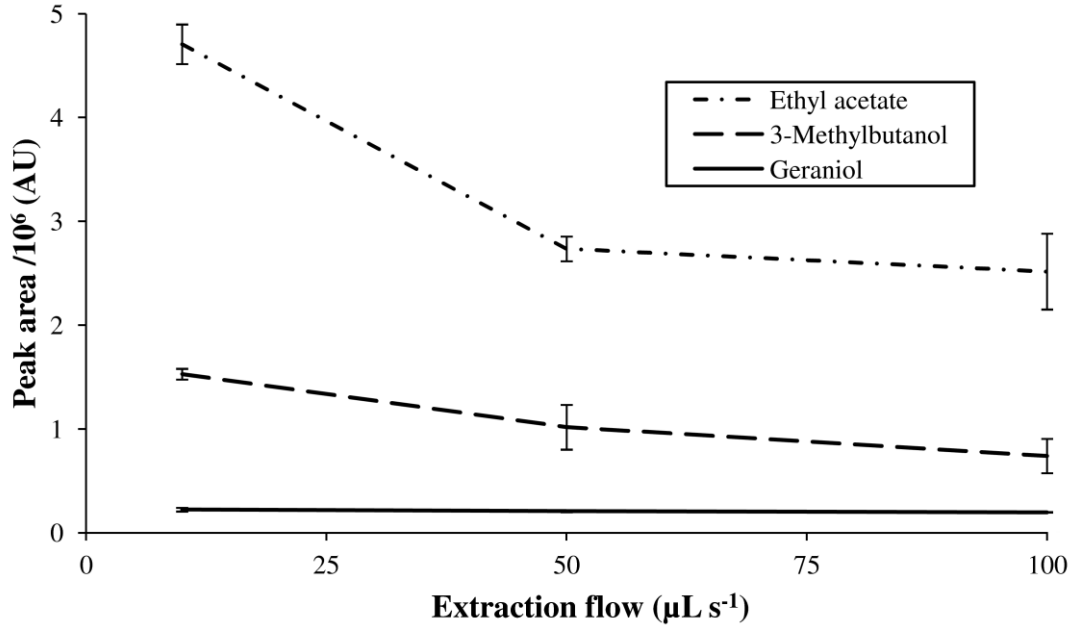

Figure 5.6 Influence of the extraction flow on the obtained peak areas of ethyl acetate, 3-methylbutanol and geraniol with a TTA trap using 75 extraction strokes from a 24-component stadard mixture with a concentration of 5 µg L^{-1}, per compound

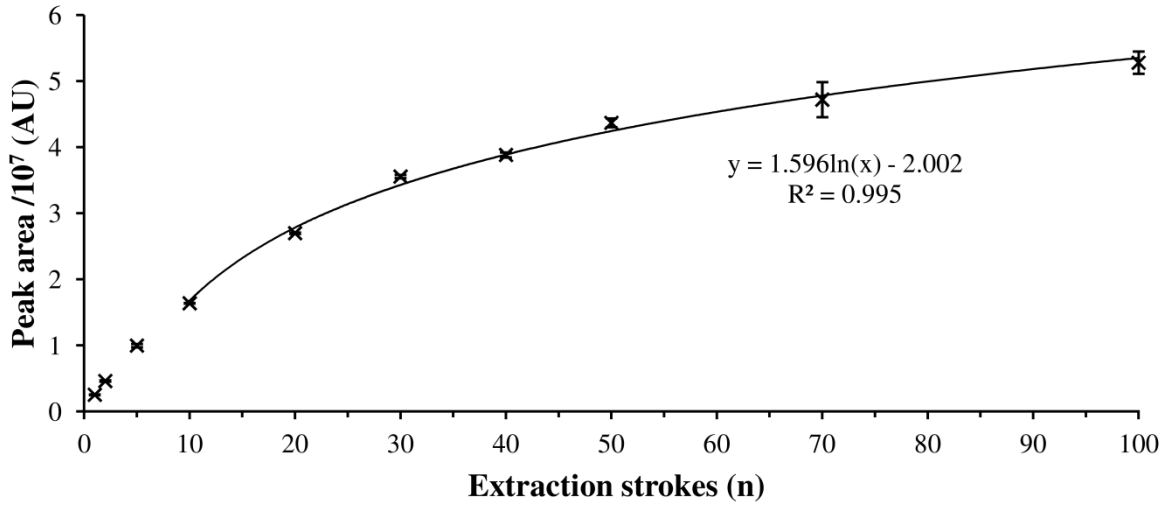

Figure 5.7 Influence of the number of extraction cycles on the extraction yield of toluene

The peak areas of nine combinations of extraction flows and extraction strokes are presented in **Figure 5.8**, accompanied by the resulting extraction time for each combination. As expected, the peak areas increased towards lower extraction flows and also towards more extraction strokes. The results for most combinations, apart from 80 extraction strokes with 30 µL s^{-1} or 20 extraction strokes with 90 and 60 µL s^{-1}, were quite similar, but the necessary extraction times varied from 18.5 to 55.6 minutes. Thus, the calculation of the extraction efficiency, as peak area obtained per second of extraction, can be a good way to identify the most suitable extraction parameters, which is given in **Table 5.2**.

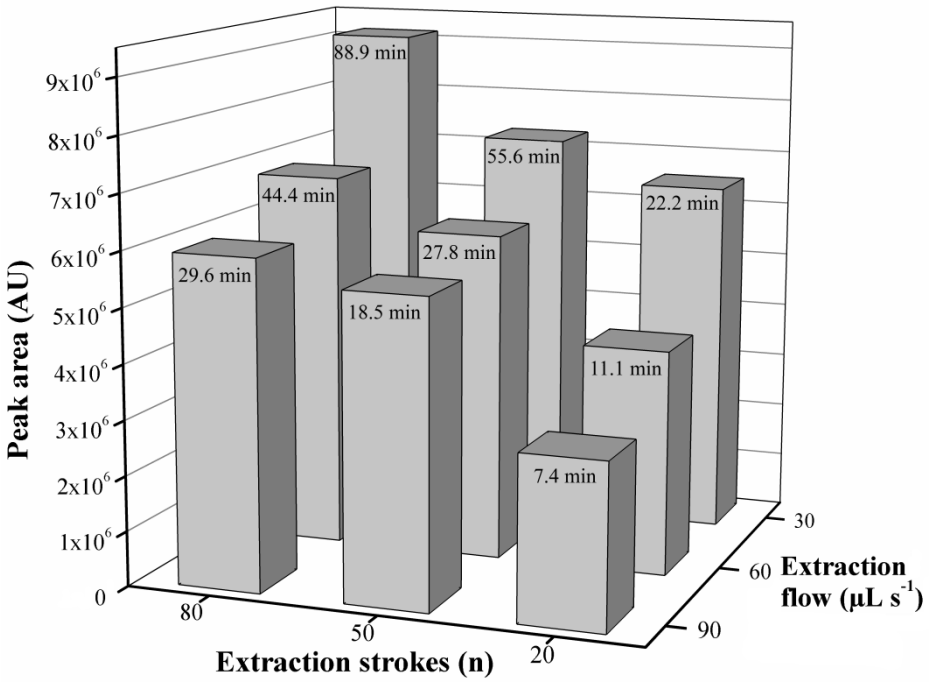

Figure 5.8 Effects of combinations of varying numbers of extraction strokes and extraction flows on the extraction yield of toluene on MWCNTs, together with the resulting extraction time of each combination (unpublished data from [20])

The highest extraction efficiencies were achieved at 20 extraction strokes with 60 and 90 µL s^{-1}, because they mainly cover the linear part of the extraction profile (see **Figure 5.7**), however with small peak areas. The most efficient extraction of the combinations with similar peak areas were achieved with 50 strokes at 90 µL s^{-1} and 20 strokes at 30 µL s^{-1} with 5.0 and 4.9 kAU s^{-1}, respectively. Four combinations which result in a constant extraction time are compared in **Figure 5.9**; here, the higher number of fast extraction strokes by far outperforms the lower extraction flows. However, these differences might diminish when longer extraction times are chosen, as was seen before.

