Development of GC-MS methods for the identification and quantification of leachables from plastic packaging in dialysis solutions

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Summary

The presence of low molecular weight substances in plastic is unavoidable. Monomers, oligomers, catalysts and solvents among others originate from its productions process. In addition, plasticizers, colorants, antioxidants, antistatic agent and others are typically added to achieve or improve certain properties of the plastic. The increasing use of plastics as packaging materials for solid and in particular liquid pharmaceuticals brought the focus onto the migration of those low molecular weight substances out of the packaging into packed good. This is of especial concern for solutions given the close contact to the packaging and the possibility that substances easily diffuse through the whole solution and contaminate it, once they are leached out of the plastic. In the case that the pharmaceutical solution is meant for an infusion these so called 'leachables' get directly transferred into the blood of a patient and could pose a safety risk to patient health. Especially when the duration of a treatment lasts years or even a lifetime long very low concentrations down to ppb (µg/kg) or even ppt (ng/kg) should be taken as an upper threshold, to ensure patient safety. European authorities are aware of this risk and therefore demand detailed information about the identity, the amounts and the toxicological properties of all leachables in a pharmaceutical.

For patients with a kidney failure dialysis with typically three treatments a week a lifetime long is the only kidney replacement therapy available beside a kidney transplantation. Depending on the treatment modality the dialysis solutions employed are packed in plastic bags. Changes in raw materials used for this plastic packaging or in particular newly developed materials have to be characterized first by an extraction study under exaggerated conditions, to force the migration of all possible leachables into a test solution. The high concentrations in the ppm (mg/kg) range achieved by this approach simplify the identification of unknown components.

This identification of such substances detected in an extraction study for a newly developed multi-layered plastic packaging material, was successfully carried out within the framework of this thesis. Eleven cyclic oligomers and one diurethan were unambiguously identified, due to the complementary use of several hyphenated chromatographic methods combined with a mass spectrometric detection. The source of all extracted components was the polyurethane adhesive that was used for the lamination of the plastic films during the production of the multi-layer packaging.

The second step, which follows the extractable study, is a leachable study, where concentrations of leachables under conditions of use are determined. Therefore, trace analysis

methods with a high sensitivity as reasoned above must be available. Hence, two methods providing this sensitivity - the stir bar sorptive extraction (SBSE) and the dynamic headspace (DHS) – were optimized and validated within this thesis for the determination of leachables in dialysis solutions. Both employ a gas chromatographic separation with a mass spectrometric detection and both methods are not limited to the analytes used in the respective standard solutions, but instead both were developed as screening methods, to be able to detect unknown leachables as well.

The optimized SBSE method utilized a sample volume of 25 mL that was extracted after the addition of 8.5 g NaCl for 4 hours at 1100 rpm with a 20 mm x 1 mm stir bar. Detection limits of 0.01 µg/kg and below were found for most of the 45 substances with repeatabilities below 23%. The method was successfully applied to five different, currently available dialysis solutions. These findings showed similarities in chemical structures and concentrations for some leachables between all suppliers, but also distinct differences. Concentrations for most of the detected leachables were in the range of 0.01 µg/kg to 10 µg/kg.

The DHS method utilized 10 mL sample volume that was extracted with a purge flow of 60 mL/min for about 13 min. The limits of quantification (LOQ) were 0.5 µg/kg or less for most of the 23 analytes with repeatabilities below 10% in the water matrix. The method was validated with similar results in four additional matrixes, where two of them represent typical dialysis solutions. Therefore the method has proven to be suitable for the determination in dialysis solutions. Its key advantage beside the low LOQ is the fully automated sample preparation, which qualifies it for quality control laboratories with a high sample throughput per day.

Depending on the analytical problem, two methods, one with a limit of quantification in the sub-µg/kg-range at a minimal workload (DHS) and the other with highest sensitivity down to the low ng/kg range at a slightly higher workload, are now available for the determination of leachables in dialysis solutions. With these tools more detailed information about leachables can be gathered in the future, which are important for profound toxicological assessments to ensure patient safety. Furthermore, these methods will probably be helpful in the development of plastic packaging materials, comprising significantly reduced leachable levels.

Zusammenfassung

Niedermolekulare Bestandteile sind in Kunststoffen unvermeidbar. Unter anderem verbleiben Monomere, Oligomere, Katalysatoren und Lösungsmittel aus dem Produktionsprozess im Kunststoff. Des Weiteren werden zusätzlich bewusst zum Beispiel Weichmacher, Antioxidantien, Farbstoffe und andere Hilfsstoffe zugesetzt, um bestimmte Eigenschaften des Kunststoffs zu erreichen oder zu verbessern.

Der zunehmende Einsatz von Kunststoffen als Verpackungsmaterial für feste und insbesondere flüssige Arzneimittel brachte die Problematik der Migration dieser niedermolekularen Verbindungen in das Arzneimittel hinein verstärkt in den Vordergrund. Dieser Prozess ist bei flüssigen Arzneimitteln aufgrund des direkten Kontakts zur Verpackung und der üblicherweise hohen Diffusivität im Wässrigen besonders problematisch. Kommt diese pharmazeutische Lösung als Infusion zum Einsatz, so gelangen die niedermolekulare Verbindungen, sogenannte „Leachables“, direkt ins Blut des Patienten und stellen somit ein potentielles Gesundheitsrisiko dar. Insbesondere wenn die Behandlung sich über mehrere Jahre oder gar Jahrzehnte erstreckt, sollten sehr geringe Leachable-Konzentrationen im ppb (µg/kg) oder sogar ppt (ng/kg) Bereich als oberer Grenzwert angenommen werden, um die Patientensicherheit zu garantieren. Die Europäische Arzneimittelbehörde verlangt auf Grund dieses Risikos seit 2005 detaillierte Informationen über die Identität, Quantität und toxikologische Eigenschaften aller Leachables, die in einer pharmazeutischen Lösung detektiert werden.

Für Patienten mit Nierenversagen ist die Dialyse mit üblicherweise drei Behandlungen pro Woche ein Leben lang die einzig vorhandene Nierenersatztherapie neben der Transplantation. Abhängig von der jeweiligen Behandlungsart kommen hier bei in Kunststoffbeuteln abgefüllte Dialyselösungen zum Einsatz. Bei Änderungen von Rohmaterialien die zur Produktion dieser Verpackungen eingesetzt werden und insbesondere bei der Neuentwicklung von Verpackungsmaterialien müssen im ersten Schritt Extraktionsstudien unter verschärften Bedingungen durchgeführt werden. Durch die Wahl extremerer Bedingungen als üblich soll eine verstärkte Migration aus der Verpackung in die Testlösung provoziert werden. Die dabei erreichten hohen Konzentrationen im ppm (mg/kg) Bereich vereinfachen die Identifizierung unbekannter Leachables.

Im Rahmen dieser Arbeit wurden 12 unbekannte Substanzen, welche bei einer Extraktionsstudie an einer neuentwickelten mehrschichtigen Folie erfasst wurden, erfolgreich identifiziert. Elf cyclische Oligomere und ein Diurethan wurden durch den kombinierten Einsatz verschiedener flüssig- (LC) und gaschromatographischer (GC) Methoden mit mas-

senspktrometrischer Detektion (MS) eindeutig identifiziert. Die Quelle aller extrahierten Verbindungen war der Polyurethankleber, der zum Verkleben der einzelnen Folienschichten verwendet wurde.

Der zweite Schritt, welcher der Extraktionsstudie folgt, ist eine Leachable-Studie, bei der die Konzentrationen der Leachables im realen Produkt unter normalen Anwendungsbedingungen bestimmt werden. Dafür müssen spurenanalytische Verfahren mit einer hohen Empfindlichkeit vorhanden sein. Daher wurden im Rahmen dieser Arbeit zwei GC-MS Methoden – die „ Stir Bar Sorptive Extraction“ (SBSE) und die „Dynamische HeadSpace“ (DHS) – für die Bestimmung von Leachables in Dialyselösungen im sub-ppb Bereich optimiert und validiert. Beide hier vorgestellten Analysenmethoden sind nicht auf die Analyten begrenzt, die in den jeweiligen Standardlösungen zum Einsatz kamen, stattdessen wurden sie als Screening-Methoden entwickelt, um auch unbekannte Leachables bestimmen zu können.

Bei der SBSE-Methode wurde ein Probenvolumen von 25 mL mit 8,5 g NaCl versetzt und für 4 h bei 1100 rpm mit einem 20 mm x 1 mm Magnetrührstäbchen extrahiert. Die Nachweisgrenze lag für die meisten der 45 gleichzeitig quantifizierten Analyte bei 0,01 µg/kg und darunter mit Wiederholpräzision kleiner 23%. Die Methode wurde erfolgreich zur Quantifizierung von Leachables in fünf verschiedenen, aktuell erhältlichen Dialyselösungen eingesetzt. Hierbei wurden sowohl Gemeinsamkeiten hinsichtlich der Art und Menge der Leachables zwischen verschiedenen Hersteller festgestellt; es gab aber auch deutliche, womöglich herstellerspezifische Unterschiede. Die Konzentration der meisten Leachables in diesen Realproben lag zwischen 0,01 µg/kg und 10 µg/kg.

Bei der DHS-Methode wurden 10 mL Probenvolumen mit einem Gasfluss von 60 mL/min für etwa 13 min extrahiert. Die Bestimmungsgrenze lag für die meiste der 23 parallel bestimmten Analyte bei 0,5 µg/kg und darunter, mit einer Wiederholpräzision unter 10% in Wasser. Die Methode wurde mit ähnlichen Ergebnissen für vier weitere Matrices validiert, wobei zwei davon typische Dialyselösungen darstellen. Die DHS ist somit sehr gut für die Leachable-Quantifizierung in Dialyselösungen geeignet. Der Hauptvorteil neben der geringen Bestimmungsgrenze liegt in der vollautomatischen Probenvorbereitung, wodurch die Methode für Labore zur Qualitätssicherung mit hohem täglichem Probenaufkommen ideal geeignet ist.

Mit diesen beiden Methoden stehen nun Analysenverfahren zur Verfügung, die je nach Fragestellung mit minimalem Arbeitsaufwand und Bestimmungsgrenzen im sub-µg/kg-Bereich (DHS) oder höchsten Empfindlichkeiten im einstelligen ng/kg Bereich bei geringfügig höherem Arbeitsaufwand (SBSE) für Leachable-Studien an Dialyselösungen zum Einsatz

kommen können. Mit diesen Verfahren können zukünftig noch detailliertere Informationen über Leachables gewonnen werden, welche für die Erstellung umfassender toxikologischer Bewertungen im Interesse der Patientensicherheit von grundlegender Wichtigkeit sind. Des Weiteren können die Methoden womöglich unterstützend bei der Entwicklung neuer, Leachable-armer Verpackungsmaterialien eingesetzt werden.

Contents

1 General Introduction

1.1 Preface

The number of patients with a kidney failure is continuously growing all over the world and reached in the European Union an overall prevalence of more than 1070 patients per million population at the end of 2012 [1]. The preferred treatment for this so called end-stage renal disease (ESRD) is a transplantation of the kidney [2], but donors are rare. Thus dialysis renal replacement therapies such as hemodialysis (HD) or peritoneal dialysis (PD) are the only life-extending treatments left. Both aim at the removal of toxic substances and excess water from the blood. The main difference between the two treatment modalities is that in HD an extracorporeal cleaning of the blood is performed, whereas PD is an intracorporeal process, which involves the peritoneum to clean the blood.

The first successful hemodialysis was already performed in 1946 by Kolff. In the meantime HD has significantly improved in regard to the knowledge about the process and its key parameters and of course in regard to the technical equipment used. Nowadays chronic HD treatment is typically performed three to four times per week. For acute renal failure a continuous HD treatment for a few days up to a few weeks is performed till the kidney's proper function recovered. During a HD treatment the blood from the patient is pumped by a dialysis machine through an external filter, the so called dialyzer. This dialyzer removes excess water but especially metabolic waste products, by passing the blood along one side of a semipermeable ultrafiltration membrane with pore sizes in the range of a few nanometers up to a few ten nanometers. Toxic waste products are filtered out by the membrane and flushed out of the dialyzer with the dialysis solution, which flows on the other side of the membrane in a counter current direction.

In contrast to HD the peritoneal dialysis is less demanding from a technical point of view as this treatment modality does not need a dialysis machine or a dialyzer. Here a dialysis solution is filled into the abdominal cavity of the patient and stays there for about four hours. The blood-rich peritoneum is surrounded by this solution and serves as a filter. Toxic products diffuse through it into the dialysis solution and get removed in addition to the excess water, when the solution is replaced with a fresh one. This regular exchange is either carried out by the patient throughout the day, which is the so called continuous ambulatory peritoneal dialysis (CAPD), or it is automatically performed every night while the patient sleeps. In this case it is called automatic peritoneal dialysis (APD).

Both renal replacement therapies, HD as well as PD, have the use of dialysis solutions in common, which are typically delivered in plastic bags. The use of solution bags for chronic HD treatments becomes more and more obsolete, due to the increasing use of online pre-

pared dialysis solutions. 'Online' means in this context, that the dialysis center produces high purity water by its own water treatment system and this water is finally used by the dialysis machine to prepare the dialysis solution online, during the HD treatment. But solutions in bags are currently and most probably in the coming years the standard for acute HD and of course for PD.

The use of plastic packaging materials for pharmaceutical solutions has several advantages such as a low weight, an inexpensive production process and an easy handling. Furthermore, the properties of plastic packaging materials, for example stability, elasticity, antioxidant effects or UV resistance, can be varied within a wide range. This is usually accomplished by adding low molecular weight substances, such as plasticizers, radical scavengers or UV absorbing components. Another source of low molecular weight substances in the plastic is the production process of the polymers used in the packaging material, where probably monomers, catalysts or organic solvents remain in the polymer. In the case that specific properties cannot be achieved with one particular plastic an even wider range of properties arises from a multi-layer plastic packaging material in which layers of different plastics are combined, with every layer having its own typical low molecular weight contaminants.

All in all there is a variety of substances in the plastic packaging, which may leach out and contaminate the pharmaceutical [3–6]. These components are typically called leachables and are of particular concern for pharmaceutical solutions compared to solid pharmaceuticals. A liquid is in close contact to the packaging material from the moment of filling and packaging till its use. During this timeframe leachables can diffuse into the solution and easily distribute in the whole volume. As soon as the solution is infused into a patient during a dialysis treatment the leachables are transferred directly into the blood and may pose a safety risk. Consequently the European Medicines Agency (EMEA) demands in their Guideline on Plastic Immediate Packaging Materials (CPMP/QWP/4359/03) from 2005 a toxicological assessment of all migrating substances regardless of their amount. This assessment includes the exact identification and quantification of leachables as the first step. In response to this extensive demand of assessing all leachables, the product quality research institute (PQRI, Arlington, Virginia, USA) proposed a safety concern threshold (SCT) of 150 ng/d for orally inhaled and nasal drug products [7]. Below this threshold even genotoxic substances – a class of substances considered to be most harmful, as they can be carcinogenic or mutagenic or can cause birth defects - are supposed to have no observable effect. The SCT concept limits the extensive requirement of the EMEA to a toxicological qualification just for leachables above this threshold and was later on adopted by the PQRI for parenteral products, which includes dialysis solutions [8].

The EMEA guideline recommends extractable studies followed by leachable studies to test plastic packaging materials within regard to migrating substances. The difference between these two types of studies is that the first one is performed under exaggerated conditions, for example at longer times and/or higher temperatures than in a typical application, to present a worst case scenario. This provoked high leaching is a criterion of extraction studies, because these tests aim at the identification of substances that may leach out of the packaging into the product. In contrast, a leachable study is performed under normal use conditions to determine the leachables and their concentration in the final product. These data typically represent a subset of the data gathered from the extractable study and are used to assess patient risk.

Due to the relatively high concentrations determined in extractable studies trace analysis methods are not essential, whereas for leachable studies highly sensitive analytical methods are necessary, especially for dialysis solutions. Here, several liters are applied per day often a lifetime long and thus even very small concentrations of a particular substance may lead to a regular high daily intake of leachables. Thus, highly sensitive analytical methods are needed; first of all as a tool for a high-level quality assurance with a focus on the patient safety but also due to the need to comply with currently applicable guidelines. In addition, trace analysis methods are valuable for the development of new raw materials or the further development of existing plastic packaging materials.

The SCT for PD or HD solutions is in the low ppt (ng/kg) range as exemplarily shown in the following, thus analytical methods need to have a limit of detection (LOD) in the same range. In the daily PD treatment typically two liters of dialysis solution are introduced into the abdomen of the patient and remain there for four hours, before they are replaced with fresh solution [9,10]. The assumption of five replacements a day results in a total infused volume of 10 L which can double, when using automated solution changers [11]. Thus the SCT of 150 ng/day corresponds to a required LOD for the leachables of 15 ng/L (10 L dialysis solution per day, CAPD) or even 7.5 ng/L (20 L per day, APD). For hemodialysis solutions, or hemofiltration solutions to be more precisely, a LOD of 35 ng/L is required. Hemofiltration is a hemodialysis treatment modality where in comparison to the standard hemodialysis higher filtrate flow rates are utilized for the cleaning of the blood. This LOD value was calculated by assuming three hemofiltration treatments per week with a four hour duration and a final exchanged volume of 10 L per treatment [12].

Trace analysis methods usually employ a preconcentration of the analytes under study in the first step. In general two approaches can be distinguished for the extraction and enrichment of the analytes from aqueous samples: liquid-liquid-extraction (LLE) and

solid-phase-extraction (SPE). For both of them exist analysis methods, which can be performed either directly in immersion or in the headspace above the sample. The decision to perform the measurement in or above the sample mainly depends on the air-water-partition coefficient K_{aw} of the analytes. Determination of highly-volatile and semi-volatile analytes is feasible in the headspace. In most cases the preconcentration is followed by a chromatographic separation via gas chromatography (GC) or, especially for low-volatile analytes, liquid chromatography (LC). The common LLE employs several mL of an organic solvent to extract an aqueous sample by mixing both phases vigorously [13–15]. In the further development of LLE the liquid-phase microextraction (LPME) evolved, which overcomes the drawback of using large volumes of potentially toxic solvents. LPME utilizes the extraction solvent either in form of a single droplet (single drop microextraction, SDME [16]) or within the pores of a membrane (membrane liquid phase microextraction, MLPME [17]) or dispersed in tiny droplets (dispersed liquid-liquid-microextraction, DLLME [18]). All of them comprises limit of detection (LOD) values in the low ng/L range and are thus well suitable for trace analysis. An alternative to these LLE-methods is SPE, with well-established solvent free variants such as solid-phase microextraction (SPME) [19] and stir bar sorptive extraction (SBSE) [20]. SBSE and most commonly SPME employ the polymer polydimethylsiloxane (PDMS) as the extraction phase. This polymer has a glass transition temperature of -120°C and therefore behaves as a gum-like, stationary phase, which is despite its non-liquid character able to dissolve the analytes. Hence the extraction still bases on the partitioning of the analytes into a non-miscible liquid phase, which is similar to LLE. The SPME method employs silica fibers coated with PDMS, which are placed in a needle of a syringe-like arrangement. In contrast, the SBSE method uses PDMS coated stir bars and thereby increases the PDMS volume by a factor of 100, which significantly raises the enrichment factor. In both methods the analytes are finally desorbed from the PDMS by a thermal desorption step and are injected into the GC column.