Table 5.2 Extraction efficiencies of nine combinations of numbers of extration strokes and extraction flow, calculated as resulting peak area per second of the extraction procedure

Extraction strokes (n)	20	50	80
Extraction flow (µL s^{-1})	**Extraction efficiency (kAU s^{-1})**		
30	4.9	2.2	1.7
60	6.1	3.6	2.6
90	6.7	5.0	3.4

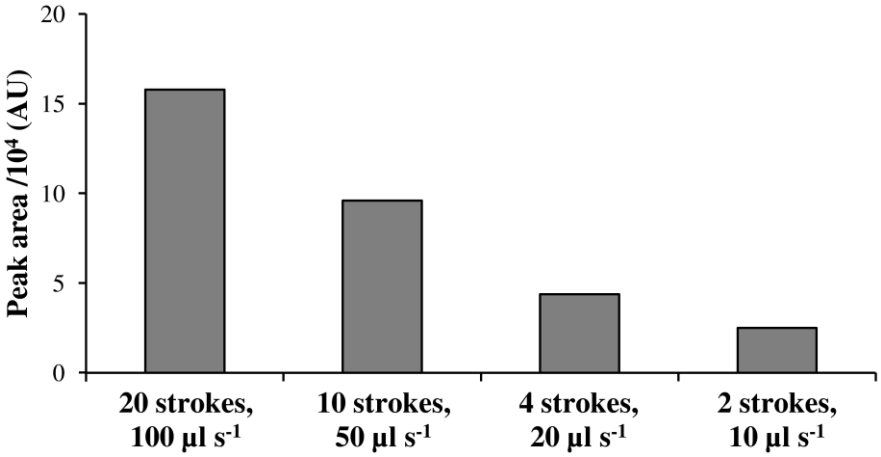

Figure 5.9 Toluene extraction yield of a TTA trap for different combinations of extraction strokes and flows, resulting in a constant extraction time of 6.7 minutes

5.3.3 ITEX Injection

The most suitable parameters for ITEX injections depend on the volatility of the analytes of interest and the technical configuration of the GC. Based on these preconditions, two general cases can be distinguished: (i) the analytes cannot be re-focused on the head of the analytical column and (ii) the analytes can be re-focused, either by a low oven temperature alone or by a cryogenic cooling trap. The consequences will be discussed in the following.

5.3.3.1 Injection without Analyte Focusing

There are two injection methods for the ITEX technique. The first (hence denoted as ITEX_inj) aspirates the defined injection volume of a desorption gas, then starts heating the trap and injects the analytes, when the predefined desorption temperature has been reached. The second (Vol_inj) aspirates a fraction of the defined injection volume (50% by default), then starts to heat the trap, while simultaneously aspirating the remaining fraction of the injection volume. Analyte injection is performed solely, when the desorption temperature and the whole injection volume have been reached. In this way, the analytes will be transported into the syringe at first and then be injected through the heated bed with a higher injection flow, similar to a classical headspace injection technique. This is used to avoid peak broadening of volatile compounds and to compensate for the thermal expansion of the gas in the trap, during the heating process, which would otherwise result in bleeding of analytes into the injection system, before the actual injection is performed.

The differences between both injection methods for desorption temperatures of 200, 250 and 300 °C are presented in **Figure 5.10**. The peaks with the ITEX_inj method displayed increased fronting, when the desorption temperature was raised, until a distinct valley developed at 300 °C. While the peak areas were similar with 204794, 221662 and 201930 AU, respectively, the intensity and signal to noise ratio decreased significantly. The peaks of the Vol_inj method did not show fronting, except for the one with 300 °C desorption temperature, where the heating took longer than the parallel aspiration of the desorption gas volume, which might be compensated by a larger desorption volume. However, the peak areas were much smaller with 133017, 139258 and 135774 AU, because the analytes were diluted to the whole extraction volume plus the void volume of the trap, which remains in the tube after the injection. The total volume of an ITEX-tube is about 300 µL, the sorbent bed takes up 160 µL, which results in about 118 µL sorbent material, if an optimal sphere packing is assumed, ensuing a total void volume of about 180 to 190 µL. With a desorption volume of 500 µL, the resulting peak area of the Vol_inj method should theoretically be around 40% lower than the ITEX_inj peak area, which is close to the actual results.

The heating times for several desorption temperatures, starting from a temperature of 30 °C, are given in **Table 5.3**, together with the theoretical expansion of the gas in the void volume. However, the theoretical expansion can only be used as an allusion to the necessary aspiration volume of the Vol_inj method, because the volume of gas released during heating is also influenced by the amount of analytes or water sorbed to the trap material. Above

200 °C, the heating rate decreases significantly, which would require larger desorption volumes with the Vol_inj method to avoid premature bleeding of the analytes to the injector, as it was observed with the 300 °C desorption temperature. On the other hand, this would also lead to stronger analyte dilution in the desorption gas and a broadened injection band. The peak width is also determined by the quotient of desorption volume and desorption flow (like in the example: 500 µL ÷ 100 µL s^{-1} = 5s), but the influence of the desorption flow is mostly insignificant for volatile compounds, while low volatiles benefit from lower flows.

In the given example, complete desorption of the analyte was achieved at 200 °C with both injection methods using a desorption volume of 500 µL and the peak areas did not further increase, when the desorption temperature has been raised. In this case, the ITEX_inj method gave a higher peak area with sufficiently good peak shape and might be the better option, when only volatile compounds are analyzed. Higher desorption temperatures may be needed, when also low volatile compounds are analyzed, necessitating the use of the Vol_inj method. Then, the analyst has to find a suitable balance between desorption temperature, desorption volume and aspiration flow for all target analytes.

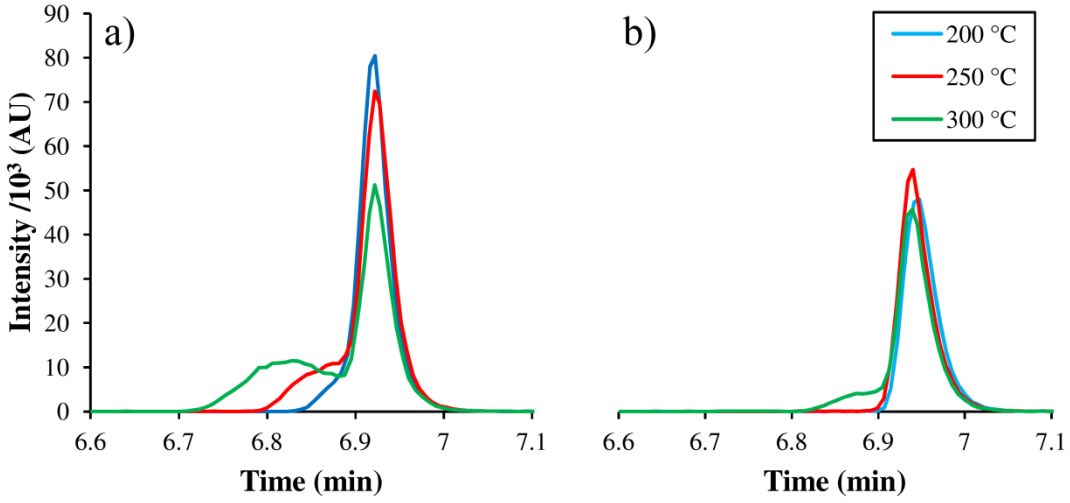

Figure 5.10 Peak shapes of 0.1 mg L^{-1} toluene from a Tenax TA trap using a) ITEX_inj and b)Vol_inj methods at different desorption temperatures, with an injection volume of 500 µL, 100 µL s^{-1} desorption flow and a 1:10 split ratio