The sorption of analytes into the PDMS is a thermodynamically driven process to attain an equilibrium state with analyte concentrations in water C_w and PDMS C_{PDMS} corresponding to the analyte's polarity. The extent of sorption is quantified by a substance specific factor – the partition coefficient $K_{PDMS/W}$. Thus equilibrium is defined by:

$$C_{PDMS} = K_{PDMS/W} \cdot C_W \tag{1-1}$$

This equilibrium is not reached instantaneously. Instead the process is kinetically hindered mainly due to the presence of an aqueous boundary layer close to the PDMS surface [21–23] and just to a minor degree due to the lower diffusion coefficients of the analytes in

PDMS compared to water. Published diffusion coefficients are just one order of magnitude lower than the corresponding values in water [24,25].
Equation (1-1) can be used to estimate the yield of extraction under equilibrium conditions. With the mass balance for the initial analyte mass in the sample (m_{W0}) and the amounts in PDMS (m_{PDMS}) and in water (m_W)

$$m_{w0} = m_{PDMS} + m_w \tag{1-2}$$

follows

$$K_{PDMS/W} = \frac{c_{PDMS}}{c_W} = \frac{m_{PDMS}}{m_w} \cdot \frac{V_W}{V_{PDMS}} = \frac{m_{PDMS}}{m_w} \cdot \beta \tag{1-3}$$

where V_W and V_{PDMS} are the volumes of the sample and PDMS, respectively. For reasons of simplification their ratio is defined as β. The theoretical recovery R, which illustrates the extraction efficiency or extraction yield, can be defined as the ratio of the analyte mass in the PDMS and the initial mass of the analyte:

$$R = \frac{m_{PDMS}}{m_{w0}} = \frac{K_{PDMS/W}}{K_{PDMS/W}+\beta} \tag{1-4}$$

This equation shows the relationship between the partition coefficient and the phase ratio. The smaller β is, the higher becomes R. The PDMS volume is usually given by the used equipment and is about 0.5 µL in SPME experiments and up to 120 µL in SBSE methods. For a sample volume of 20 mL this leads to β values of 40,000 and 170, respectively. Calculated recoveries as a function of $K_{PDMS/W}$ are shown in figure 1-1.

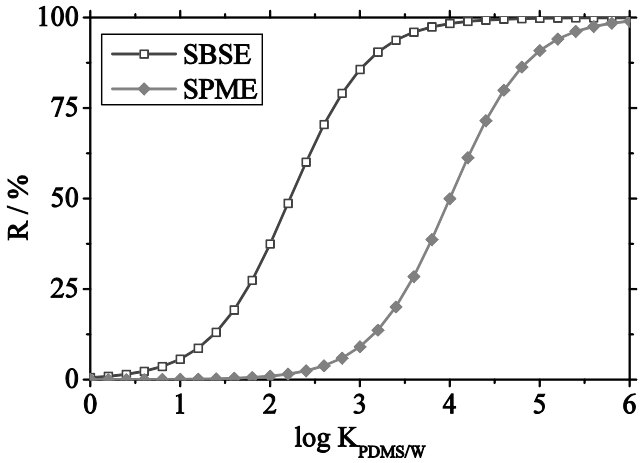

Figure 1-1 Recovery *R* as a function of $K_{PDMS/W}$ for SBSE and SPME, employing PDMS volumes of about 120 µL and 0.5 µL, respectively

It is obvious, that the higher PDMS volumes employed in SBSE dramatically improve the extraction yield for analytes with a low $K_{PDMS/W}$. In addition, small sample volumes, which lead to small values of β, seem to be advantageous. However, this conclusion is misleading. The percentaged recovery relative to the initial amount becomes higher for smaller sample volumes, but the absolute amount of analyte in the PDMS decreases. Thus a reduction of the sample volume from 20 mL to 5 mL roughly doubles the recovery from 37% to 70% for a PDMS volume of 120 µL, but the absolute amount sorbed in the PDMS divides in half by doing so.

When Baltussen at al. established SBSE they assumed a proportionality between $K_{PDMS/W}$ and the already established octanol-water partition coefficient $K_{O/W}$ [20]. Meanwhile an approximately linear relationship between these two K-values was experimentally and theoretically determined [26].

As mentioned before the analyte extraction can either be done by immersion of the extraction phase into the aqueous sample or by placing it in the headspace above the sample. Headspace sampling seems to be a promising alternative for leachables in pharmaceutical solutions as most of the typical leachables are either high-volatile or at least semi-volatile. Its advantage is the cleaner and simpler analyte extraction combined with a high degree of automation. Headspace sampling can be performed in a static headspace (SHS) setup, where the sorbent remains in the headspace till equilibrium is reached or in a dynamic setup (dynamic headspace, DHS), where the headspace is continuously purged and the purge flow is passed along the sorbent. The purging is done either by bubbling gas

though the sample (the so called 'purge and trap' approach) or by sweeping it over the aqueous sample [27]. The advantages of DHS are on the one hand its higher sensitivity due to exchanging the headspace typically several ten times during the enrichment step and on the other hand that it does not base on equilibrium conditions, which removes the need to wait till its establishment. Several sorbents are available for the DHS such as PDMS, polyacrylate, Tenax TA (a polymer basing on 2,6-diphenylene-oxide), Carbopack (a graphitized carbon black) and Amberlite (a quaternary ammonium resin). The apolar PDMS and the polar polyacrylate were utilized by Perestrelo et al. [28] in a DHS-SPME experiment. Despite these absorbers, which dissolve the analyte, Tenax, Carbopack and Amberlite adsorb the analytes on the surface and are typically applied in DHS for the extraction and enrichment of the analytes.

Similar to equation (1-1) the mass balance for DHS is given by the total amount of the analyte m_{w0}, which is the sum of the amount of analyte in the sample (m_w) and the amount on the sorbent (m_s), assuming the complete adsorption of the analytes in the purge gas :

$$m_{w0} = m_S + m_w \tag{1-5}$$

Due to the constant gas flow during the DHS extraction no equilibrium will be reached between the sample and the whole headspace above it, but it is reasonable to assume the establishment of an equilibrium between the liquid and a gas layer with an arbitrary thickness in the direct proximity of the sample. This equilibrium is comparable to the thermodynamic equilibrium under SHS conditions, where the resulting concentrations in the sample C_w and headspace C_a are given by the Henry's law constant K_{aw}.

$$K_{aw} = \frac{C_a}{C_w} \tag{1-6}$$

Due to the continuous extraction for a certain time t at a flow F, the amount of analyte m_w will decrease exponentially starting at m_{w0} for $t = 0$.

$$m_w = m_{w0} \cdot exp\left(-\frac{F \cdot t}{b}\right) \tag{1-7}$$

The variable b is defined as [29,30]

$$b = \frac{V_w}{K_{aw}} + V_a \tag{1-8}$$

where V_w is the sample volume and V_a is the headspace volume above the sample. Summarizing equations (1-5) to (1-8) gives the theoretical recovery for the DHS:

$$R = 1 - exp\left(-\frac{F \cdot t}{\frac{V_w}{K_{aw}} + V_a}\right) \quad (1\text{-}9)$$

Compared to the recovery for SBSE in equation (1-4) once more the volume ratio of the sample volume V_w and extraction phase volume (flow *F* times extraction time *t*) is one of the key parameters.

1.2 Scope of this study

This study focusses on the identification and quantification of leachables in dialysis solutions, emerging from its packaging.

The first aim of this work, described in chapter 2, was the identification of 12 unknown components, which were observed in an extractable study for the qualification of a new multilayer gas barrier film. A sterilisation-resistant polyurethane (PUR) laminating adhesive was used for the lamination of a supporting film (for stability) and a silicon-oxide-coated film (as the gas barrier) in the production process of this multilayer film. The film was intended to be used as a primary packaging material for dialysis solutions. The study to identify the 12 unknown substances was conducted as an extractable study under stringent conditions and therefore high analyte concentrations in the 0.1 to 1 ppm (mg/kg) range were achieved. As mentioned above, this extractable study is performed as the first step for the characterization of plastic packaging materials for pharmaceutical solutions. The subsequent leachable study, performed under conditions of use, will lead to much lower concentrations of leachables. These are most probably not quantifiable with the LLE method employed in the extraction study. Therefore, the second aim of this work was the development and validation of trace analysis methods for the simultaneous quantification of a multi-component mixture of analytes representing potential leachables in dialysis solutions. From the trace analysis methods described before two were chosen: SBSE used in immersion mode as described in chapters 3 and 4 and DHS in combination with the Tenax sorbent for the headspace analysis as shown in chapter 5. SBSE is a method that combines high sensitivity with a simple, partly automated handling and due to the high PDMS volumes up to 120 µL it is superior to SPME. A simple search for 'stir bar sorptive extraction' as a keyword in www.webofknowledge.com (Thomson Reuters, formerly ISI) shows a linear increase in annual publications from 1 publication in 1999 to about 60 publications in 2007. This level remained approximately the same for the last five years. The main field of application is the water and environmental analysis [31–36], followed by food and biomedical [37, 38] analysis. Food and beverage are mainly analyzed in regard to aroma and taste [39–42] compounds or degradation [43] products and just a few publications were about leachables emerging from the packaging [44–46]. But there are virtually no SBSE studies regarding leachables from packaging materials into pharmaceutical solutions, although this topic is recognized as an important one [33,47–49].

As a complementary method to SBSE, DHS was chosen by virtue of its higher sensitivity compared to SHS or SPME-SHS and due to its high degree of possible automation. The latter is a key aspect for quality assurance laboratories with a high daily sample throughput. Tenax was used as the sorbent because its suitability for a wide range of analytes having different polarities from the polar to the non-polar region [50, 51]. PDMS was consciously not taken again, because it was already employed in the SBSE method. A literature research reveals that a similar situation exists as described above for SBSE. There are some studies, which employ DHS to determine volatile components in plastic packaging materials for pharmaceutical products [52,53], but there are no DHS studies that aimed at the determination of leachables directly in pharmaceutical solutions and especially not in dialysis solutions.

For both methods, SBSE as well as DHS, the same GC-MS analysis device was employed and for the development of both methods a multi-component mixture was used. This mixture of analytes comprises phenols, alcohols, carboxylic acids, carboxylic acid amides, phthalates and hydrocarbons among others, whereas both methods are not limited to a determination of these leachables.

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2

Migrating components in a polyurethane laminating adhesive identified using GC-MS

Redrafted from B. Athenstädt, M. Fünfrocken, T. C. Schmidt: Migrating components in a polyurethane laminating adhesive identified using gas chromatography/mass spectrometry, Rapid Commun Mass Spectrom. (2012), 26, 1810-1816

2.1 Introduction

Plastics have become an integral part of the daily life, with more than a third of packaging materials being made of plastic [1-3]. Plastics are light, easy to mould, and amenable to modifications that confer properties such as stability, elasticity or permeability to gases matched to requisite limits within a wide range of possibilities. Packaging made of plastics frequently protects food [4, 5] or pharmaceutical and medical products [6] from light, humidity or oxygen, thus improving the shelf life and stability of these products during storage.

Given that the required properties of packaging (e.g., gas barrier effect, stability and transparency) can frequently not be achieved using a single-layer film of plastic, multilayer films have become increasingly important. Laminating adhesives, including PUR variants, can permanently bond adjacent plastic layers. The polymerization involved in the formation of a PUR usually involves the reaction of a diol and a diisocyanate. These two components are either combined immediately before the bonding process, or in separate steps. Excess diol reacts with the diisocyanate in a first step, with the resulting prepolymer then reacting with additional diisocyanate for bonding.

The potential of packaging components to interact with the enclosed food [7-9] or pharmaceuticals [10] is always a key consideration in selecting primary packaging materials for these commodities. The possibility that low molecular weight substances, such as monomers, oligomers, plasticizers or catalyst components left after polymerization might migrate from the packaging into the product [11-15] is of particular relevance, as they might pose a health threat to consumers. This consideration is more problematic for liquid goods, given the closer proximity of the packaging to the product and the likelihood that soluble substances derived from the packaging are likely to rapidly diffuse into the solution. Since the end of 2005, European health authorities have consequently required the exact identification and quantification of migrating substances to comply with the Guideline on Plastic Immediate Packaging Materials (CPMP/QWP/4359/03). The ability to identify and quantify these substances is essential for assessment of their toxicities and to regulate the levels of potentially harmful substances. Given that the extraction studies most commonly used for this purpose involve promoting the migration of leachable substances under conditions of increasing stringency, they usually present a worst-case scenario.

The identification of the source of migrating substances becomes more complicated for packaging materials with a multilayer structure, as it is sometimes unclear whether the migrating substances are derived from the films or from the laminating adhesive. Reactions of substances derived from either of these sources might generate migrating products not present in any of the multilayer components.

In this study, three hyphenated mass spectrometric methods were employed - GC-MS with electron impact ionization (EI), GC-MS with chemical ionization (CI), and high resolution LC-MS with electrospray ionization (ESI) - to identify migrating substances in a sterilisation-resistant PUR laminating adhesive. The PUR adhesive studied was proposed to be used for the production of a multilayer gas barrier film comprising a supporting film (for stability) and a silicon-oxide-coated film (as the gas barrier). A schematic cross-cut of the multilayer film is shown in figure 2-1. The film was intended to be used as a primary packaging for aqueous pharmaceutical preparations. The aim of this work was to develop an approach to identify components capable of migration from the PUR adhesive. Despite the range of compositions of PUR laminates, the approach presented here may be applicable for the qualitative assessment of all PURs. Quantification and toxicological evaluation of the identified components was beyond the aim of this chapter.

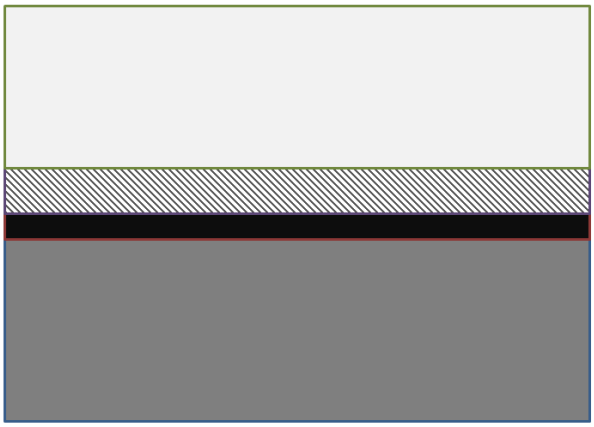

Figure 2-1 Sketch of a cross-cut of the investigated multilayer packaging material. Layers from top to bottom: supporting layer in light grey, PUR adhesive shaded, the SiOx layer in black and the carrier film for the SiOx in dark grey.

2.2 Experimental

Samples

Test bags were made from the laminated film, filled with 100 mL of phosphate buffer solution (pH 6), sealed, and autoclaved for 1 hour at 121°C. The test bags were approximately 17 × 17 cm, generating a surface area of the film in contact with the buffer solution of approximately 600 cm². The ratio of surface area to volume was thus 6 cm² per 1 mL.

For the GC measurements, 100 g of the autoclaved aqueous solution (the so-called autoclavate) were extracted with 4 g of chloroform; the chloroform phase was removed and injected.

For the LC measurements, the autoclaved aqueous solution was injected directly.

Chemicals

Chloroform for gas chromatography (stabilized with ethanol), isophorone diisocyanate (synthesis grade), methanol p.a., and ethyl acetate p.a. were purchased from Merck. The water used for all experiments was purified using a Milli-Q Gradient system from Millipore.

Gas chromatography-mass spectrometry with electron impact ionization

Most of the GC-MS measurements were performed using a GC 6890 system from Agilent equipped with a split/splitless injector and a mass spectrometer (type 5973) with an electron impact (EI) source. Mass-to-charge ratios (m/z) were recorded at values between 50 and 500. The column used was a Zebron (ZB-5) capillary column (length 30 m, diameter 0.25 mm, film thickness 0.25 µm, stationary phase: 5% diphenyl polysiloxane, 95% dimethyl polysiloxane). Helium 5.0 was used as the carrier gas at a constant flow of 0.9 mL/min. Splitless sample injection (injection volume 3 µL) was carried out at a temperature of 250°C with a transfer line temperature of 280°C. The temperature programme used for chromatographic separation involved increasing the temperature from 50°C to 150°C at a rate of 20°C/min, and then maintaining it at this level for 1.5 min before increasing the temperature further at the same rate to 300°C, and then maintaining the temperature at this level for 17 min. The ion source was held at a temperature of 270°C.

Gas chromatography-mass spectrometry with chemical ionization

The GC-MS (CI) measurements were made using a 6890 GC system (Agilent) in combination with a chemical ionisation (CI) source, a mass spectrometer of type 5975, methane as the ionisation gas, and an Agilent HP-5ms column (length 30 m, diameter 0.25 mm, film

thickness 0.25 µm, 5% phenyl methyl siloxane). The carrier gas, its flow, the sample injection volume, the temperatures and the temperature programme for the chromatographic separation were identical to those used for the GC-MS (EI) measurements. These measurements were carried out at CBA GmbH, Kirkel.

Liquid chromatography-mass spectrometry with electrospray ionization

The LC-MS measurements were carried out by Dr. Steinbach at the Department for Mass Spectrometry of Philipps University (Marburg, Germany) using a type-1100 HPLC system (Agilent) in combination with an electrospray ionisation source (ESI) and an LTQ/FT high resolution mass spectrometry system (Thermo), which combines an ion trap and a fourier transform ion cyclotron resonance mass spectrometer. The chromatographic separation took place on a Nucleosil 120-3 C-18 column (Macherey Nagel, length 250 mm, diameter 3 mm, particle size 3 µm). The eluent was changed from pure water to a 50:50 mixture of water and acetonitrile after 30 min and finally to pure acetonitrile after 60 min. The flow was 0.3 mL/min.