Table 5.3 Heating time and theoretical void gas expansion during the desorption process, starting from 30 °C

T (°C)	30	50	100	150	200	250	300	350
Heating time (s)	0	5	10	16	24	34	47	63
Volume expansion (%)	0	7	23	40	56	73	89	106

5.3.3.2 Injection with Analyte Focusing

The initial width and shape of the injection band are of less importance, when the analytes can be re-focused on the column. Therefore, the ITEX_inj method can be used with higher desorption temperatures, which will result in larger peak areas of low volatile compounds or when stronger sorbent materials than Tenax TA in the example above are used. In this case, the desorption temperature is either limited by the thermal stability of the analytes and the sorbent material or by the maximum temperature of the ITEX heater. Variations of the desorption volume only cause small effects on the resulting peak areas, because a fraction of the analytes will be transported into the GC injector by the thermal expansion during the heating process and only the remaining void volume needs to be flushed. Generally, the results with 100 and 500 µL desorption volume were very similar and a further increase to 1 mL only resulted in inferior repeatability for most compounds.

In contrast to the non-focused injection, low desorption flows do not result in peak broadening and therefore, also low flows can be applied for injections when analyte focusing is possible. As mentioned before, the desorption flow had only low influence on the resulting peak areas of volatile compounds and was practically insignificant for highly volatiles like vinyl chloride, while a decrease of the desorption flow from 50 µL s^{-1} to 10 µL s^{-1} almost doubled the obtained peak area of geosmin (see **Figure 3.3 (d)**)

5.3.4 Trap Conditioning

Before the first use, the traps should be conditioned to remove possible impurities from packing, transport and storage. The conditioning is straightforward, the nitrogen flow is recommended to be about 5 mL min^{-1} and the conditioning temperature should be just below the maximum tolerable temperature of the sorbent material, to achieve complete desorption of possible residual compounds. An initial conditioning time around 30 minutes should be sufficient. The conditioning time between analyses depends on the sorbent strength, the volatility of analytes and the sample concentration. A flushing time of ten minutes is usually long enough to avoid carryover of volatiles in the µg L^{-1} range, while the complete removal of semi-volatiles in the mg L^{-1} range can require over 20 minutes.

5.3.5 Improving the ITEX-procedure

A relatively simple way to improve the system is to add a cooling-fan to the autosampler (**Figure 5.11**), to reach lower trap temperatures, because this makes the sorption process more efficient. Therefore, a 12 V, 6 cm axial-flow fan has been attached directly to the

autosampler head using duct-tape. The fan was operated with a 5 V DC power adapter, originally intended for an USB hub, which gave sufficient air-flow for the cooling task. A trap temperature of 24 °C could be achieved with active cooling, but the lowest software controlled temperature of the ITEX trap heater, to maintain steady enrichment conditions, is 30 °C. This lower temperature limit could not be reached without active cooling, even in an air-conditioned laboratory. Active cooling also shortens the cooling time of the trap. **Figure 5.12** shows the difference in cooling time between the standard passive cooling and active cooling by a fan, attached to the autosampler. With a cooling fan, suitable trap temperatures for analyte enrichment can be reached in about ⅓ of the time.

Figure 5.11 Combi PAL modified with a 6 cm fan for trap cooling

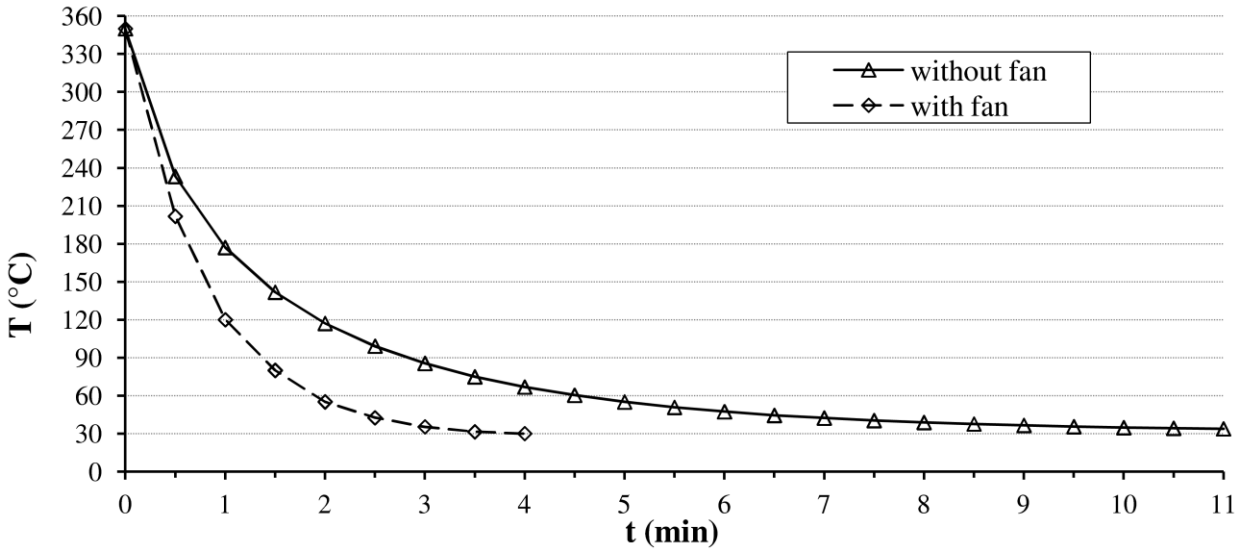

Figure 5.12 Influence of active cooling of the trap on cool-down time

The most time consuming steps in the ITEX-procedure are typically sorption, sample conditioning and trap cleaning with cooling. As seen before, the number of extraction strokes performed during the sorption step is the most important parameter, defining the sensitivity of the method and it is therefore desirable, to use as much time as possible on this step, when trace analysis is required. This can be achieved by the modification of the standard procedure to perform the trap cleaning in parallel to the sample conditioning, before the extraction of the next sample begins (see **Figure 5.13**). When the whole extraction procedure is conducted in parallel to the GC-run of the previous sample, it is possible to perform about 50 extraction strokes in a total analysis time of 30 minutes, with an incubation time of 15 minutes, while the trap is flushed for 10 minutes, to allow a safe cooling time. The standard procedure would only allow 10 to 20 strokes, when the flushing time is shortened.

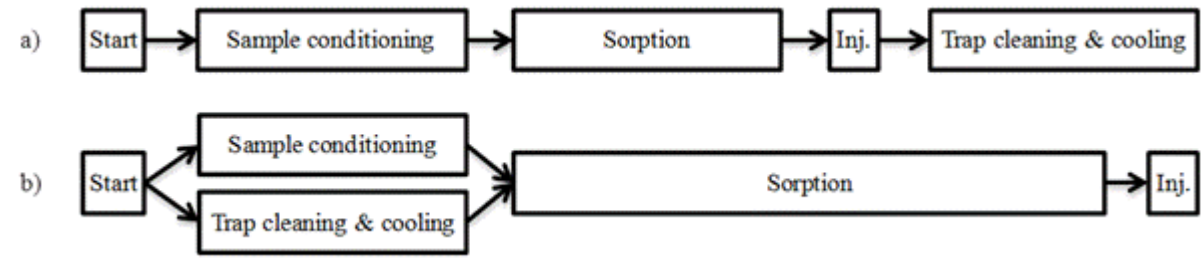

Figure 5.13 Basic steps of the ITEX-procedure; a) standard procedure, b) runtime optimized procedure

Furthermore, the Vol_inj method by default aspirates 50% of the desorption volume before heating the trap, which does not leave enough aspiration volume/time to achieve high desorption temperatures without analytes bleeding to the column, unless large desorption volumes are used. A modification to the macro, that was performed without negative effects,

was to skip the pre-heating aspiration and to begin the aspiration of the whole desorption volume at the same time as the heating process. The aspiration flow can then be adjusted in a way that the required heating time (see **Table 5.3**) is slightly shorter than the aspirating time.

5.3.6 Possible Sources of Error

The main reasons for unsatisfactory results often are non-optimum extraction and injection conditions, like a small number of slowly performed extraction strokes or a too large desorption volume for volatile compounds. However, there are also a few mechanical issues that can cause the diminishing of extraction performance over time and need to be observed.

The most important is the plunger of the syringe. It should be checked for leak tightness regularly because a failure will result in less gas pumped through the sorbent bed, lowering the extracted analyte amount. Although the plunger usually lasts for several thousand movements, this limit might be reached within several weeks, when a very large number of extraction strokes is performed per analysis. Another fault can occur at the connection of the sorbent tube to the syringe. When the connection nut has not been screwed in well enough, it can loosen over time, due to thermal stress during desorption and trap cleaning, which will result in a leakage, too. Finally, a problem that has only occurred once so far in our lab, in over four years of continuous use, was the blocking of the needle by scraped septum particles, which has most likely been caused by too much force on the septum nut.

5.4 Conclusions

Based on previous experiences, the time needed for the development of appropriate methods for certain analytical tasks can be shortened drastically. Therefore, all characteristics of the sample and the analytes have to be taken into account. **Figure 5.14** presents a flow chart, in which the previously discussed parameters are summarized and which gives recommendations for efficient method development, for different analyte volatilities and sample compositions. This should enable new ITEX users to develop suitable methods in less time, avoiding unfavorable extraction and injection conditions. Currently, a characterization of ITEX-traps is undergoing with several test compounds, to build a database, which can be used to predict optimal extraction conditions by simulation.

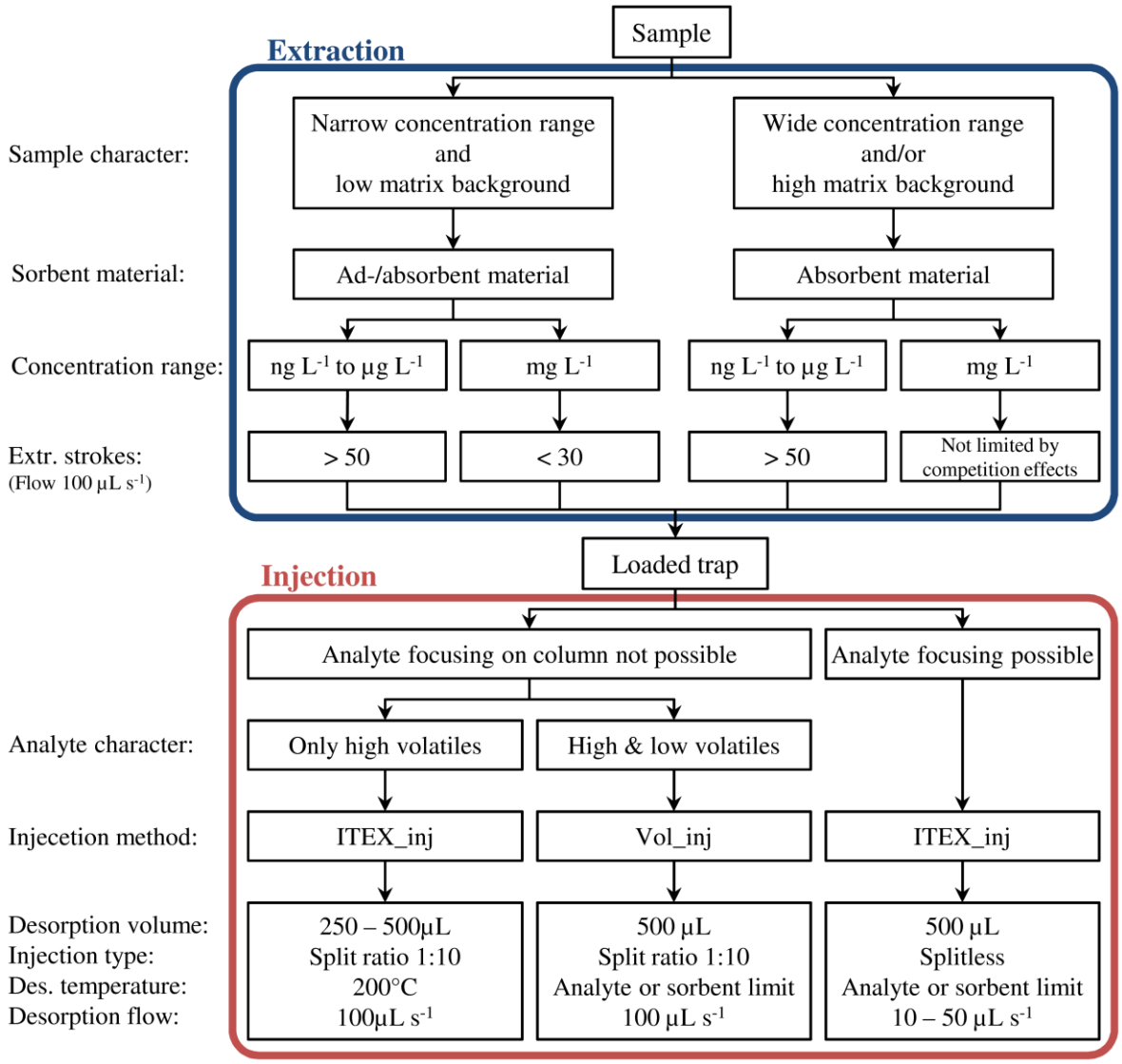

Figure 5.14 Flow chart of the ITEX procedure for accelerated method development, showing exemplary extraction and injection parameters for different sample conditions and analyte compositions

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5.6 Supporting Information

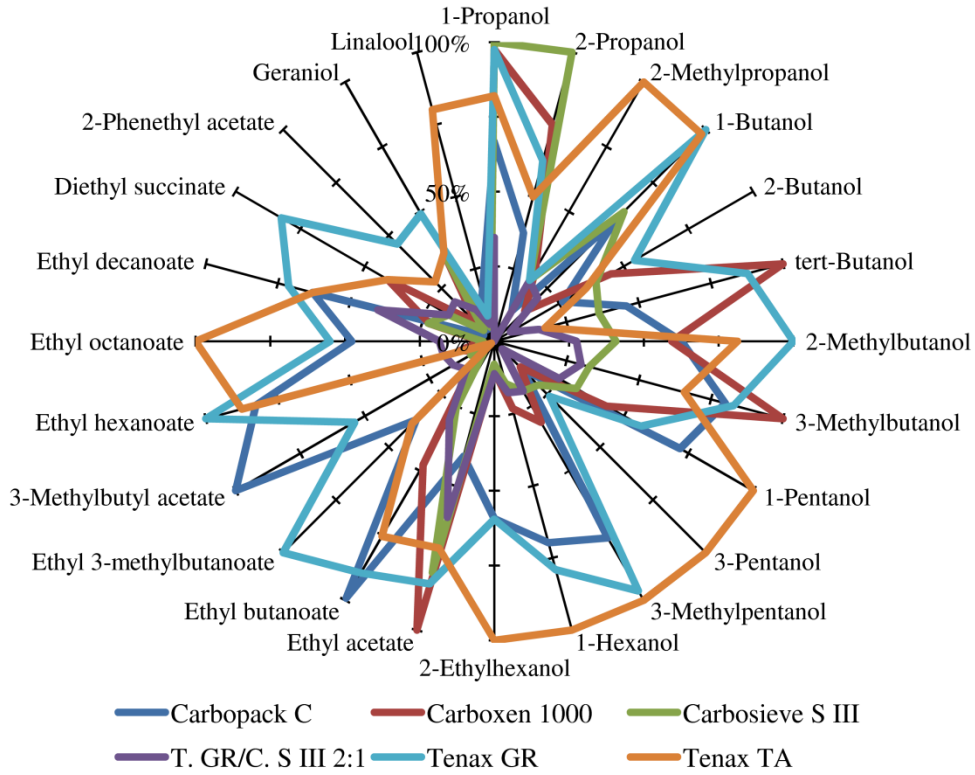

Figure S 5.1 Relative extraction yields of six tested standard sorbent traps for the analysis of beer aroma compounds, result for each compound was normalized to the most efficient sorbent from Figure S 5.1 and Figure S 5.2