2.3 Results and discussion

Initial GC-MS screening

A representative chromatogram obtained by analysis of autoclaved material by GC-MS (EI) is shown in figure 2-2. Thirteen prominent signals in this chromatogram were reproducibly observed in all experiments.

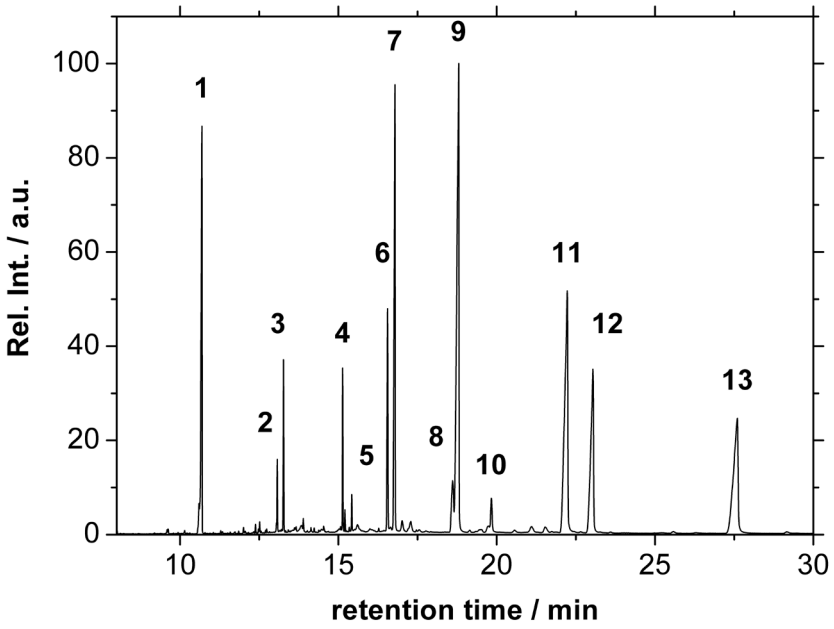

Figure 2-2 GC-MS TIC chromatogram of the autoclavate.
The thirteen dominant peaks are numbered consecutively.

Separate extraction of the individual components of the laminated film - the supporting film, the adhesive and the gas barrier film - proved that all migrating components originated from the laminating PUR adhesive (data not shown). As mentioned above, PURs are generated following the reaction of a diol with a diisocyanate. These components are not necessarily used as monomers. For the PUR adhesive studied here, the two components are a prepolymer with free hydroxy groups (the polyol component) and isophoron diisocyanate (IPDI, Figure 2-3).

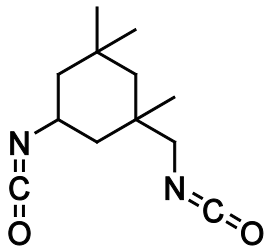

Figure 2-3 Structure of isophoron diisocyanate (IPDI).

Identification of the main leachables

The polyol component of the PUR adhesive consists of esters of adipic acid (AA) and isophthalic acid (IA) with monoethylene glycol (MEG) and diethylene glycol (DEG), which have been prepolymerised before further reaction with IPDI. A methoxysilane compound, which ensures strong bonding to the SiOx layer, is also included.

Because the migrating substances are chemically stable and obviously do not react with isocyanates most probable eliminate the involvement of the monomers IA, AA, MEG, or DEG. Moreover, the leachables cannot possess free hydroxy groups due to the observed lack of reactivity with isocyanate. Based on these considerations, linear oligomers can be excluded. Therefore, the observed peaks in the chromatogram represent presumable cyclic ester oligomers (CO), as these can be formed as undesirable by-products during the production of the polyester component [16-18]. All conceivable cyclic oligomers with a molar mass less than 500 g/mol (the upper mass range limit of the GC-MS used here) are listed in table 2-1, together with their respective monomer units. The corresponding chemical structures are given in figure 2-4. Some of these compounds were previously reported to be by-products generated during polyester synthesis (Table 2-1).

Table 2-1 Possibilities for combining adipic acid (AA), monoethylene glycol (MEG), isophthalic acid (IA) and diethylene glycol (DEG) for the formation of cyclic oligomers.
'Peak number' refers to substances that were synthesized and unambiguously assigned to a peak in the chromatogram of the autoclavate. References list previous literature reports of the corresponding compound

Cyclic Oligomer	Monomer units	Formula	Peak number	Mass / g/mol	References
CO-1	AA-DEG	$C_{10}H_{16}O_5$	1	217.00	[19,20]
CO-2	AA-MEG-AA-MEG	$C_{16}H_{24}O_8$	4	344.36	[21-23]
CO-4	AA-MEG-AA-DEG	$C_{18}H_{28}O_9$		388.41	
CO-5	IA-MEG-AA-MEG	$C_{18}H_{20}O_8$	7	364.35	
CO-6	AA-DEG-AA-DEG	$C_{20}H_{32}O_{10}$	8	432.47	
CO-7	IA-MEG-AA-DEG	$C_{20}H_{24}O_9$		408.40	
CO-8	IA-MEG-IA-MEG	$C_{20}H_{16}O_8$	10	384.34	[24-30]
CO-9	IA-DEG-AA-DEG	$C_{22}H_{28}O_{10}$	11	452.46	
CO-10	IA-MEG-IA-DEG	$C_{22}H_{20}O_9$		428.39	
CO-11	IA-DEG-IA-DEG	$C_{24}H_{24}O_{10}$	13	472.45	[31]
CO-13	IA-MEG	$C_{10}H_8O_4$		192.17	
CO-12	IA-DEG	$C_{12}H_{12}O_5$		236.22	
CO-14	AA-MEG	$C_8H_{12}O_4$		172.18	[21,22]

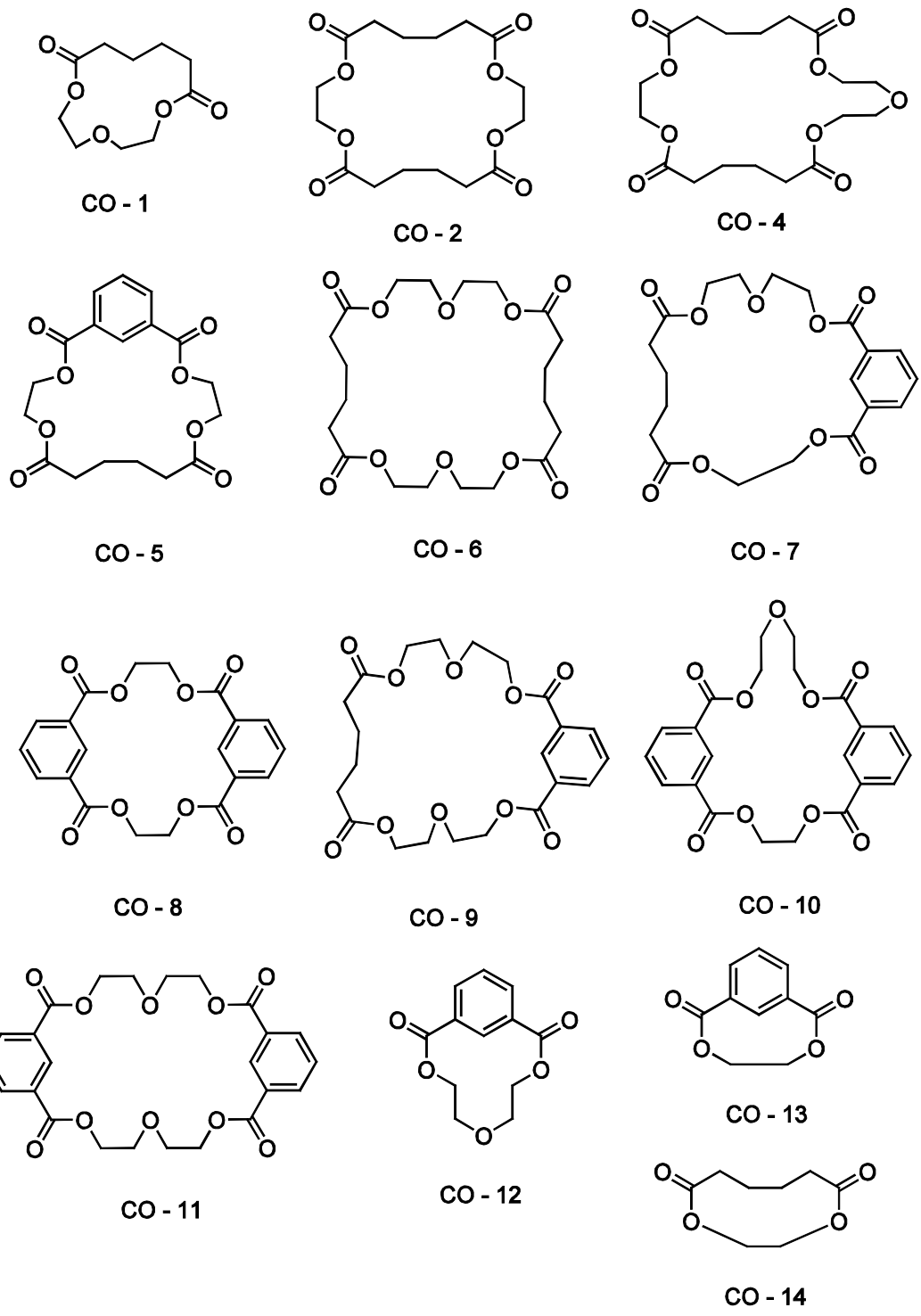

Figure 2-4 Molecular structures of the conceivable cyclic oligomers.

Unequivocal elucidation of the structures of compounds in the autoclavate requires comparisons with authentic standards. Given that the oligomers are not commercially available, they were synthesised commercially (Taros Chemicals GmbH & Co. KG, Dortmund) to generate pure samples of the esters CO-1, CO-2, CO-5, CO-6, CO-8, CO-9, and CO-11. The synthesis of CO-14 was not successful as it dimerised to CO-2 under the reaction conditions used. None of the esters CO-4, CO-7 and CO-10 could be synthesised as there was no suitable synthesis strategy available for these compounds consisting of two different glycol monomers. Furthermore, CO-13 and CO-12 could not be synthesised due to their high ring tension.
The presence of all seven synthesised cyclic oligomers could be confirmed in the autoclavate by virtue of their GC retention times and mass spectra. Corresponding peak numbers are given in table 2-1, and the mass spectra and fragmentation patterns obtained are discussed below.

Comparison of the mass spectra of the synthesised compounds shows that all spectra reveal evidence of fragments with an m/z value of 44. Given the monomeric building blocks of the esters, these fragments must represent C_2H_4O units. The generation of relatively large fragments comprising an acid fragment (IA or AA), including one or several C_2H_4O units, is also evident. The characteristic decomposition patterns of the AA and/or IA fragments in the low molecular mass range enable categorization of cyclic ester oligomers in terms of their composition on the basis of these two acids. Fragmentation involving m/z values of 173, 155, 99 and finally 55 is typical for cyclic esters of AA (CO-1, CO-2 and CO-6). An exemplary mass spectrum for CO-6 is shown in figure 2-5. In contrast, the compounds comprising IA (CO-8 and CO-11) exhibit characteristic fragments at m/z values 149, 104 and 76, as shown in figure 2-6 for CO-8. As expected, mixed fragmentation patterns are found in the mass spectra of the oligomers CO-5 and CO-9, because both contain one IA and one AA moiety. The typical fragmentation patterns described here are similar to EI mass spectra of cyclic ethers, the so-called crown ethers, and/or lactones (i.e., cyclic esters) [32-37].

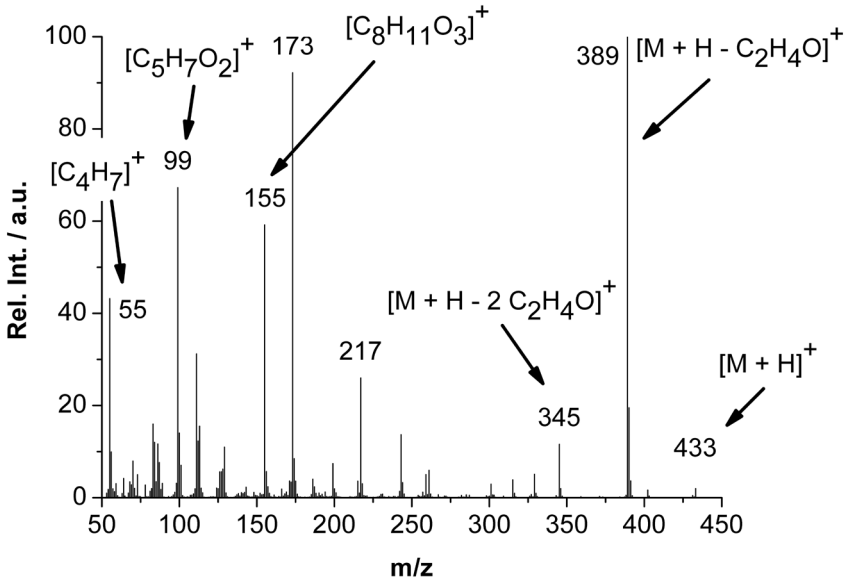

Figure 2-5 EI mass spectrum of ester CO-6 consisting of two AA and two DEG units. For molecular structure, see figure 2-4

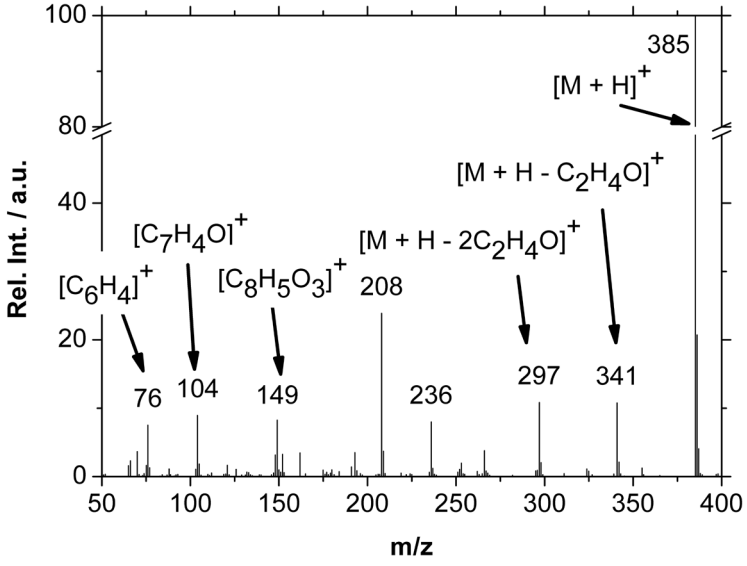

Figure 2-6 EI mass spectrum of CO-8 consisting of two IA and two MEG units. For molecular structure see figure 2-4

The six remaining unknown peaks were identified by comparing their mass spectra with mass spectra of the identified esters. It was striking that in the mass spectra for peaks 2 and 3, neither the cleavage of C_2H_4O nor the fragmentation pattern of at least one of the acid groups was evident. Moreover, the identical mass spectra obtained for the two substances suggest the involvement of isomers. In contrast to the mass spectra of peaks 2 and 3, the mass spectra of the four remaining peaks (peak nos. 5, 6, 9 and 12) exhibited the EI fragmentation patterns described above, thus enabling allocation of the acid groups shown in table 2-2.

Table 2-2 Known fragments and molar masses of unknown oligomers determined using electron impact (EI) and chemical ionization (CI), respectively
AA and IA denote adipic acid and isophthalic acid.

Peak	**Fragment comparison (EI)**	**Molar mass (CI)**
Peak 5	only IA	324.33 g/mol
Peak 6	only AA	388.41 g/mol
Peak 9	AA and IA	408.40 g/mol
Peak 12	only IA	428.39 g/mol

In order to elucidate the molecular masses of these four compounds, additional measurements involving chemical ionisation were performed. As a result of the soft ionisation used, the mass spectra exhibited hardly any fragmentation and and therefore allowed the determination of the molecular masses basing on the mass of the observed adduct ions with a proton and/or methane, which was the ionisation gas used. The molar masses of the unknown substances are given in table 2-2. This information enabled unequivocal allocation of the conceivable ester oligomers presented in table 2-1 to peaks 6 (CO-4, AA-DEG-AA-MEG), 9 (CO-7, IA-MEG-AA-DEG) and 12 (CO-10, IA-MEG-IA-DEG). The approach failed only for peak 5. The molar mass and the mass spectrum that points merely to an IA and several ethylene groups implies that only an ester of IA and tetraethylene glycol can be involved. Given that tetraethylene glycol is not a component of the adhesive, this was rather unexpected. However, the same structure – named CO-3 in this study - has been proposed before [37] based on an identical mass spectrum as obtained for peak 5 in this work. Subsequent analysis of the DEG used during prepolymer production corroborated this re-

sult because it contained small traces of tetraethylene glycol. The results clearly show (i) the relevance of oligomeric cyclic esters as migrating compounds from PUR adhesives, and (ii) the potential presence of previously unexpected migrating compounds derived from impurities of the monomers.

Identification of diurethanes

The GC-MS (CI) measurements were not able to identify the two remaining substances (peaks 2 and 3). Despite the soft ionisation, the distinct fragmentation observed prevented derivation of the molecular mass. For this reason, LC-MS measurements were employed using ESI and a high-resolution mass spectrometer. The allocation of the two substances to be identified in the LC-MS chromatogram was possible by means of the characteristic fragments with m/z 287 and 255 that had already been shown to occur reproducibly in the EI and CI spectra. As in the previous GC-MS measurements, the mass spectra of both peaks were identical in LC-MS. A molar mass of 287.1971 ± 0.0006 g/mol was determined for the protonated molecule $[M+H]^+$; after deducing the proton, the structural formula $C_{14}H_{26}N_2O_4$ is thus obtained for both substances.

The presence of two nitrogen atoms clearly points to the presence of a diisocyanate group. However, neither of the substances can be either IPDI used for the production of the prepolymer (molar mass of 222 g/mol) or a hydrolysis product of IPDI because the mass of the unknown compounds (approximately 286 g/mol) is substantially higher. The mass also suggests that the compounds can also not be derived from the reaction of IPDI with one of several acids (IA, AA) or alcohols (MEG, DEG). This leaves methoxysilane as the only other compound present in the prepolymer.