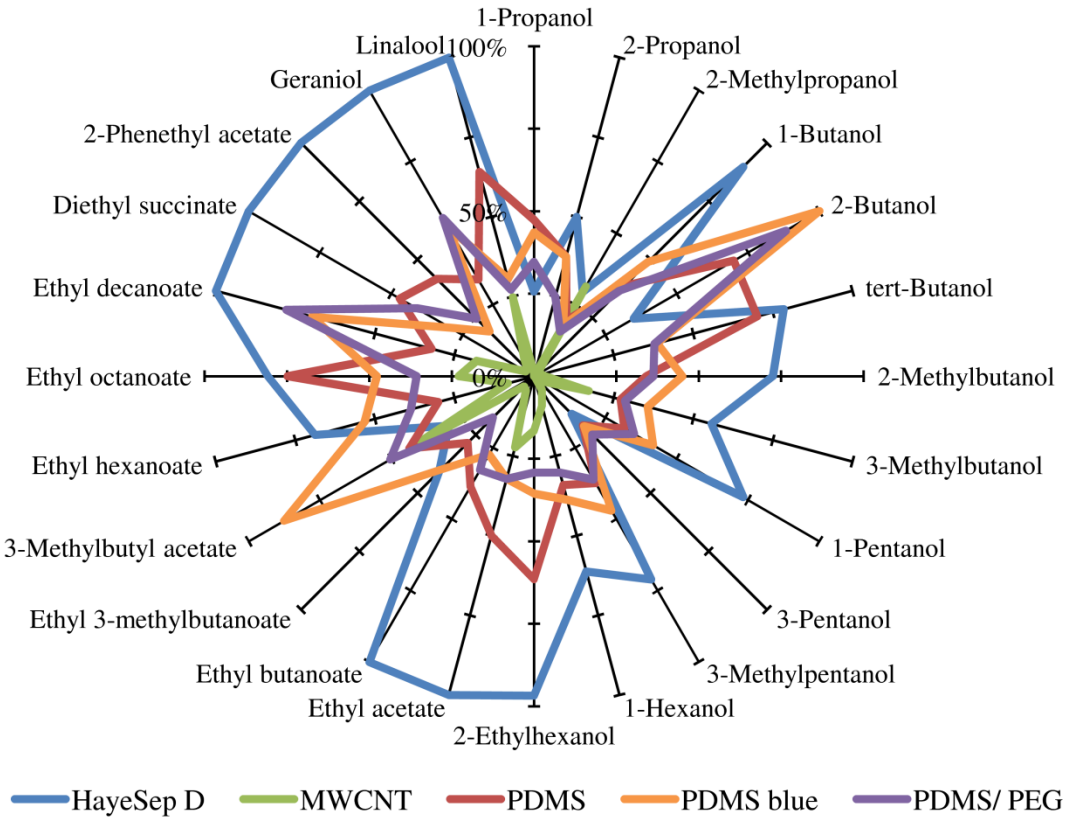

Figure S 5.2 Relative extraction yields of five tested custom packed traps for the analysis of beer aroma compounds, result for each compound was normalized to the most efficient sorbent from Figure S 5.1 and Figure S 5.2

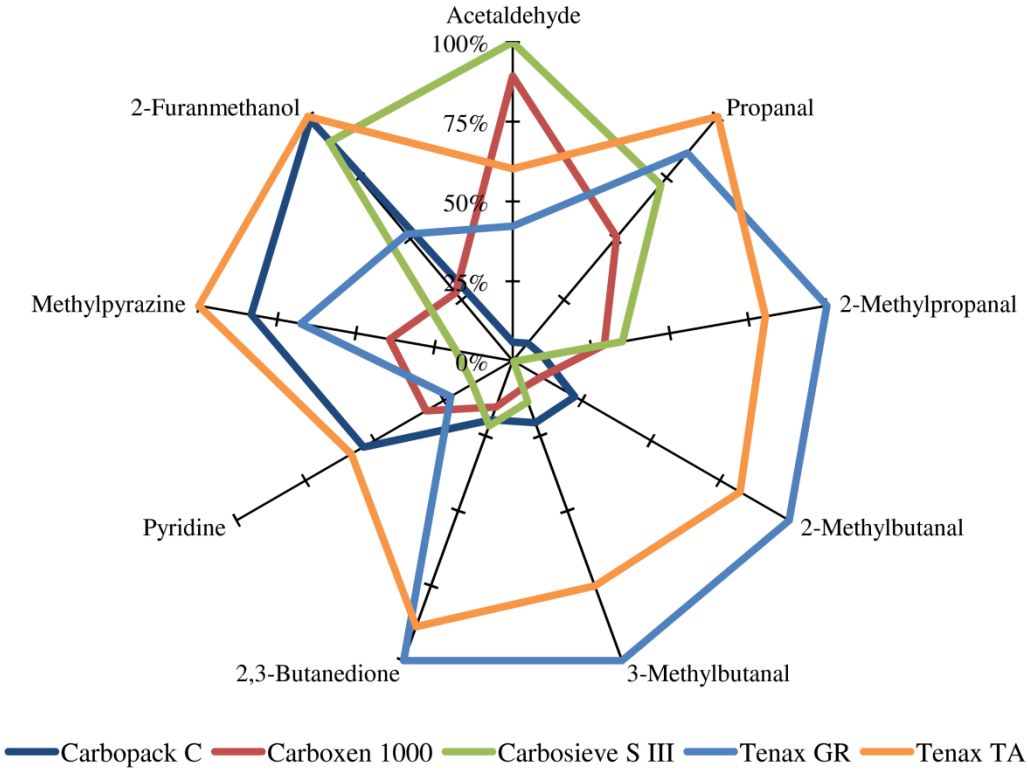

Figure S 5.3 Relative extraction yields of five tested standard sorbent traps for the analysis of coffee aroma compounds, result for each compound was normalized to the most efficient sorbent from Figure S 5.3 and Figure S 5.4

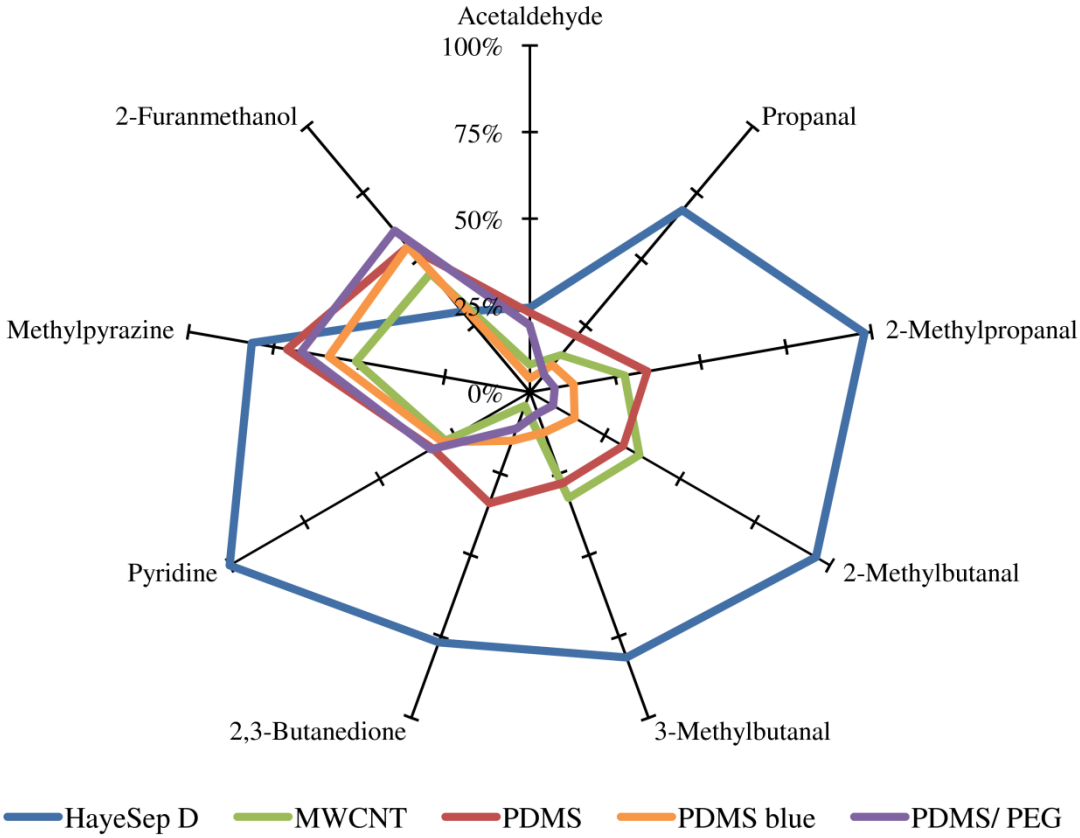

Figure S 5.4 Relative extraction yields of five tested custom packed traps for the analysis of coffee aroma compounds, result for each compound was normalized to the most efficient sorbent from Figure S 5.3 and Figure S 5.4

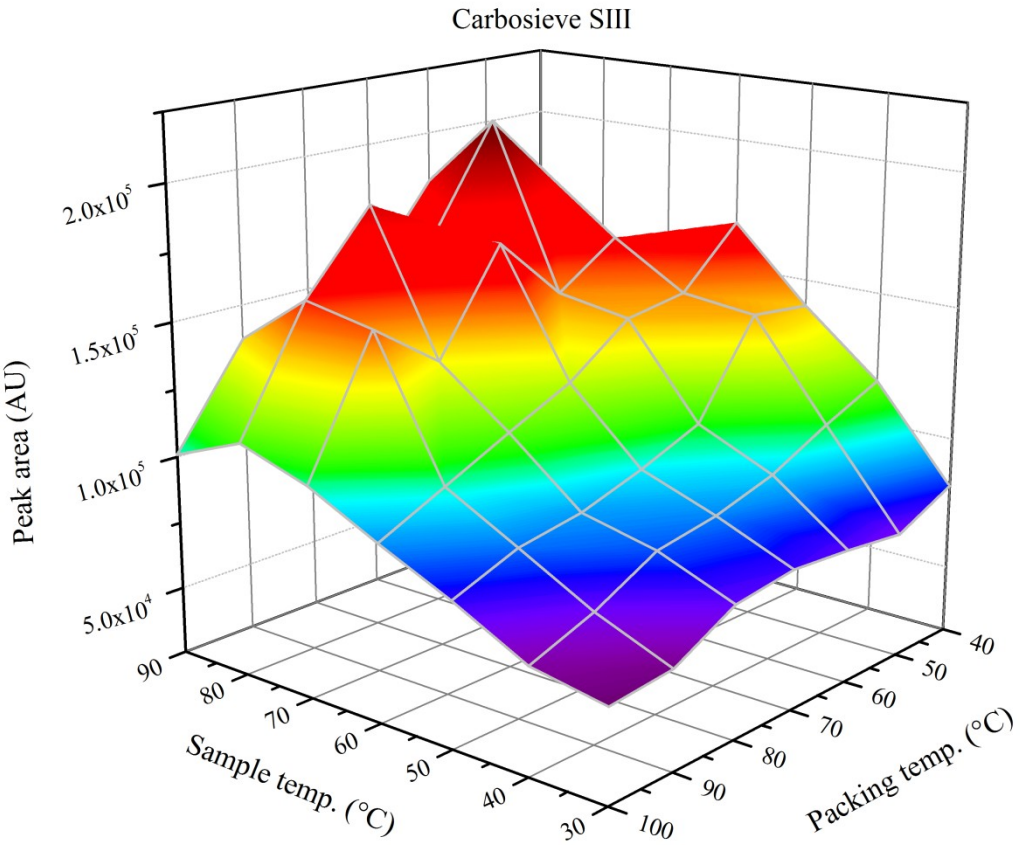

Figure S 5.5 Influence of sample and packing temperature on the resulting peak area of toluene for Carbosieve S III

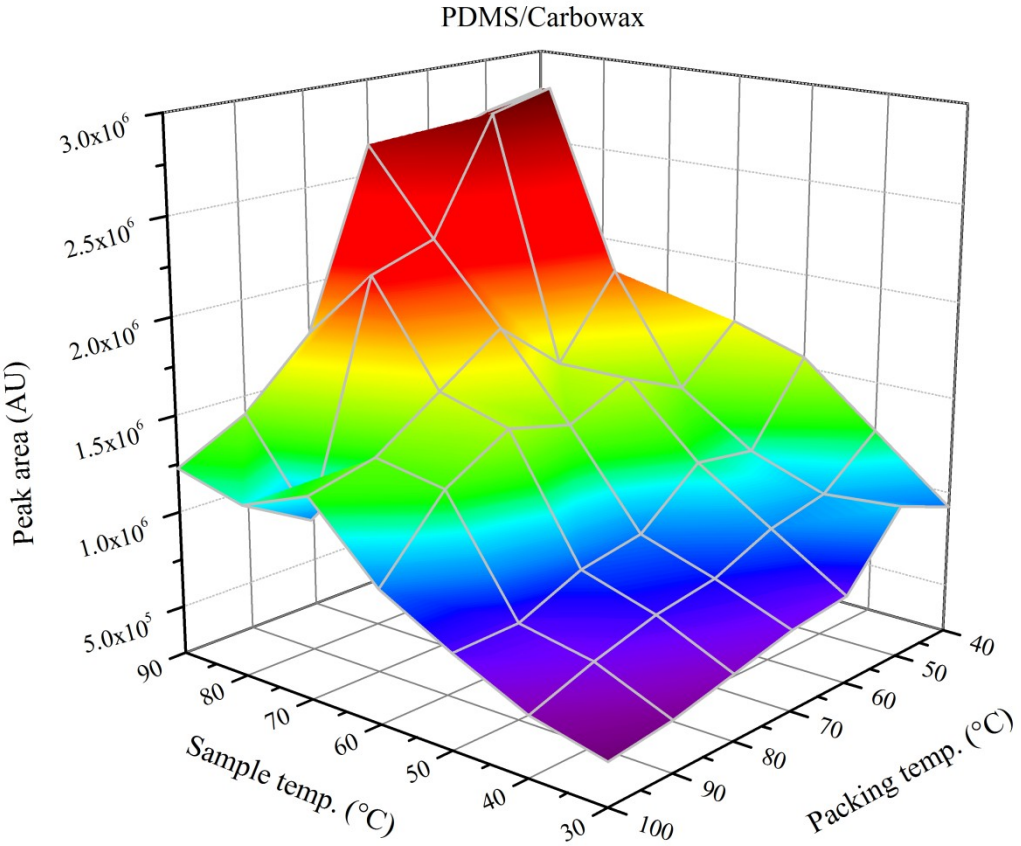

Figure S 5.6 Influence of sample and packing temperature on the resulting peak area of toluene for PDMS with 10% Carbowax