To support identification of the compound(s), the manufacturer of the PUR adhesive provided samples of different production steps that clearly showed that these two substances can only be found after the addition of methoxysilane. This silane cannot react with IPDI, but reacts with water liberated during the formation of the polyester. Even if this reaction is carried out at high temperatures above 100°C, small amounts of water are sufficient for the partial hydrolysis of the methoxysilane. Figure 2-7 shows that the methanol that is subsequently formed from this process reacts with IPDI. The reaction product matches exactly the structural formula of the compounds found. To confirm the presumed reaction, a few drops of IPDI were added to approximately 10 mL of methanol. The mixture was stirred for 1 hour, and a dilute sample was analysed by GC-MS (EI). The chromatogram thus obtained exhibited two distinct peaks whose retention times and mass spectra match

those for peaks 2 and 3 in the chromatogram of the autoclavate. The occurrence of two peaks can be explained by *cis-trans* isomerism of the cyclohexane ring, which has already been described in the literature for IPDI derivatives [38-43].

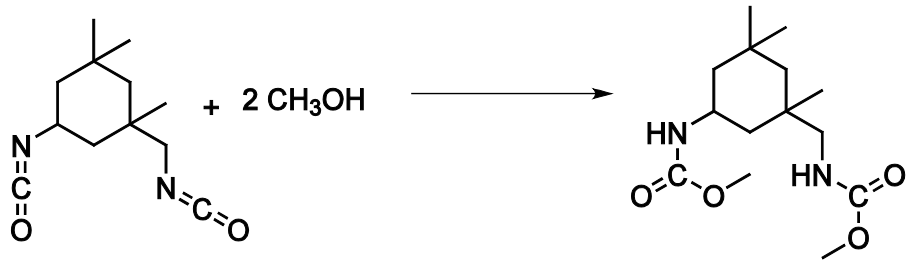

Figure 2-7 Reaction of IPDI with methanol.

The starting compound IPDI possesses two stereocenters, and can therefore be present as four stereoisomers. Given that the reaction with methanol is not stereoselective, the resulting diurethane is also present as four isomers, as shown in figure 2-8, where (R,R) and (S,S) and/or (R,S) and (S,R) represent enantiomers, which of course cannot be separated on the achiral GC column used.

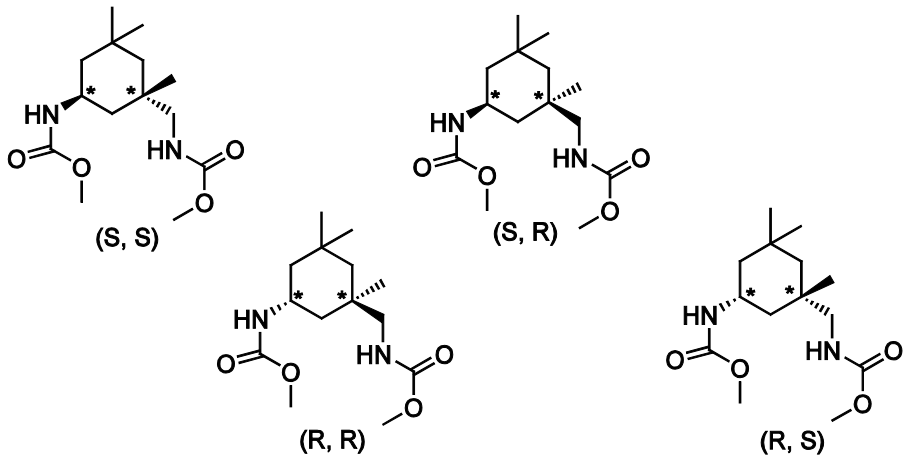

Figure 2-8 Four stereoisomers of the reaction of IPDI with methanol; stereocenters are marked by an asterisk*.

In contrast, the two pairs of diastereomers of the diurethane are clearly separated. The allocation of the two peaks to the diastereomers is highly controversial in the literature. The only agreement to be found among published reports is that both pairs are present in a ratio of approximately 70:30 with regard to their peak area ratios, and that the NCO group of IPDI, which is bound directly to the cyclohexane ring, is in an equatorial position

due to steric hindrance. Earlier publications [38-40] attribute the more intense signal to the stereoisomer where the CH_2-NCO group is in the axial position and both NCO groups are consequently in a trans position vis-à-vis each other (corresponds to (R,R) or (S,S) in figure 2-8). However, this is contradicted in more recent publications [41-43], which report that the cis form (in which the CH_2-NCO group is also in the equatorial position) is the dominant IPDI isomer. The data confirm the 70:30 ratio of the stereoisomers, although it was beyond the scope of this investigation to elucidate the exact conformation of these two minor migration products.

2.4 Conclusions

This study has shown that in a worst-case scenario by-products formed during the production of PUR adhesive migrate to aqueous solutions in contact with the multilayer polymer film. Complementary hyphenated MS-techniques identified these compounds. Further work will quantify the amount of migrating substances, which is crucial for a subsequent toxicological evaluation.

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3

Quantification of leachables from packaging in pharmaceutical solutions in the low ppt range by ‘stir bar sorptive extraction’-GC-MS
-
method development

3.1 Introduction

Plastics have become an integral part of our daily life, with more than a third of packaging materials being made of plastic [1–3]. Plastics are light, easy to mold, and amenable to modifications that confer properties such as stability, elasticity or permeability to gases matched to particular requirements within a wide range of possibilities. Amongst others packaging made of plastics frequently protect food or pharmaceuticals [4–7] from light, humidity or oxygen and thus improve the shelf life and stability of these products during storage.

The potential of packaging components to interact with the enclosed goods [8] is always a key consideration in selecting primary packaging materials. The possibility that low molecular weight substances, such as monomers, oligomers, plasticizers or catalyst components migrate from the packaging into the product [9–12] is of particular relevance, as they might pose a safety risk to consumers. This consideration is especially problematic for aqueous pharmaceutical solutions due to the direct contact of the packaging with the product and the likelihood that soluble substances derived from the packaging are rapidly diffusing into the solution. Since the end of 2005, the European Medicines Agency (EMEA) has consequently required the exact identification and quantification of migrating substances to comply with the Guideline on Plastic Immediate Packaging Materials (CPMP/QWP/4359/03). In order to successfully implement this guideline analytical methods with a high sensitivity are required. The necessary limits of detection (LOD) are in the ppb (µg/kg) to ppt (ng/kg) range. To achieve these low limits an extraction and enrichment of the analyte is essential as part of the sample preparation. A well-established technique is liquid-liquid-extraction (LLE) [13] that employs organic solvents non-miscible with water for the extraction of aqueous samples. While mixing both phases vigorously the analyte partitions into the organic solvent, which is afterwards either directly analyzed for example via gas chromatography (GC) or the analyte is further concentrated by evaporating the solvent partly before the GC analysis. The main disadvantages of LLE are its time-consumption, the high costs and the usage of large volumes of potentially toxic solvents, which are hazardous to health as well as to environment. These drawbacks brought the focus on solvent free and simple to perform sample preparation techniques like solid-phase microextraction (SPME) [14] and stir bar sorptive extraction (SBSE) [15], which were both developed in the 1990s and where most frequently the polymer polydimethylsiloxane (PDMS) is used as the extraction phase. SPME employs silica fibers coated with PDMS, which are placed in a needle of a syringe-like arrangement to extract and enrich the analytes. The SBSE method, in contrast, uses PDMS coated stir bars and thereby increases the PDMS volume by ca. two orders of magnitude, which significantly raises the enrichment

factor. The extraction principle for both methods is similar to LLE because they also base on an extraction of the analytes into a non-miscible liquid phase. But contrary to LLE, SPME and SBSE utilize the PDMS above its glass transition temperature of -120°C as the sorptive extraction phase. Here, the key advantage is that the polymer is a gum-like, stationary phase, which is despite its non-liquid character able to dissolve the analytes. PDMS is a well-known stationary phase for GC columns, it is inert, thermally stable and has specific degradation products, which may form during the repeated use due to aging of the material. The analytes can easily be desorbed from PDMS by a thermal desorption step and injected into the GC column.

SPME and SBSE have been compared in several publications [16–18] and all came to similar conclusions: SBSE is the method of choice for trace and ultra-trace analysis. Several review article [19–21] show that its main field of application is the water and environmental analysis, followed by food and biomedical analysis. Food and beverage are mainly analyzed in regard to aroma and taste compounds or degradation products and just a few publications were about leachables emerging from the packaging into the food. Two publications during the last years had a medical background: Armstrong et al. determined 15 structurally related leachables from implantable medical devices made of ultra-high molecular weight polyethylene [22] and Huang et al. developed a method for the determination of five halogenated phenol and anisol compounds in solid drug product samples [23]. But there are virtually no SBSE studies regarding leachables from packaging materials into pharmaceutical solutions, although this topic is recognized as an important one [24–27]. Only Sun et al. investigated pharmaceutical solutions so far. They determined four kinds of phthalate esters in polyvinyl chloride infusion bags by the use of SBSE [28].

The objective of the current work was to establish a method for the quantification of potential leachables in pharmaceutical solutions such as peritoneal or hemodialysis solutions used for the treatment of patients with renal disease. These solutions are in particular demanding with regard to the required LOD down to the low ppt (ng/kg) range since in contrast to other pharmaceutical solutions several liters may be infused per day and patient. With a restricted acceptable mass uptake per day the concentration levels of leachables to be monitored is very low due to these high volumes. This chapter describes the development and optimization of a SBSE-GC-MS method for the simultaneous quantification of more than 40 analytes, which are known to be possible leachables emerging from plastic packaging materials. This multi-component mixture comprised, among others, phenols, alcohols, carboxylic acids, carboxylic acid amides, phthalates and hydrocarbons. However, the method is not limited to these target analytes.

3.2 Experimental

Solvents, Chemicals

The following 39 compounds were used as analytes in this study: Phenol, 2'-hydroxyacetophenone, 2-*tert*-butylphenol, 4-*tert*-butylphenol, 2,6-di-*tert*-butylphenol, 2,4-di-*tert*-butylphenol, bisphenol A, butylhydroxytoluene, cyclohexanol, 2-ethylhexanol, benzyl alcohol, dodecanol, octadecanol, diethylphthalate, di-*iso*-butylphthalate, di-butylphthalate, dicyclohexylphthalate, undecane, 2-(2-butoxyethoxy)ethyl acetate, methyl-*iso*-butylketone, cyclohexanone, toluene, ethylbenzene, styrene, divinylbenzene, benzaldehyde, 1,2-dicyanobenzene and chlorobenzene were purchased from Merck KGaA (Darmstadt, Germany). 4-*tert*-amylphenol, oleamide, erucamide and 1,4-diacetylbenzene were purchased from Sigma-Aldrich Co. LLC (Steinheim, Germany). Decanamide, dodecanamide, hexadecanamide, stearamide and 4-methyl-2-heptanone were purchased from ABCR GmbH & Co. KG (Karlsruhe, Germany). Tetradecanamide, 5,5-dimethyl-2,4-hexandione and 1,3-diacetylbenzene were purchased from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany). The standard stock solution was prepared by dissolving these substances in ethanol with a final concentration of 250 mg/kg for each of the analytes. A second standard stock solution in ethanol was prepared for ten carboxylic acids with a concentration of about 540 mg/kg: 2-ethylhexanoic acid, heptanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid and docosanoic acid were purchased from Merck and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid was purchased from Alfa Aesar. All substances were purchased in the highest purity available. The stock solutions were stored in the darkness at room temperature. A summary of all analytes with their retention times and log $K_{O/W}$ values are given in table 3-1.

Four of the standards given above, namely cyclohexanol, docosanoic acid, di-*iso*-butylphthalate and 2-(2-butoxyethoxy)ethyl acetate, were excluded from this study as it turns out that these components were not properly quantifiable. Docosanoic acid was not detectable under the conditions given below. Cyclohexanol was detected but not quantifiable due to its simultaneous elution with styrene, while the latter one had partly similar fragments with more intensive signals. Di-*iso*-butylphthalate as well as 2-(2-butoxyethoxy)ethyl acetate showed concentration independent high target ion peak areas, suggesting a background contamination. The source of 2-(2-butoxyethoxy)ethyl acetate could not be identified, but the analysis of phthalates is known to be troublesome, due to their background level in every laboratory [29,30].

In addition, a solution of phenanthrene-D_{10} (Sigma Aldrich) at 2 mg/kg in ethanol (p.a., Merck KGa) was used for quality assurance. All samples were spiked with this component, to provide a final concentration of 75 µg/kg phenantrene-D_{10}.
The phenanthrene-D_{10} served as a control compound to assure an accurate and error free sample extraction procedure. Deviations from the usually obtained target ion peak area for phenanthrene-D_{10} pointed to problems in the stirring process during extraction or to an advanced aging of the stir bar.
From the two standard stock solutions a 20 µg/kg standard with all compounds including the carboxylic acids was freshly prepared for every SBSE measurement by dilution with water. A further dilution resulted in samples with 13 concentrations ranging from 20 µg/kg down to 10 pg/kg, which were used for the determination of the LOD.
The water used for all experiments was purified with a Milli-Q Gradient system from Millipore. Methanol (p.a., Merck KGaA) and dichloromethane (GC-grade, VWR) were used to clean the stir bar. Sodium chloride in p.a quality was purchased from Bernd Kraft (Duisburg, Germany).
All chemicals were used without any further purification.
The PDMS coated Twister^{TM} stir bars were purchased from Gerstel GmbH (Mülheim, Germany). The stir bars were cleaned and conditioned before every measurement by stirring them in 6 mL of a 50:50 mixture of methanol and dichloromethane for 4 hours, with a renewal of the solvents after 2 hours. Afterwards, the stir bars were dried under pure nitrogen at 30°C for 30 min and then conditioned at 300°C for 4 hours. The stir bars were allowed to cool down to room temperature for 4 hours under nitrogen. The heat conditioning procedure with the additional cool down to room temperature was repeated once more.

Sample Extraction Procedure

For SBSE optimization the following procedure was performed, whereby one of the parameters like sample volume, stirring time or speed was varied as indicated in the particular section, while the other parameters were kept constant as described in the following. A sample volume of 25 mL was placed in glass head space vials with a nominal volume of 25 mL. A concentration of 20 µg/kg was chosen for most of the optimization experiments. Then 8.5 g sodium chloride and 100 µL of the phenantrene-D_{10} solution were added and a 2 cm long PDMS stir bar with a 1 mm thick PDMS coating (= 126µL PDMS phase volume) was placed into each vial. Subsequently, the vials were sealed with a crimp cap. The extraction was performed for 4 h at a stirring speed of 1100 rpm at 25°C. Afterwards, the stir bars were removed with a magnetic stir bar retriever, rinsed with water and shortly

purged with nitrogen to remove the water from the surface. Rinsing with water does not influence the analytes, because they are absorbed within the PDMS and are not located on the surface [21]. Finally, the stir bars were transferred into a desorption tube and placed into the auto sampler. These desorption tubes were cleaned after every three measurements by rinsing them first with water and afterwards with acetone. Subsequently, they were dried over night at 70°C.
The entire stir bar handling was done with tweezers, to avoid possible contamination by direct skin contact.

TD-GC-MS analysis

The GC/MS measurements were performed using a GC 7890 system from Agilent equipped with a thermal desorption unit "TDU" (Gerstel) and a cold injection system "CIS" (Gerstel). In addition, a multipurpose autosampler "MPS" (Gerstel) was used to introduce the stir bars into the TDU. The desorption took place in solvent vent mode at 280°C for 10 min. Helium 5.0 at a flow of 50 mL/min was used to transfer the analytes into the CIS where they were cryo-focused at -120°C. Finally the CIS was heated up to 280°C at a speed of 12°C/s and the analytes were injected into the GC column. The helium carrier gas had a constant flow of 1 mL/min. The temperature program used for chromatographic separation involved a one minute waiting time at 50°C after the injection. Afterwards the temperature was increased from 50°C to 150°C at a rate of 10°C/min, and then maintained at this level for 5.5 min before increasing the temperature further at a rate of 50°C/min rate to 300°C, and then maintaining the temperature at this level for 10 min. The ion source was held at a temperature of 270°C. The detector was an Agilent 5973 quadrupole mass spectrometer (MS) with an electron impact (EI) source and was used in scan mode. Mass-to-charge ratios (m/z) were recorded at values between 25 and 700. The column used was a Zebron (ZB-50) capillary column (length 30 m, diameter 0.25 mm, film thickness 0.50 µm, stationary phase: 50% diphenyl polysiloxane, 50% dimethyl polysiloxane) purchased from Phenomenex (Aschaffenburg, Germany).

3.3 Results and Discussion

Chromatographic separation

As the focus of this work was on the optimization of the stir bar extraction just a short overview about variations of the parameters related to the gas chromatographic (GC) separation is given in the following. Three different GC columns were tested for their suitability to separate the multi-component mixture: a ZB5 column (length 30 m, diameter 0.25 mm, film thickness 0.25 µm, stationary phase: 5% phenyl, 95% dimethylpolysiloxane, supplier: Phenomenex), a HP Innowax column (length 15 m, diameter 0.25 mm, film thickness 0.25 µm, stationary phase: polyethylene glycol (PEG), supplier: Agilent) and a ZB50 column. The ZB5 turned out to be not suitable for the analytes under study here, as some of them were not eluted due to an inadequate polarity of the column. The HP Innowax gave a very good separation for most of the compounds, but had a limited thermo stability. Thus, the temperature could not be increased up to 300°C, which was necessary to achieve a proper elution of the amides. Finally, the ZB50 column was chosen. This column had partially an inferior separation compared to the HP Innowax column. For example, styrene and cyclohexanol were baseline separated when using the HP Innowax column, but were eluted nearly simultaneously in the case of the ZB50 column. Therefore cyclohexanol was not properly quantifiable in the study here. But the key advantage of the ZB50 column was its higher thermo stability that allowed a heating up to 300°C. This ensured a complete elution of the amides. The temperature program of the column oven was optimized for the ZB50. The optimal settings are given in the experimental section and were kept constant during the following SBSE optimization experiments.

Stir Bar Conditioning

A crucial requirement for the detection of compounds in the ng/kg range is a stir bar void of any background contamination. It turned out that a usually performed, simple conditioning step such as heating the stir bars for a certain time at temperatures up to 300°C [31–33] was insufficient in that regard. Different methods were tested to minimize background contamination. All of them have a washing in a 1:1 (v/v) mixture of methanol and dichloromethane in common. One procedure was similar to the one described in [13, 34, 35]. Here the stir bars were placed in 6 mL of this mixture and were then sonicated for 15 min. This step was repeated 4 more times with a renewed washing solution. Afterwards the stir bars were dried under pure nitrogen at 30°C for 30 min and then baked out at 300°C for 4 hours. Subsequently the stir bars were allowed to cool down to room tempera-

ture under nitrogen. A second procedure consisted of stirring the stir bar in 6 mL of the cleaning solution for 4 hours and drying them as already described above. This procedure was further varied by exchanging the cleaning solution after 2 hours and additionally repeating the drying step twice.

The results for the three conditioning methods are shown exemplary for some components in figure 3-1. Data for all analytes are given in the supplement chapter 3.6.4. The third procedure described above showed the lowest remaining contaminations and was therefore used as the standard conditioning method for the used as well as for the new stir bars.

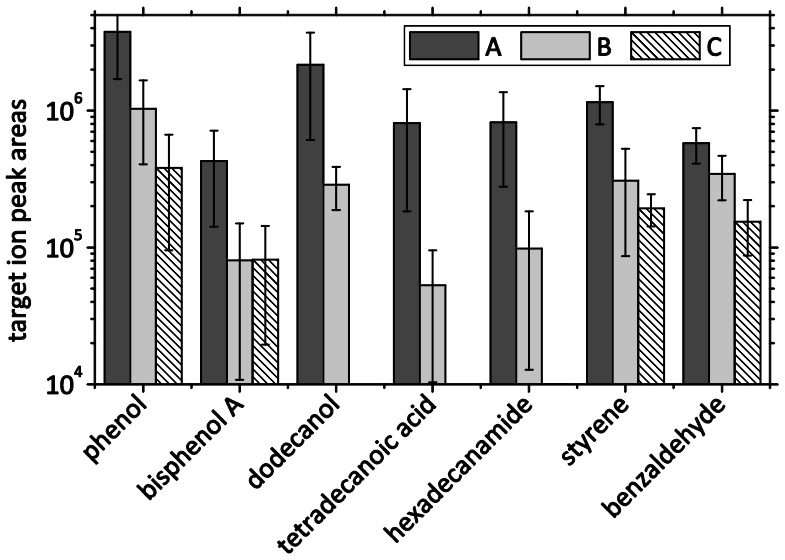

Figure 3-1 Comparison of the efficiency of the three conditioning procedures on some exemplary components.
4 h washing with 4 h drying (A), 4 times 15 min sonication with 4 h drying (B), 2 times 2 h washing with 2 times 4 h drying (C). Error bars represent the standard deviation for N=3.

Stirring Time and Speed

The stirring or extraction time in combination with the stirring speed mainly influences the kinetics of the sorption process and thus determines the total duration of the sample analysis. For a short analysis time a fast establishment of the phase equilibrium between water and PDMS is important. This can be achieved by high stirring rates. Usual stirring rates are between 600 rpm and 1200 rpm, whereby a higher stirring speed increases the transfer coefficient from the aqueous solution into the PDMS, because it minimizes the thickness of the diffusion layer [36–39]. This thin layer, which is also called concentration boundary layer, is a region close to the PDMS surface, where the concentration of the analytes is lower than in the bulk solution. Using high stirring speeds minimizes the thickness of this boundary layer and thus minimizes the resistance for the solute transport into the PDMS. There are two main disadvantages of high stirring rates significantly exceeding 1000 rpm. First of all, it is challenging to have high and stable stirring conditions. As the magnetic bar is quite small in comparison to standard lab equipment stirring bars, they easily lose contact to the rotating magnetic field and finally just "jump" up and down in the vials with a slow rotating movement [40]. This behavior must be avoided under any circumstances as this dramatically reduces the extraction efficiency. For about a third of the compounds the target ion peak areas droped below 1%, for some of them even below 0.1% and for most of the other analytes a peak area of 10% or less was observed in this case. A second disadvantage is the higher mechanical stress for the PDMS at high stirring rates, which is in direct contact with the bottom of the vial.

The stirring rate was varied in 5 steps at a stirring time of 4 hours; 200 rpm, 400 rpm, 600 rpm, 800 rpm and 1100 rpm. The effect on the extraction yields, represented as the target ion peak areas, is shown in figure 3-2 and summarized for all components in the supplement chapter 3.6.5.

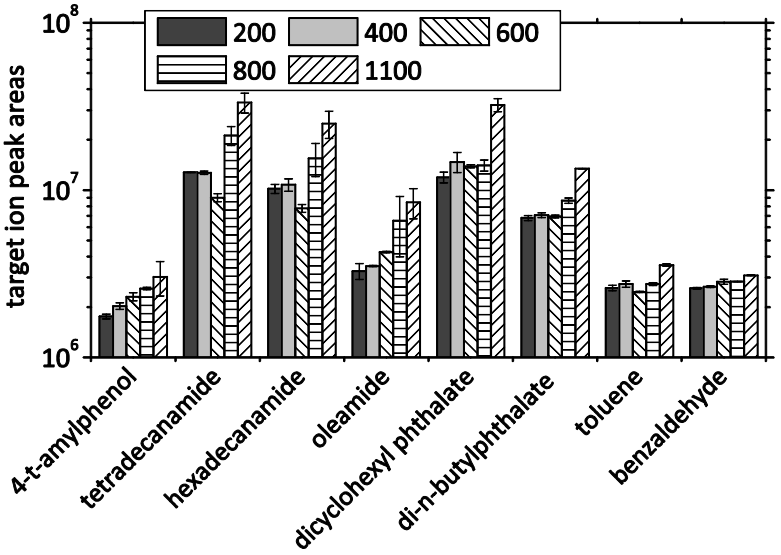

Figure 3-2 Influence of the stirring speed on the extraction efficiency of some exemplary components.
Stirring speed is given in rpm. Error bars represent the standard deviation for N=3.

Noticeable is the increase in the target ion peak areas for the amides and phthalates, which double when increasing the stirring speed from 200 rpm to 1100 rpm. These compounds are probably more distant from the PDMS-water equilibrium at low stirring speeds and thus the largest effects of stirring enhancement were seen for them; an effect that was already observed and described [38]. For most of the remaining substances the extraction efficiency increases about 30%. Despite the probably shorter lifetime of the stir bar the highest stirring speed of 1100 rpm was determined to be optimal. Even higher stirring rates were not suitable as no steady stirring was possible.

To ensure equilibrium conditions usually extraction times between 60 min and 24 h are chosen. The exact time till equilibrium is reached depends e.g. on the diffusivity of the solute, on the phase ratio and the temperature, which was not changed within this work and kept constant at room temperature.

The extraction time was varied in 7 steps; 30 min, 60 min, 120 min, 240 min, 360 min, 960 min and 1440 min while stirring the samples at 1100 rpm. The last two times were chosen from a practical point of view as a 16 hours or even 24 hours stirring time is easily realized in overnight measurements.

After 240 min the equilibrium between PDMS and water is reached for almost all compounds as shown in figure 3-3 and summarized in the supplement chapter. A prolonged extraction does not have any advantages; it just prolongs the analysis time. Figure 3-3

shows that there is even a disadvantage for the carboxylic acids, as their concentration in the PDMS decreased considerably for times much longer than 240 min. Interestingly, the reduction of the extracted amount of acids by time seemed to have an inverse correlation with the number of carbon atoms and thus with the hydrophobicity and $K_{O/W}$ value, respectively. The decrease from 4 h to 24 h was most pronounced for dodecanoic acid and tetradecanoic acid, whereas nearly no change in target ion peak area was observed for the octadecanoic acid. The variation of the extraction yield by time for the carboxylic aids shown in figure 3-3 can just be explained by assuming the presence of a process, which slowly but steadily removes the carboxylic acids from the solution within several hours. The carboxylic acids under study here are known for their preference to form micelles [41, 42] and to adsorb on the glass and on the water surface [43, 44]. The critical micellization concentrations (CMC) in pure water of these acids are above the concentrations used here. Thus no micelles should be formed. But as soon as the salt is added to the sample, the salting-out effect leads to a decrease of the CMC [45], which in turn may lead to the formation of micelles. Possibly, the decrease of the extracted amount by time is a result of the interplay between adsorption on the glass and the water surface, formation of micelles and sorption into the PDMS; processes which are related by the concentration of the acid monomers in solution. Additionally, dimerization of a protonated and an unprotonated fatty acid molecule through a hydrogen bond is known to occur in water [46], which might be an additional parameter that influences the system.

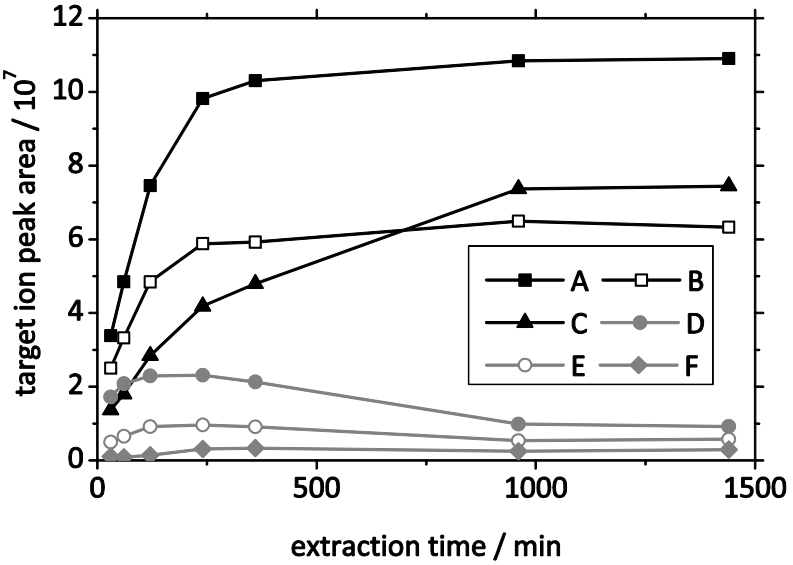

Figure 3-3 Influence of the extraction time on the target ion peak areas.
Results are shoen exemplarily for dodecanamide (A), diethyl phthalate (B), decanamide (C), tetradecanoic acid (D), hexadecanoic acid (E) and octadecanoic acid (F). Standard deviations (N=3) were below 25% for the acids and below 10% for the other analytes.

Sample volume, stir bar dimensions

The stir bar dimensions - the PDMS volume to be more precise – and the sample volume directly influence the extraction efficiency. Both variables are connected via the partition coefficient as shown in the well-known equation (3-1):

$$K_{PDMS/W} = \frac{C_{PDMS}}{C_{Water}} = \frac{m_{PDMS}}{m_{Water}} \cdot \frac{V_{Water}}{V_{PDMS}} = \frac{m_{PDMS}}{m_{Water}} \cdot \beta \qquad (3\text{-}1)$$

The partition coefficient $K_{PDMS/W}$ for the solute distribution in PDMS and water is defined as the ratio between the solute concentrations in PDMS (C_{PDMS}) and water (C_{Water}), which is equal to the mass ratio (m_{PDMS}/m_{Water}) times the phase ratio β. When Baltussen at al. established SBSE they assumed a proportionality between $K_{PDMS/W}$ and the already established octanol-water partition coefficient $K_{O/W}$ [15]. Meanwhile an approximately linear relationship between these two *K*-values was experimentally and theoretically determined [47]. Regarding formula (3-1), which describes the equilibrium situation, both volumes have obviously an opposite effect on the final concentration of the solute in the PDMS. Currently available commercial stir bars have PDMS volumes of 23.5 µL (10 mm long stir

bar with a 0.5 mm thick PDMS coating, herein afterwards referred to as “10x0.5”), 47 µL (“20x0.5” stir bar), 63 µL (“10x1” stir bar) and 126 µL (“20x1”). As higher PDMS volumes result in higher extraction values, the 20x1 stir bar seems to be the first choice. But beside the absolute PDMS volume, the thickness of the PDMS layer can also have an effect on the extraction as it influences the kinetics of the absorption [48]. Thus a stir bar with a thinner PDMS-coating might be promising. To find the most suitable stir bar size for the multicomponent mixture under study here, three different stir bars were tested: 10x1, 20x0.5 and 20x1.

These were used for the extraction of 10 mL sample with a concentration of 10 µg/kg for 4 h. Salt was not added and the stirring speed was 850 rpm. Figure 3-4 shows the results for 10x1 and 20x0.5 stir bars, where both target ion peak areas were normalized to the target ion peak areas of the 20x1 stir bar. The compounds are sorted in order of their $K_{O/W}$ values.

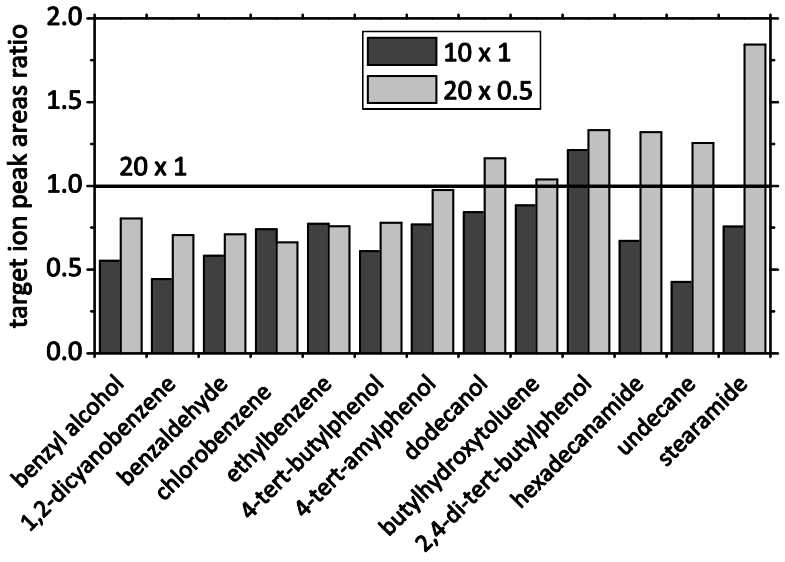

Figure 3-4 Influence of the stir bar dimensions on the detected target ion peak areas for exemplary analytes, sorted in order of their $K_{O/W}$ values (see Table 3-1). Results are shown for the 10x1 stir bar and 20x0.5 stir bar normalized to target ion peak areas obtained with a 20x1 stir bar (denoted as the line at a value of 1). Standard deviations of the target ion peak areas were below 10% (N=3).

Obviously the extraction yield is smaller when using a 10x1 stir bar compared to a 20x1 stir bar. This is not surprising as the second stir bar has twice the PDMS volume compared to the first one. Furthermore, the results shown in figure 3-4 are in good qualitative accordance with theoretical results. Equation (3-1) can be used to calculate the ratios of the ab-

solute analyte amounts in the 10x1 and 20x1 stir bars. Plotting these ratios against log $K_{O/W}$ results in a sigmoidal curve. For low log $K_{O/W}$ values the ratio between the absolute masses of the analyte in 10x1 stir bars and 20x1 stir bars is 0.5. For increasing log $K_{O/W}$ values this ratio reaches 1, which is qualitatively shown in figure 3-4 as well.

Figure 3-4 also shows the normalized target ion peak areas for the 20x0.5 stir bar. Regarding equation (3-1) the extraction yield should be even lower for the low log $K_{O/W}$ compounds than with the 10x1 stir bar, because the 20x0.5 stir bar has an even smaller PDMS volume. But figure 3-4 does not show a lower relative extraction yield. In contrast, it shows that for compounds with a high log $K_{O/W}$ value the extracted amount is even higher than in the 20x1 stir bars. A similar behavior was observed by Doong et al. [49]. The authors determined and compared partition coefficients for different hydrocarbons by solid-phase microextraction (SPME) with 7 µm and 100 µm thick fibers. They observed for high molecular weight compounds a much higher apparent partition coefficient for the thinner fiber. Beside possible differences in the production process of the thick and thin fibers, they assumed this is due to not having achieved equilibrium conditions in the thicker fiber. Difilippo et al. also mentioned an effect of the coating thickness [47]. They assumed that the differences in the PDMS layer thickness might be the source for variations in experimentally determined partition coefficients. However, in this work a higher extraction efficiency was just seen for a few substances, while for more compounds a decreased extraction yield for the thinner coating was observed. Consequently, the 20x1 stir bars were considered to be more suitable for the method under study here.

To study the influence of the sample volume four different volumes of 5 mL, 10 mL, 20 mL and 29 mL were extracted with 20x1 stir bars for 4 h at 850 rpm without the addition of salt. The resulting target ion peak areas are shown in figure 3-5. Despite equation (3-1), which predicts lower extraction efficiencies for compounds with log $K_{O/W}$ values below 4, figure 3-5 shows an increasing target ion signal with increasing sample volumes for nearly all compounds. This can be attributed to the higher absolute amount of the analyte in a larger sample volume and is in accordance with several publications [50–52], which also describe a higher target ion signal for increasing sample volumes. An additional positive effect of the largest volume of 29 mL was the minimized head space in the vial above the sample solution.

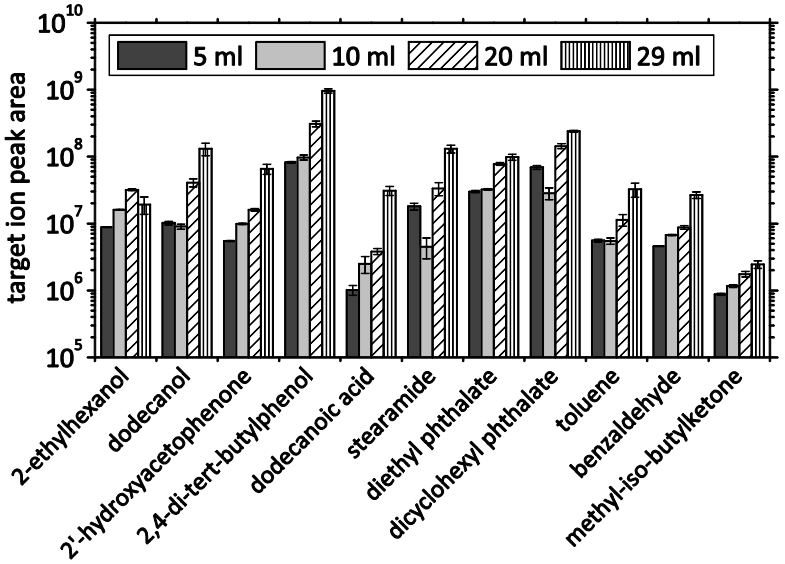

Figure 3-5 Influence of the sample volumes from 5 mL to 29 mL on some exemplary components.
The error bars represent the standard deviation for N=3.

NaCl addition, salting out

Usually extraction efficiency of polar solutes is enhanced by salting out, an effect that was described in several publications, for example [53–57]. The increase in ionic strength reduces the solubility of organic compounds and increases their partition coefficient between PDMS und water. Consequently, the extraction yields become higher. But there are also contradictory, not directly SBSE related publications [58, 59], which describe the opposite effect: salting out effects are small for polar components and increase with increasing log $K_{O/W}$. As the standard solution under study here comprises a wide variety of different substances ranging from very polar (log $K_{O/W}$ = -2.9 for the dissociated form of the ethylhexanoic acid) to nonpolar (log $K_{O/W}$ = 8.4 for erucamide) salting out effects should be taken into account as a factor which may strongly influence the extraction. Thus salting out was studied by comparing the extraction of samples without addition of NaCl and with addition of 3 g, 6 g, 8 g, 8.5 g and 9 g salt. The addition of 9 g results in a salt concentration of 360 g/L which is above the water solubility limit of NaCl and thus finally results in a saturated solution. The idea behind this high concentration was a simplification of weighing the salt. As soon as the solution is saturated a small inaccuracy in the amount of salt added should not influence the salting out effect.