6 Concluding Remarks and Outlook

Parts of this chapter have been published in modified form in *Laaks, J.; Jochmann, M. A.; Schmidt, T. C., Solvent-free microextraction techniques in gas chromatography. Analytical and Bioanalytical Chemistry 2012, 402, 565-571,* © Springer-Verlag 2012

Although microextraction techniques have come a long way since they have first been presented about 25 years ago, there is still room for improvement, due to their importance in the overall analytical process. Automated solventless extraction techniques are now commercially available for routine GC-based analysis of almost all typical volatile and semi volatile compounds. In the coming years, such methods surely will also be included to a larger extent in standardization efforts, thereby replacing more and more outdated liquid-liquid extraction methods. Among the numerous different devices of solvent-free microextraction techniques for gas chromatography are sorbent coated rods, bars and needles, sorbent packed needles and bulk sorbent techniques. However, most newly presented techniques are minor adaptations of already existing approaches and fundamentally new developments are not on the horizon. Yet, all of these techniques possess their individual advantages and limitations and until now, no truly universal microextraction device has been presented, which is equally useable for liquid and gas phase extractions and provides an unrestricted choice of extraction phase material, at the same time. In this way, the user is either limited to liquid polymer coatings that can be applied to a surface or to particulate sorbents for packed beds.

An increase in the amount of applied extraction phase material, which determines the enrichment capability of a microextraction device, does not necessarily result in a linearly increased sensitivity and might lead to other problems during automation or sample introduction to the chromatographic system. In this thesis, method detection limits for VOCs were achieved, which were in the same range as with typical purge and trap systems. While the sorbent bed of purge and trap systems has a volume of more than 1 mL, the bed volume of the ITEX system is just 0.16 mL and the sample size for the extraction procedure is smaller, also. The method detection limits of the ITEX method for alcohols and esters was, on the other hand, depending on the sorbent material applied, in the same range as or higher than with the presented SPDE method or a SPME method using a sol-gel coating. The extraction phase volumes are about 4.5 µL for SPDE and 0.6 µL for SPME, respectively and the ITEX method required a cryogenic trap, to re-focus the analytes on the column head to

prevent peak broadening. Peak broadening can result from the larger void volumes of the ITEX system, that come along with the larger sorbent amount, but also from the relatively slow heating rate of the ITEX heater. On the other hand, the heater makes ITEX independent from possible injection port temperature profiles, which can influence desorption of other needle based techniques. A further optimization of the ITEX system to reduce the void volumes and to increase the heating rate might solve these problems.

It is apparent that the choice of sorbent material is the decisive factor for the sensitivity and dynamic range of the method and that lower amounts of a well suited sorbent material can give better results than larger amounts of an inept sorbent. The method detection limits with the adsorbent Tenax TA were about one tenth of those with the partitioning material PDMSb, but the opposite could be observed for the linear range, which was about five times larger with PDMSb, due to saturation effects of the adsorbent. While practically all kinds of particulate standard adsorbents can be used straightforward for ITEX, the situation for absorbents is much different, where until now only PDMS is usable. Another interesting absorbent would be the PEG material, which was also used in the SPDE needle, but so far, it could only be applied as a fraction of 10% in PDMS with ITEX. This small fraction had no significant influence on the extraction performance for polar compounds and cannot be increased, because PEG turns liquid at desorption temperatures and could drip out or block the needle. This problem might be solved, when a suitable support material is found, on which the PEG can be grafted. Therefore, more research should be undertaken to adapt more absorbent materials for the use in ITEX traps or to develop and evaluate new extraction phase materials to further enhance the scope of target compounds and to increase selectivity, especially for the analysis of polar compounds. In the focus are, for instance, several types of nanomaterials (gold nanoparticles, porous carbon, carbon nanotubes), ionic liquids, sol-gel coatings or molecularly imprinted polymers.

Another important aspect of microextraction techniques, which has made little progress since the beginning, is the more systematic development and optimization of analytical methods, which is currently still dominated by mere trial-and-error approaches. These require much time and experimental effort to find appropriate extraction materials and operational parameters. A first step, which has been made here, was to summarize the experiences from method development for multiple analytes, including aromatics, heterocyclic aromatics, halogenated hydrocarbons, fuel oxygenates, alcohols, esters and aldehydes. This should give future users general guidelines for a more efficient method development, i.e. which

parameters are the most important and where to start with their optimization. Currently, a characterization of ITEX-traps is undergoing with several test compounds, which can give further evidence for the choice of appropriate sorbent materials. This should result in the future development of predictive tools for the optimization of extraction parameters by design of experiment approaches and integration of such tools in software platforms, to minimize remaining experiments and to facilitate a rapid optimization of analytical methods.

7 Appendix

7.1 Abbreviations and Symbols

A_{cal}	:	Calibration peak area
A_{cor}	:	Corrected peak area
A_{max}	:	Max. peak area
amu	:	Atomic mass unit
A_t	:	Auto-tune peak area
AU	:	Arbitrary unit
Axx	:	Altbier
β	:	Phase ratio
BTEX	:	Benzene, toluene, ethylbenzene, xylenes
C	:	Analyte concentration
C_0	:	Initial analyte concentration
C1000	:	Carboxen 1000
CAS	:	Chemical Abstracts Service
CC	:	Carbopack C
CME	:	Capillary microextraction
CSIII	:	Carbosieve SIII
d_c	:	Core diameter
DC	:	Direct current
d_f	:	Film thickness
DLLME	:	Dispersive liquid-liquid microextraction
DVB	:	Divinylbenzene
EI	:	Electron ionization
EPA	:	Environmental Protection Agency
ETBE	:	Ethyl *tert*-butyl ether
EU	:	European Union
eV	:	Electron Volt
F_e	:	Extracted fraction
FPN	:	Fiber-packed needle
GC	:	Gas chromatography
HF-Esy	:	Hollow fiber extraction syringe
HS	:	Headspace
HSD	:	HayeSep D
HSSE	:	High capacity sorptive extraction
Hxx	:	Helles
i.d.	:	inner diameter
INCAT	:	Inside needle capillary absorption trap

ITEX	:	In-tube extraction
K_{aw}	:	Air-water partitioning constant
K_{EH}	:	Extraction phase-headspace distribution constant
K_{ES}	:	Extraction phase-sample distribution constant
K_{HS}	:	Headspace-sample partitioning constant
K_{ow}	:	Octanol-water partitioning constant
Kxx	:	Kölsch
LD	:	Linear discriminant
LDA	:	Linear Discriminant Analysis
LLE	:	Liquid-liquid extraction
LLME	:	Liquid-liquid microextraction
LP	:	liquid phase
LPME	:	Liquid phase microextraction
m	:	Mass
m_0	:	Initial analyte mass
MDL	:	Method detection limit
MEPS	:	Microextraction in packed syringe
MESI	:	Membrane extraction with sorbent interface
MHE	:	Multiple headspace extraction
MIB	:	2-Methylisoborneol
MS	:	Mass spectrometry
MTBE	:	Methyl *tert*-butyl ether
MWCNTs	:	Multi-walled carbon nanotubes
NT	:	Needle trap
OSF	:	Organic solvent film extraction
OTT	:	Open-tubular trapping
P&T	:	Purge and trap
PA	:	Poly(acrylate)
PAH	:	Polycyclic aromatic hydrocarbon
PCB	:	Polychlorinated biphenyl
PDMS	:	Polydimethylsiloxane
PEG	:	Polyethylene glycol
PES	:	Polyethersulphone
PFTBA	:	Perfluorotributylamine
pp-LFER	:	Polyparameter-linear free energy relation
PTFE	:	Polytetrafluorethylen
Pxx	:	Pilsener beer
Pxx\alc	:	Alcohol free Pilsener beer
RF	:	Linear range factor
R_i	:	Retention index
RSD	:	Relative standard deviation