Detailed results are given in the supplement chapter 3.6.5. Figure 3-6 shows examples of substances whose extraction efficiency increases or decreases by the addition of salt. It is

quite striking that the extraction of most substances with a log $K_{O/W}$ below 4 is enhanced by the addition of salt. Compounds with a log $K_{O/W}$ from 4 to 5 show a negligible change in extraction behavior and for substances with higher log $K_{O/W}$ values the extraction efficiency becomes worse when adding salt. This observation correlates with the solute concentration ratio in PDMS and water calculated by equation (3-1). Around log $K_{O/W}$ values of 4 to 5 it reaches a nearly 100% extraction into the PDMS. For the high log $K_{O/W}$ compounds the target ion signal decreased when adding 3 g or 6 g NaCl but even higher salt concentrations did not lead to a further considerable decrease. Undecan with a log $K_{O/W}$ of 5.74 showed a strong decrease in extraction yield, which dropped to 10% for an addition of 8.5 g NaCl. Surprisingly substances with an even higher log $K_{O/W}$ such as octadecanol (log $K_{O/W}$ 7.19) or stearamide (log $K_{O/W}$ 6.70) did not exhibit such a pronounced decrease. Here the target ion signals just dropped to about 30% at 8.5 g NaCl. On the other hand the extracted amount for low log $K_{O/W}$ substances further increases when adding 8.5 g salt. In contrast to existing publications no hindrance of the solute diffusion into the PDMS was observed [21, 60, 61]. An even further increase in salt concentration above the solubility limit did not lead to a further increase in extraction yield. But it turns out that saturated solutions have the disadvantage that the undissolved salt grains on the bottom of the vial were a severe interference to the stirring. Quite often the stir bar lost contact to the magnetic field and was not stirring properly anymore. Therefore 8.5 g NaCl in 25 mL, which does not lead to a saturated solution, was finally used.

The salting out experiments also revealed the special properties of the carboxylic acids compared to the other compounds of the mixture. As their pK_a values with about 4.8 are below the sample pH of 7, the carboxylic acids exist predominantly in their dissociated form. The polarity and therefore the water solubility are much higher in this case. Correspondingly, the log $K_{O/W}$ values are much lower compared to the protonated form and a positive salting out effect is observed for the carboxylic acids with log $K_{O/W}$ values below 1.75 (dodecanoic acid). For dodecanoic acid no salting out effect is observed and for the acids with higher log $K_{O/W}$ values the typical decrease in extraction efficiency was observed. A similar turning point in salting out behavior was also observed by Pan et al. [62] who observed no salting out effect for decanoic acid in water.

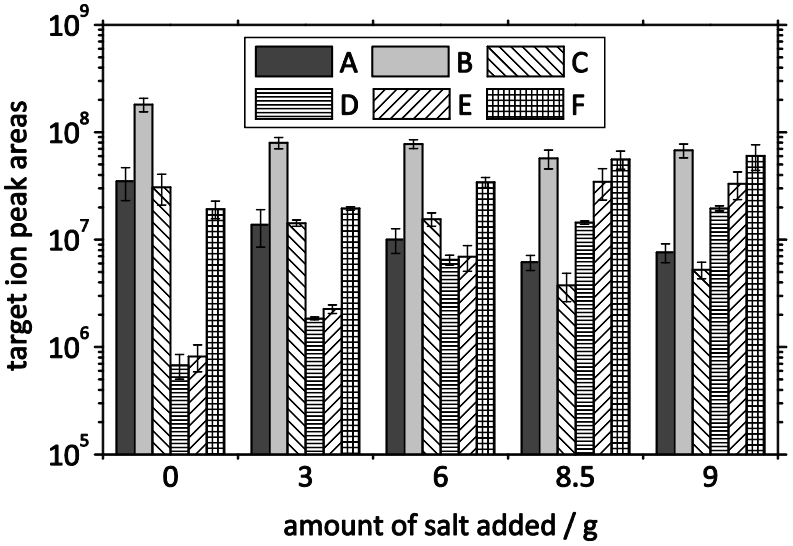

Figure 3-6 Influence of salt addition on the detected target ion peak areas.
Results are shown exemplarily for tetradecanoic acid (A), butylhydroxytoluene (B), undecane (C), bisphenol A (D), 2-ethylhexanoic acid (E) and 2-ethylhexanol (F). The error bars represent the standard deviation for N=3.

Desorption Process

Up to now parameters were discussed, which influence the kinetics and thermodynamics of the sorption process into the PDMS. For the analysis via GC the analytes were thermally desorbed from the PDMS. Here two parameters – time and temperature - have the major influence on the desorption efficiency. In addition, the effect of two different heating ramps of the CIS was studied.

In general, higher temperatures favor the desorption process, but may decrease the lifetime of the stir bar. Two desorption temperatures were compared: 250°C und 280°C at a desorption time of 10 min. No significant difference between 250°C and 280°C was determined, but for most of the compounds a desorption temperature of 280°C gave slightly higher signals. No thermal degradation of substances was observed. As the higher temperature also minimizes carry over effects, a temperature of 280°C was chosen as the preferred setting.

Subsequently the desorption time was varied in 4 steps (2.5 min, 5 min, 7.5 min, 10 min) at a constant desorption temperature of 280°C. The comparison in figure 3-7 shows an increase in the target ion peak area up to a factor of 2.2 when prolonging the time from 2.5 min to 10 min. Therefore, a 10 min desorption time was favored.

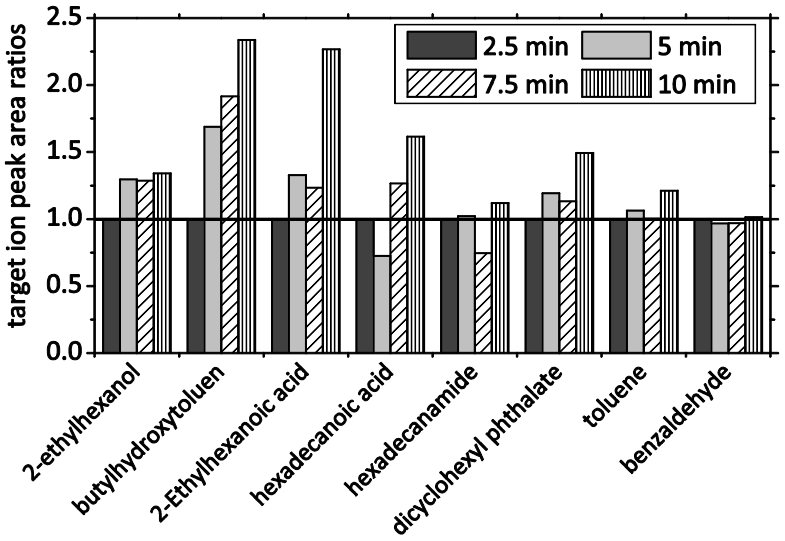

Figure 3-7 Effect of the desorption time on the detected target ion peak areas for some exemplary analytes.
Results are shown for the 5 min, 7.5 min and 10 min normalized to target ion peak areas obtained at 2.5 min (denoted as the line at a value of 1). Standard deviations (N=3) of the target ion peak areas were below 20% for the acids and below 10% for the remaining components.

Finally the CIS heating ramp was varied from the normal heating ramp (12°C/s) to a fast ramp with 16°C/s. This was meant to have a faster transfer of the analytes onto the GC column und to have finally sharper peaks in the chromatogram [63]. But this was not the case. No improvement in the peak shape could be observed (not shown here), which is also reported for example by Cajka et al. [64]. The normal ramp gave about two times higher signals and was thus preferred for this method. A similar behavior for some of their analytes was seen by Godula et al. [65], who optimized a CIS for the analysis of pesticide residuals in food samples. They varied the heating rate between 100°C/min and 500°C/min and observed a signal increase for some substances and but also a signal decrease for others. In the current study this phenomenon was not further investigated, as the main goal of an increased heating rate – the improvement of the peak shape – was not reached.

Limit of Detection

To check the suitability of the optimized SBSE method for the monitoring of leachables, the LODs were determined for the analytes in the multi-component mixture. Serial dilutions from 20 µg/kg down to 10 pg/kg were made to prepare 13 samples with continuously lower concentrations. As expected, for most of the components the target ion peak areas decreased linearly with concentration. But for some of the analytes a constant signal independent of concentration was observed below a critical threshold as shown in figure 3-8. Surprisingly, this threshold was higher than the respective background signals determined for cleaned and conditioned stir bar. For these substances the LOD values were defined as the mean of the plateau value plus the threefold standard deviation of the plateau value. For the remaining analytes, the LOD was defined in a similar way; it was either three times the S/N ratio or the averaged background signal plus its threefold standard deviation. The LOD values are summarized in table 3-1. The LODs for undecane, 1,2-dicyanobenzene and the carboxylic acids with exception of dodecanoic acid and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid were between 1.0 µg/kg and 0.1 µg/kg, but were not further investigated by serial dilutions between these two values and therefore set to 1.0 µg/kg. The high LODs for undecane and 1,2-dicyanobenzene can be reasoned for the former with the pronounced negative salting-out effect as discussed above and for the latter with the rather high polarity with a log $K_{O/W}$ of 0.99. However, the partition coefficient cannot explain the low LOD values for the dodecanoic acid and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid. Their log $K_{O/W}$ values are in the middle of the range of log $K_{O/W}$ values from -2.9 to 4.7 covered by the carboxylic acids under study here. In [66] SBSE experiments with octanoic, tetradecanoic and octadecanoic acid are described. Here, octanoic and octadecanoic acid had partially much lower recoveries at pH 7 than tetradecanoic acid, which has a log $K_{O/W}$ between the other two acids. This also indicates that the partition coefficient is at least for the carboxylic acids not the only key parameter that determines the extraction efficiency. In particular for these components effects like adsorption, micellization or dimerization as described above may influence the sorption process as well. For the remaining 35 components the LOD roughly decreases for increasing log $K_{O/W}$. However, a correlation between the logarithmized LOD and log $K_{O/W}$ shows a poor R^2 of 0.1 for a linear fit, which at least partially could be attributed to the salting-out effect. When assuming a considerable increase in extraction efficiency for polar substances (a decrease of their LOD) and just a minor decrease for the non-polar substances (an increase of their LOD) a trend between LOD and partition coefficient may become difficult to observe.

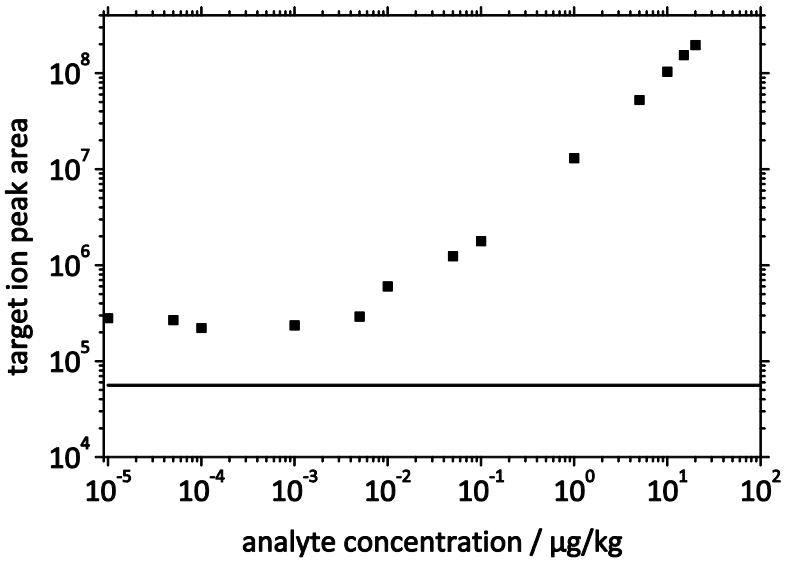

Figure 3-8 Threshold behavior for dodecanamide in a serial dilution.
The solid line represents the background contamination with dodecanamide plus its standard deviation for conditioned stir bars.

Table 3-1 Components used within this study, their retention times (rt), log $K_{O/W}$ values and limits of detection.

substances	rt / min	log $K_{O/W}$	LOD / µg/kg
phenol	8.21	1.46[a]	0.014
2'-hydroxyacetophenone	11.56	1.92[a]	0.004
2-*tert*-butylphenol	12.60	3.31[a]	0.001
4-*tert*-butylphenol	13.52	3.31[a]	0.001
4-*tert*-amylphenol	16.23	3.91[*]	0.001
2,6-di-*tert*-butylphenol	16.10	4.92[a]	0.001
2,4-di-*tert*-butylphenol	18.63	5.19[b]	0.003
bisphenol A	36.53	3.32[a]	0.006
butylhydroxytoluene	18.26	5.03[*]	0.005
2-ethylhexanol	7.64	2.73[*]	0.004
benzyl alcohol	9.41	1.10[a]	0.011
dodecanol	15.36	5.13[a]	0.007
octadecanol	31.31	7.19[*]	0.006
2-ethyl hexanoic acid[#]	9.13	-2.90[*]	1.0 [~]
heptanoic acid[#]	8.59	-0.70[*]	1.0 [~]
octanoic acid[#]	10.20	-0.21[*]	1.0 [~]

Table 3-1 continued

substances	rt / min	log $K_{O/W}$	LOD / µg/kg
decanoic acid[#]	14.08	0.77[*]	1.0 [~]
dodecanoic acid[#]	19.61	1.75[*]	0.002
tetradecanoic acid[#]	28.73	2.73[*]	1.0 [~]
hexadecanoic acid[#]	30.69	3.72[*]	1.0 [~]
octadecanoic acid [#]	32.07	4.70[*]	1.0 [~]
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid [#]	32.11	0.96[*]	0.014
decanamide	26.25	2.77[*]	0.001
dodecanamide	30.16	3.75[*]	0.003
tetradecanamide	31.69	4.73[*]	0.009
hexadecanamide	33.50	5.71[*]	0.008
stearamide	36.03	6.70[*]	0.010
oleamide	36.09	6.48[*]	0.011
erucamide	38.82	8.44[*]	0.005
diethylphthalate	28.80	2.42[c]	0.256
di-butylphthalate	31.99	4.50[c]	0.035
dicyclohexylphthalate	38.38	5.60[*]	0.004
undecane	7.16	5.74[*]	1.0 [~]
toluene	3.98	2.73[a]	0.035
ethylbenzene	5.37	3.15[a]	0.048
styrene	6.18	2.95[a]	0.004
divinylbenzene	9.85	3.80[*]	0.001
benzaldehyde	8.31	1.48[a]	0.100
1,4-diacetylbenzene	23.41	1.34[a]	0.013
1,3-diacetylbenzene	22.39	1.43[a]	0.009
1,2-dicyanobenzene	19.07	0.99[d]	1.0 [~]
chlorobenzene	5.51	2.84[e]	0.013
methyl-*iso*-butylketone	3.63	1.31[f]	0.008
4-methyl-2-heptanone	6.42	2.15[*]	0.005
cyclohexanone	6.98	0.81[a]	0.006
5,5-dimethyl-2,4-hexandione	7.08	1.67[a]	0.016

3.4 Conclusions

The optimization of a GC-MS-method for the simultaneous determination of more than 40 potential leachables emerging from plastic packaging using SBSE was described. The optimized procedure utilized a sample volume of 25 mL that was extracted after the addition of 8.5 g NaCl for 4 hours at 1100 rpm with a 20 mm long stir bar coated with 1 mm PDMS. The detection limits for most of the components were 0.01 µg/kg or below. Only the carboxylic acids had a higher LOD of 1 µg/kg due to their low partition coefficients for the dissociated forms that dominate at the sample pH.

Due to its simplicity and its ability to determine multiple compounds in MS scan mode at a high sensitivity the method described here is an ideal tool for the determination of leachables in pharmaceutical solutions, for example used for peritoneal or hemodialysis. The following chapter will present the validation of this method and will quantify the amount of leachables in dialysis solutions currently available on the market.

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3.6 Supplement

3.6.1 Solutions

Tab. S 3-1 Exemplary preparation of the used stock solution "screening standard (SC)" by weighing and solving all standards without the carboxylic acids in 92.7487 g of ethanol

Substance	Weight / mg	Concentration / mg/kg
phenols		
phenol	25.00	266.9
2'-hydroxyacetophenone	27.80	296.8
2-*tert*-butylphenol	25.90	276.5
4-*tert*-butylphenol	25.00	266.9
4-*tert*-amylphenol	25.30	270.1
2,6-di-*tert*-butylphenol	24.40	260.5
2,4-di-*tert*-butylphenol	24.20	258.3
bisphenol A	26.40	281.8
butylhydroxytoluene	27.50	293.6
alcohols		
2-ethylhexanol	25.10	267.9
benzyl alcohol	25.90	276.5
dodecanol	23.80	254.1
octadecanol	27.50	293.6
carboxamides		
decanamide	23.20	247.7
dodecanamide	23.40	249.8
tetradecanamide	23.90	255.1
hexadecanamide	23.50	250.9
stearamide	23.80	254.1
oleamide	24.00	256.2
erucamide	27.00	288.2

Tab. S 3-1: Continued

Substance	Weight / g	Concentration / mg/kg
phthalates		
diethylphthalate	22.90	244.5
di-butylphthalate	23.00	245.5
dicyclohexylphthalate	26.10	278.6
misc		
undecane	23.80	254.1
toluene	25.30	270.1
ethylbenzene	25.20	269.0
styrene	23.80	254.1
divinylbenzene	25.00	266.9
benzaldehyde	25.10	267.9
1,4-diacetylbenzene	24.60	262.6
1,3-diacetylbenzene	25.10	267.9
1,2-dicyanobenzene	25.60	273.3
chlorobenzene	26.30	280.8
ketones		
methyl-*iso*-butylketone	23.70	253.0
4-methyl-2-heptanone	26.50	282.9
cyclohexanone	24.80	264.7
5,5-dimethyl-2,4-hexandione	26.80	286.1

Tab. S 3-2 Exemplary preparation of the used stock solution for the carboxylic acids "carboxylic acids standard (CS)" by weighing and solving the acids in 43.9379 g of ethanol

Substance	Weight / mg	Concentration / mg/kg
2-ethyl hexanoic acid	23.9	541.1
heptanoic acid	24.5	554.7
octanoic acid	23.9	542.0
decanoic acid	23.6	533.8
dodecanoic acid	24.2	547.9
tetradecanoic acid	24.3	550.6
hexadecanoic acid	22.7	514.6
octadecanoic acid	24.1	546.3
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	23.3	527.0

Tab. S 3-3 Exemplary preparation of the 20 µg/kg mixed standard "standard I" and the 1 µg/kg mixed standard "standard II" including the SC and CS.

SC weight / mg	SC average concentration / mg/kg	CS weight / mg	CS average concentration / mg/kg	solvent	solvent weight / g	SC final concentration / µg/kg	CS final concentration / µg/kg	name
49.2	0.2672	27.3	0.5398	water	701.52	18.74	21.00	standard I
1000.00	0.2672			ethanol	9.00	0.0267		SC II
		1000.00	0.5398	ethanol	19.00		0.02699	CS II
22.1	0.0267 (SC II)	20.8	0.0270 (CS II)	water	594.96	1.02	0.94	standard II

Tab. S 3-4 Exemplary preparation of the diluted standards with concentrations from 15 µg/kg to 1µg/kg

standard I weight / g	water weight / g	SC final concentration / µg/kg	CS final concentration / µg/kg
150.00	50.00	14.1	15.8
100.00	100.00	9.4	10.5
50.00	150.00	4.7	5.3
10.00	191.00	0.9	1.0

Tab. S 3-5 Exemplary preparation of the diluted standards with concentrations from 100 ng/kg to 1ng/kg

standard II weight / g	water weight / g	SC final concentration / µg/kg	CS final concentration / µg/kg
20.00	180.00	0.1018	0.0941
10.00	190.00	0.0509	0.0471
2.00	198.00	0.0102	0.0094
1.00	199.00	0.0051	0.0047
0.20	200.50	0.0010	0.0009

3.6.2 Analytes

Tab. S 3-6 Summarized overview of the components used within this study, their CAS number, molecular weight, retention time, the target ion used for quantification and their log $K_{O/W}$ values.

Susbstances	CAS Nummber	Molecular weight / g/mol	Retention time / min	m/z target ion	log $K_{O/W}$
phenols					
phenol	108-95-2	94.11	8.21	94	1.46[a]
2'-hydroxyacetophenone	118-93-4	136.15	11.56	136	1.92 a
2-*tert*-butylphenol	88-18-6	150.22	12.60	135	3.31 a
4-*tert*-butylphenol	98-54-4	150.22	13.52	150	3.31 a
4-*tert*-amylphenol	80-46-6	164.24	16.23	164	3.91*
2,6-di-*tert*-butylphenol	128-39-2	206.32	16.10	191	4.92 a
2,4-di-*tert*-butylphenol	96-76-4	206.32	18.63	191	5.19 b
bisphenol A	80-05-7	228.29	36.53	213	3.32 a
butylhydroxytoluene	128-37-0	220.35	18.26	220	5.03*
alcohols					
2-ethylhexanol	104-76-7	130.23	7.64	83	2.73*
benzyl alcohol	100-51-6	108.14	9.41	108	1.10 a
dodecanol	112-53-8	186.34	15.36	83	5.13 a
octadecanol	112-92-5	270.49	31.31	83	7.19*
carboxylic acids					
2-ethyl hexanoic acid [#]	149-57-5	144.21	9.13	88	-2.90*
heptanoic acid [#]	111-14-8	130.18	8.59	60	-0.70*
octanoic acid [#]	124-07-2	144.21	10.20	101	-0.21*
decanoic acid [#]	334-48-5	172.26	14.08	129	0.77*
dodecanoic acid [#]	143-07-7	200.32	19.61	129	1.75*
tetradecanoic acid [#]	544-63-8	228.38	28.73	129	2.73*
hexadecanoic acid [#]	57-10-3	256.43	30.69	129	3.72*
octadecanoic acid [#]	57-11-4	284.48	32.07	129	4.70*
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid [#]	20170-32-5	278.39	32.11	263	0.96*

Tab. S 3-6: Continued

Susbstances	CAS Nummber	Molecular weight / g/mol	Retention time / min	m/z target ion	log $K_{O/W}$
carboxamides					
decanamide	2319-29-1	171.28	26.25	59	2.77*
dodecanamide	1120-16-7	199.33	30.16	72	3.75*
tetradecanamide	638-58-4	227.39	31.69	59	4.73*
hexadecanamide	629-54-9	255.44	33.50	59	5.71*
stearamide	124-26-5	283.49	36.03	59	6.70*
oleamide	301-02-0	281.48	36.09	59	6.48*
erucamide	112-84-5	337.58	38.82	59	8.44*
phthalates					
diethylphthalate	84-66-2	222.24	28.80	177	2.42 c
di-butylphthalate	84-74-2	278.34	31.99	150	4.50 c
dicyclohexylphthalate	84-61-7	330.42	38.38	167	5.60*
misc					
undecane	1120-21-4	156.31	7.16	43	5.74*
toluene	108-88-3	92.14	3.98	91	2.73 a
ethylbenzene	100-41-4	106.17	5.37	91	3.15 a
styrene	100-42-5	104.15	6.18	104	2.95 a
divinylbenzene	91-14-5	130.19	9.85	130	3.80*
benzaldehyde	100-52-7	106.13	8.31	106	1.48 a
1,4-diacetylbenzene	1009-61-6	162.19	23.41	162	1.34 a
1,3-diacetylbenzene	6781-42-6	162.19	22.39	162	1.43 a
1,2-dicyanobenzene	91-15-6	128.13	19.07	128	0.99 d
chlorobenzene	108-90-7	112.56	5.51	112	2.84 e
ketones					
methyl-*iso*-butylketone	108-10-1	100.16	3.63	100	1.31f
4-methyl-2-heptanone	6137-06-0	128.21	6.42	85	2.15*
cyclohexanone	108-94-1	98.15	6.98	98	0.81 a
5,5-dimethyl-2,4-hexandione	7307-04-2	142.20	7.08	85	1.67 a

Symbols and letters (#, *, a, b, c, d, e, f) are the same used within the publication and explained there.

3.6.3 Chromatograms

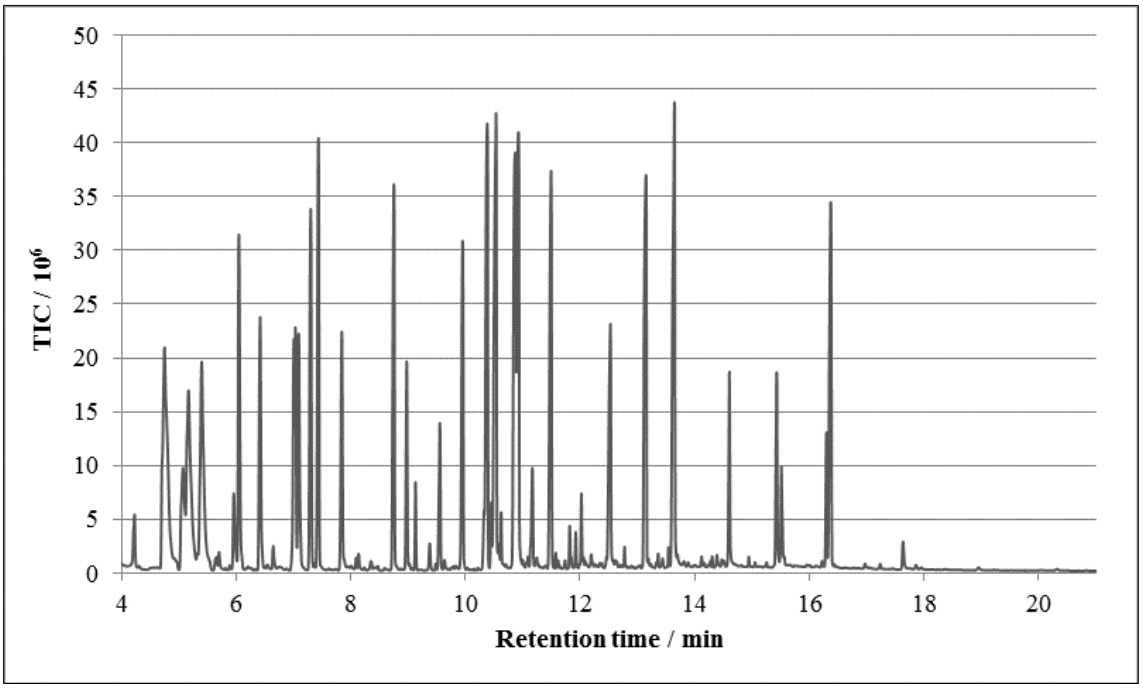

Fig. S 3-1 Total ion current chromatogram in scan mode for the complete standard solution with the carboxylic acids using a ZB5 column.
Although this column had the same length than the ZB50 column analytes were eluted earlier due to a different temperature program.

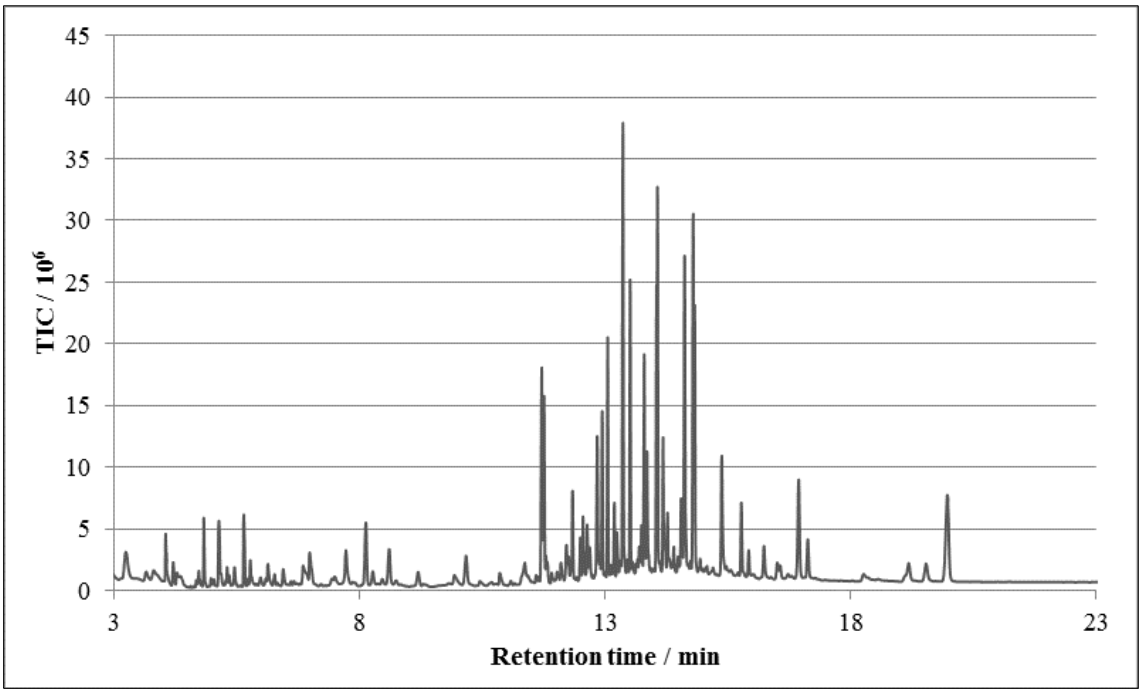

Fig. S 3-2 Total ion current chromatogram in scan mode for the for the complete standard solution with the carboxylic acids using a HP Innowax column

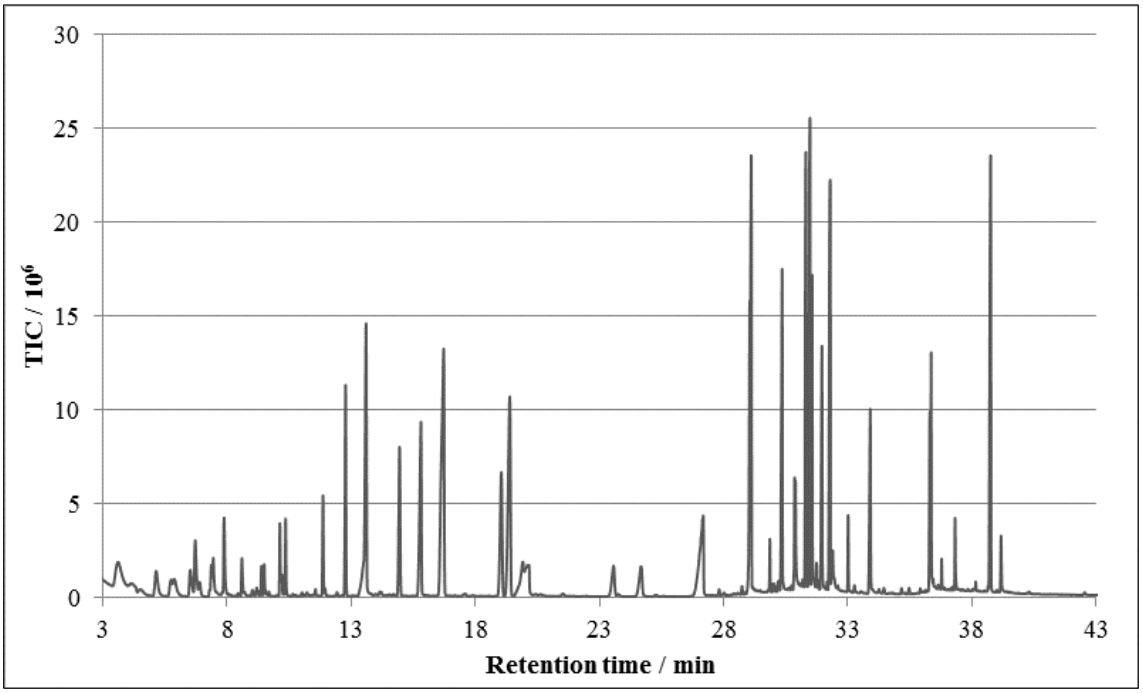

Fig. S 3-3 Total ion current chromatogram in scan mode for the complete standard solution with the carboxylic acids using a ZB50 column

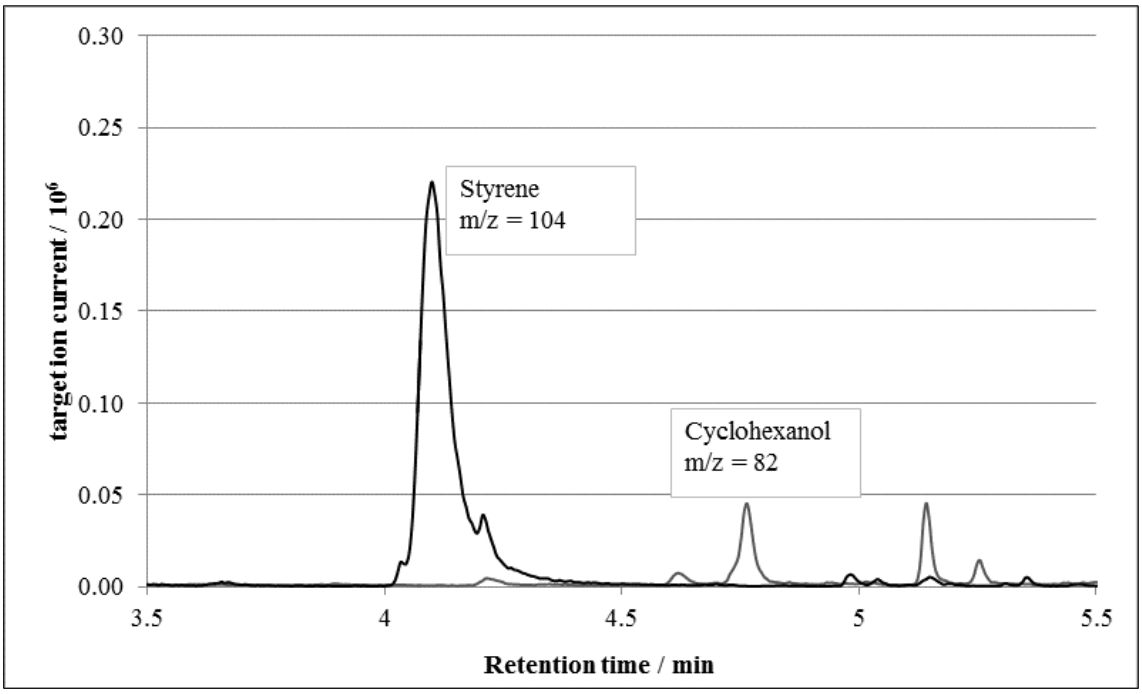

Fig. S 3-4 Target ion current chromatogram for styrene (m/z = 104) and cyclohexanol (m/z = 82) using a HP Innowax

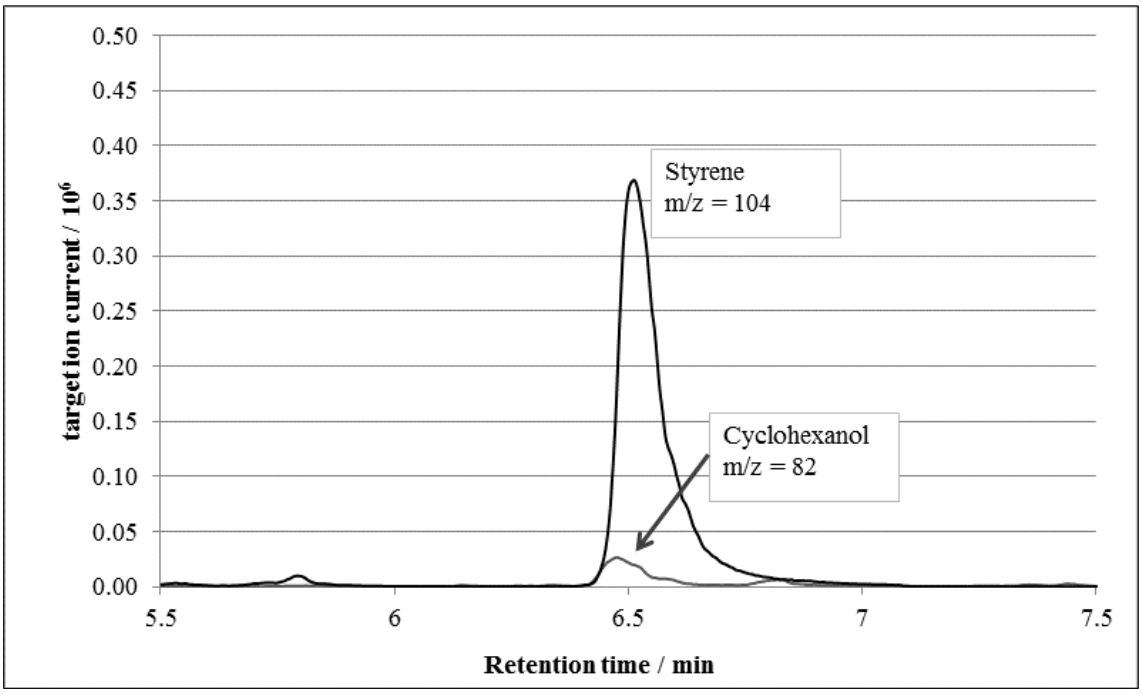

Fig. S 3-5 Target ion current chromatogram for styrene (m/z = 104) and cyclohexanol (m/z = 82) using a ZB 50 column