R_t	:	Retention time
S/N	:	Signal to noise ratio
S/SL	:	Split/splitless injector
SBSE	:	Stir bar sorptive extraction
S_c	:	Standard deviation
SDME	:	Single drop microextraction
S_E	:	Adsorbent surface concentration
S-HS	:	Static headspace
SMM	:	Single magnet mixer
SPDE	:	Solid phase dynamic extraction
SPE	:	Solid phase extraction
SPME	:	Solid phase microextraction
SR	:	Silicone rod
S_r	:	Relative sensitivity
ST	:	Silicone tube
Sxx	:	Schwarzbier
$t_{(N-1)}$	:	Student's *t*-value
TAME	:	*tert*-amyl methyl ether
TCMC	:	Tubular cylindrical microconcentrator
T_g	:	Glass temperature
TGR	:	Tenax GR
THM	:	Trihalomethanes
TTA	:	Tenax TA
$u(c_{cal})$	:	Uncertainty of calibration
$u(c_m)$	:	Total measurement uncertainty
$u(c_s)$	:	Sample uncertainty
USB	:	Universal Serial Bus
V	:	Volume
V	:	Volt
VOC	:	Volatile organic compound
WHO	:	World Health Organization
Wxx	:	Wheat beer
Wxx\alc	:	Alcohol free Wheat beer

7.2 List of Figures

7.3 List of Tables

7.4 List of Publications

7.4.1 Publications in Peer-reviewed Journals

1. **Laaks, J.**; Jochmann, M. A.; Schilling, B.; Schmidt, T. C.,
   In-tube extraction of volatile organic compounds from aqueous samples: An economical alternative to purge and trap enrichment.
   Analytical Chemistry 2010, 82, (18), 7641-7648.

2. Akinlua, A.; Jochmann, M. A.; **Laaks, J**.; Ewert, A.; Schmidt, T. C.,
   Microwave-assisted nonionic surfactant extraction of aliphatic hydrocarbons from petroleum source rock.
   Analytica Chimica Acta 2011, 691, (1-2), 48-55.

3. Bernstein, A., Shouakar-Stash, O., Ebert, K., Laskov, C., Hunkeler, D., Jeannottat, S., Sakaguchi-Söder, K., **Laaks, J**., Jochmann, M. A., Cretnik, S., Jager, J., Haderlein, S. B., Schmidt, T. C., Aravena, R., Elsner, M.
   Compound-specific chlorine isotope analysis: A comparison of gas chromatography/isotope ratio mass spectrometry and gas chromatography/quadrupole mass spectrometry methods in an interlaboratory study
   Analytical Chemistry 2011, 83, (20), 7624-7634**.**

4. **Laaks, J**.; Jochmann, M. A.; Schmidt, T. C.
   Solvent-free microextraction techniques in gas chromatography
   Analytical and Bioanalytical Chemistry 2012, 402 (2): 565-571

5. **Laaks, J**.; Letzel, T.; Schmidt, T. C.; Jochmann, M. A.
   Fingerprinting of red wine by headspace solid-phase dynamic extraction of volatile constituents
   Analytical and Bioanalytical Chemistry 2012, 403 (8): 2429-2436

6. **Laaks, J**. Jochmann, M. A., Schilling, B.; Molt, K.; Schmidt, T. C.
   In-tube Extraction-GC/MS as High Capacity Enrichment Technique for the Analysis of Alcoholic Beverages
   Journal of Agricultural and Food Chemistry 2014, DOI: 10.1021/jf405832u

7.4.2 Book Chapter

1. Jochmann, M. A.; **Laaks, J**.; Schmidt, T. C.
   Chapter 12: Solvent Free Injection Techniques in
   Dettmer-Wilde, K.; Engewald, W. (Eds.) “Practical Gas Chromatography: A Comprehensive Reference” 2014, Springer, Berlin

7.4.3 Other Publications

1. **Laaks, J**.; Jochmann, M. A.; Schmidt, T. C.
   Empfindlich, automatisch und ohne Lösungsmittel
   Nachrichten aus der Chemie 2013, 61 (1): 54-56

7.4.4 Oral Presentations

1. **J. Laaks**, M. A. Jochmann:
   MSChromSearch – Anwendungsbeispiele aus der Weinanalytik
   Axel Semrau GC/ GCMS Seminare 2008 (invited)
   21.10.08 Sprockhövel, 04.11.08 Berlin, 06.11.08 Jena

2. **J. Laaks**, M. A. Jochmann, T. C. Schmidt:
   In-tube Extraction of volatile organic hydrocarbons from aqueous samples with purge and trap sensitivity, Seminar "20. Doktorandenseminars des AK Separation Science in Hohenroda 2010", 11.01.2010 Hohenroda

3. **J. Laaks**, M. A. Jochmann, T. C. Schmidt:
   In-tube extraction of volatile organic compounds from aqueous samples: an economical alternative to purge and trap enrichment
   Conference „28th International Symposium on Chromatography", 14.09.2010 Valencia

4. **J. Laaks**, M. A. Jochmann, T. C. Schmidt
   Applikation der In-tube Extraction bei der Analyse von Aromastoffen in Lebensmitteln
   Sigma-Aldrich Seminar "Gaschromatographie: Aktuelle Entwicklungen, Tipps und Tricks" 2012 (invited)
   06.03.2012 Mannheim, 08.03.2012 Dortmund

5. **J. Laaks**, M. A. Jochmann, T. C. Schmidt
   ITEX II als Anreicherungsmethode in der Lebensmittelanalytik - Aromaanalytik von Bier
   Axel Semrau Seminar „PAL Anwendertreffen" (invited)
   25.06.2013 Rheingau

7.4.5 Posters

1. **J. Laaks**, M. A. Jochmann, T. Letzel, T.C. Schmidt:
   Headspace analysis of volatile wine aroma compounds and fingerprinting of German red wines, "27th International Symposium on Chromatography", 21.–25.09.2008 Münster
2. **J. Laaks**, M. A. Jochmann, B. Schilling, T.C. Schmidt:
   In-tube Extraction of volatile organic hydrocarbons from aqueous samples with purge and trap sensitivity, "Analytica Conference", 23.–26.03.2010 München
3. **J. Laaks**, M. A. Jochmann, B. Schilling, T.C. Schmidt:
   In-tube extraction (ITEX) in the analysis of volatile aroma compounds from food matrices, "ANAKON 2011", 22.–25.03.2011 Zürich
4. **J. Laaks**, J. Brüning, M.A. Jochmann, T.C. Schmidt:
   Multiple headspace in-tube Extraction of aroma compounds from ground coffee, "Analytica Conference", 17.–20.04.2012 München
5. **J. Laaks**, J. Brüning, M. A. Jochmann, T.C. Schmidt:
   Multiple headspace in-tube Extraction of aroma compounds from ground coffee (v2), "ExTech 2012", 24.–26.09.2012 Messina

7.5 Curriculum Vitae

The CV was removed for online publishing for the protection of personal data

7.6 Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel

„Development and Validation of Novel Solventless Microextraction Techniques in Gas Chromatography"

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Essen, im Dezember 2013

Jens Laaks

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