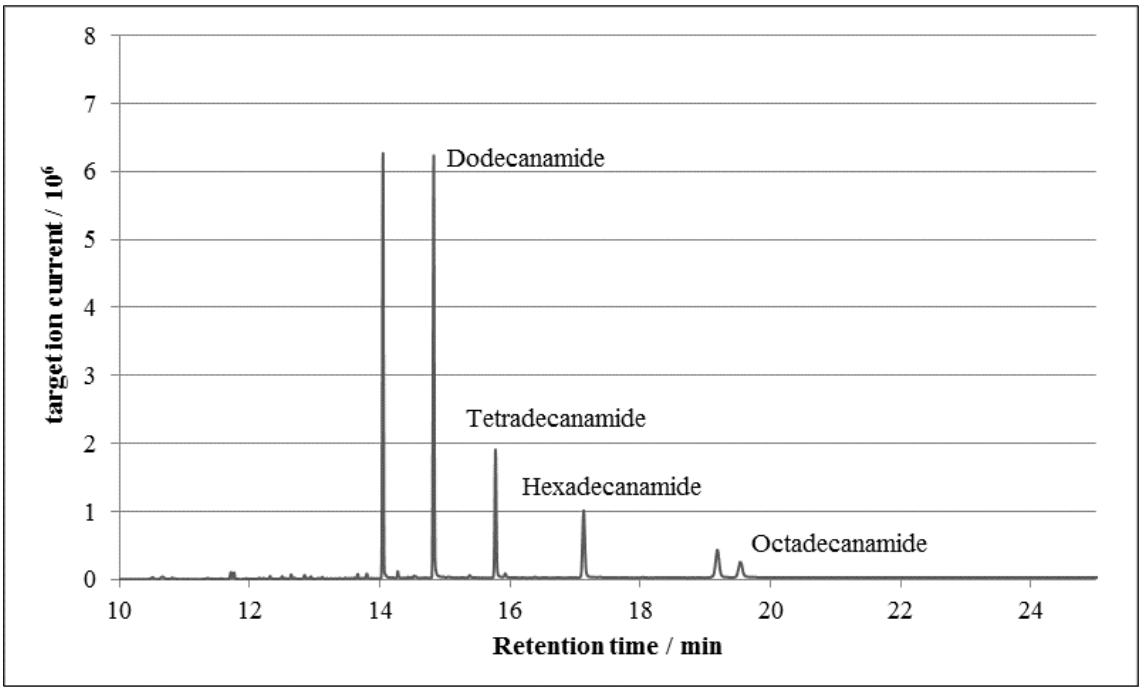

Fig. S 3-6 Target ion chromatogram for the amides (m/z = 59) using a HP Innowax

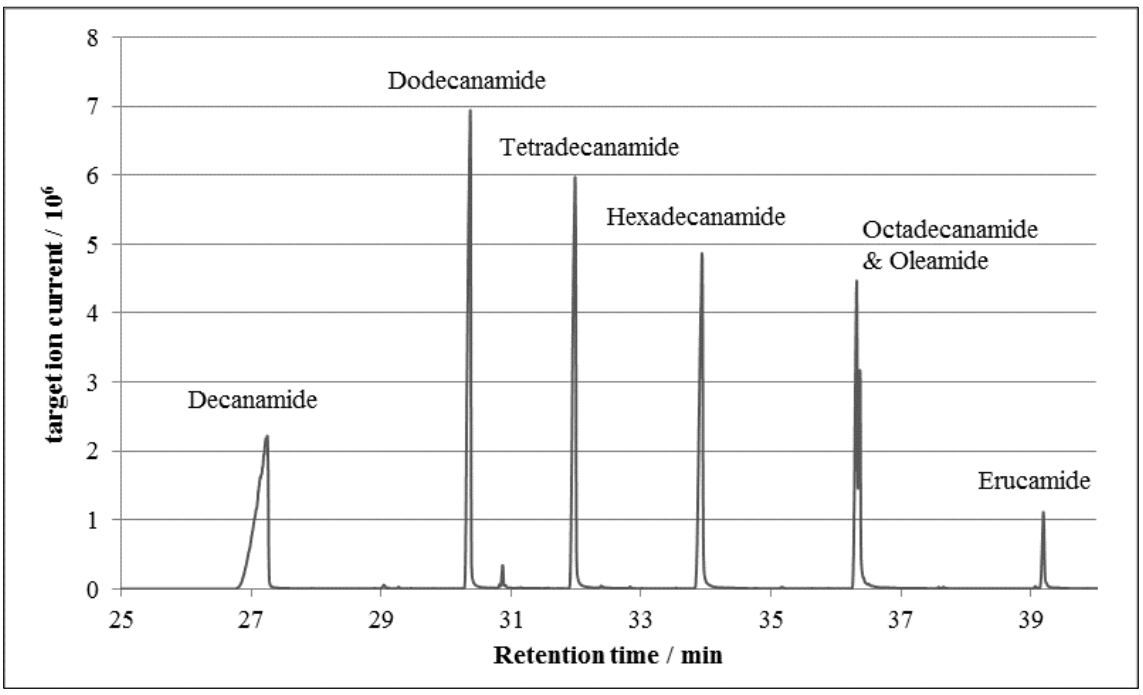

Fig. S 3-7 Target ion chromatogram for the amides (m/z = 59) using a ZB50 column

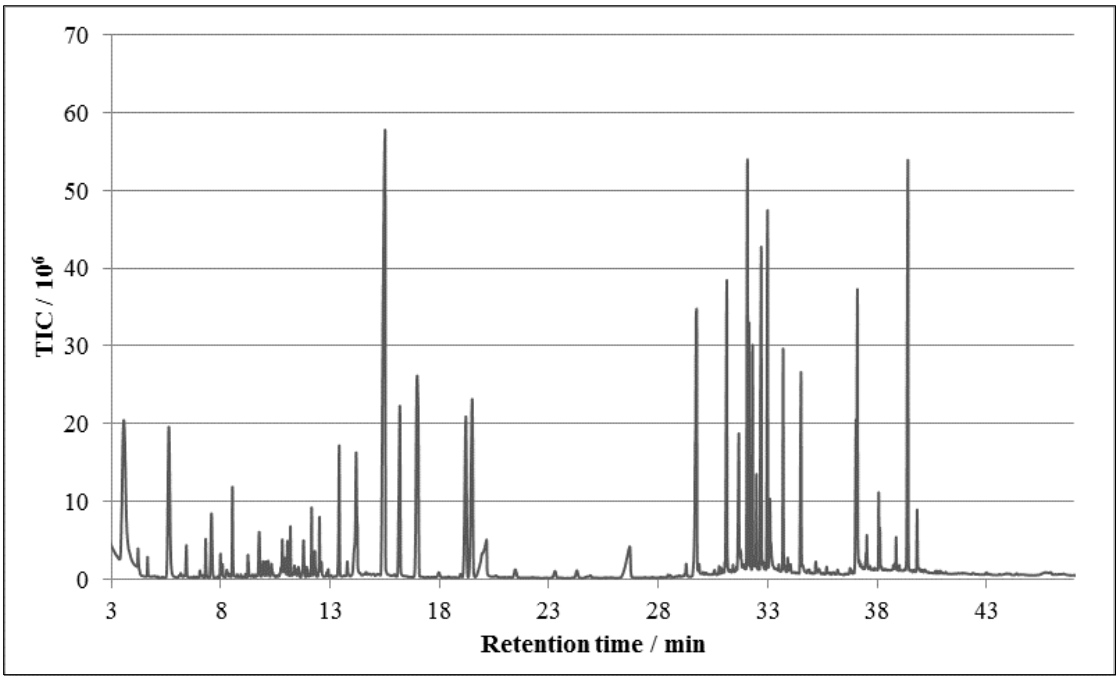

Fig. S 3-8 Total ion current chromatogram in scan mode for the complete standard solution with the carboxylic acids using a ZB50 column.
The start temperature was 40°C for the column oven. Low signal intensities were observed for early eluted substances up to a retention time of 10 min.

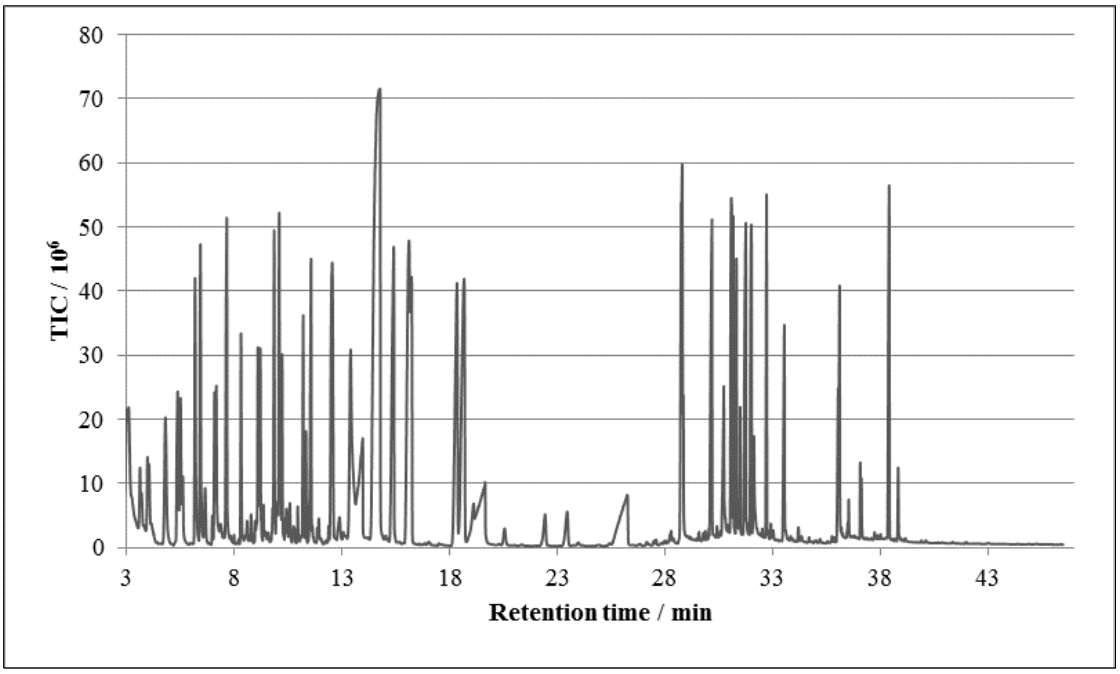

Fig. S 3-9 Total ion current chromatogram in scan mode for the complete standard solution with the carboxylic acids using a ZB50 column.
The starting temperature was 50°C for the column oven.

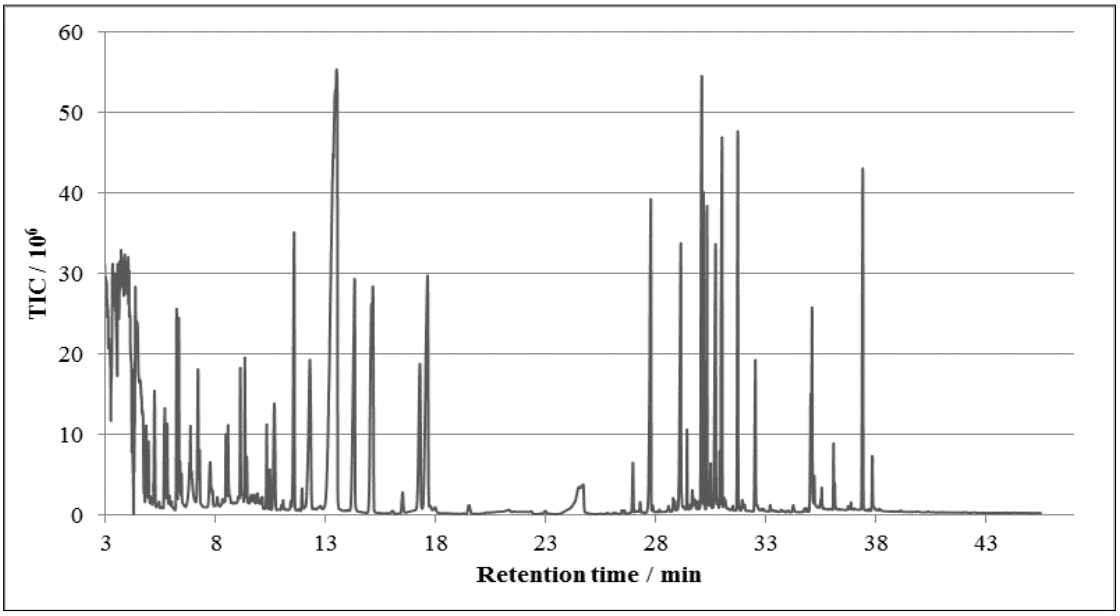

Fig. S 3-10 Total ion current chromatogram in scan mode for the complete standard solution with the carboxylic acids using a ZB50 column.
The start temperature was 60°C for the column oven. High signal intensities with an inferior signal resolution compared to 50°C is observed for the early eluted substances up to a retention time of 10 min.

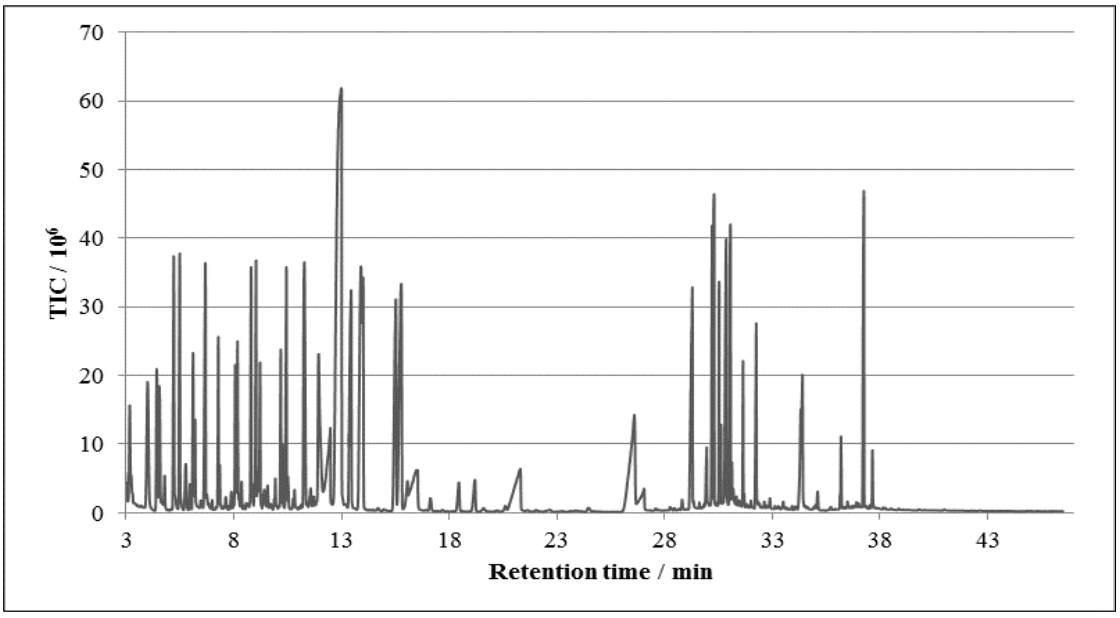

Fig. S 3-11 Total ion current chromatogram in scan mode for the complete standard solution with the carboxylic acids using a ZB50 column.
The start temperature was 60°C for the column oven and a carrier gas flow of 2 mL/min instead of 1 mL/min. The expected faster elution of all components could not be achieved, instead resolution got worse.

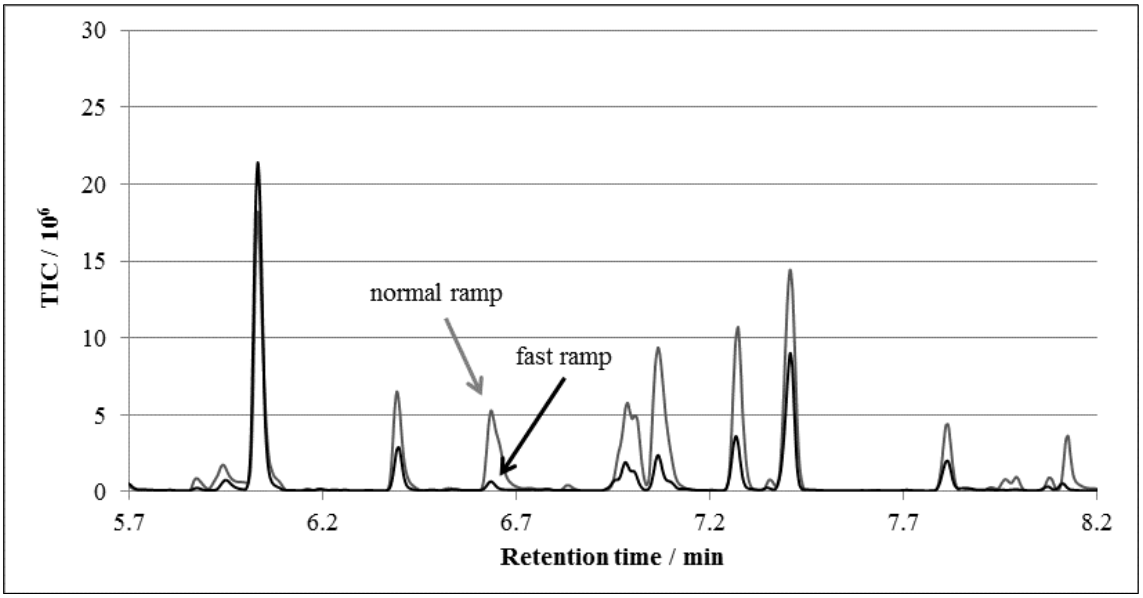

Fig. S 3-12 Total ion current chromatogram in scan mode for the complete standard solution with the carboxylic acids using the 'fast ramp' (16°C/s) of the CIS versus the 'normal ramp' (12°C/s)

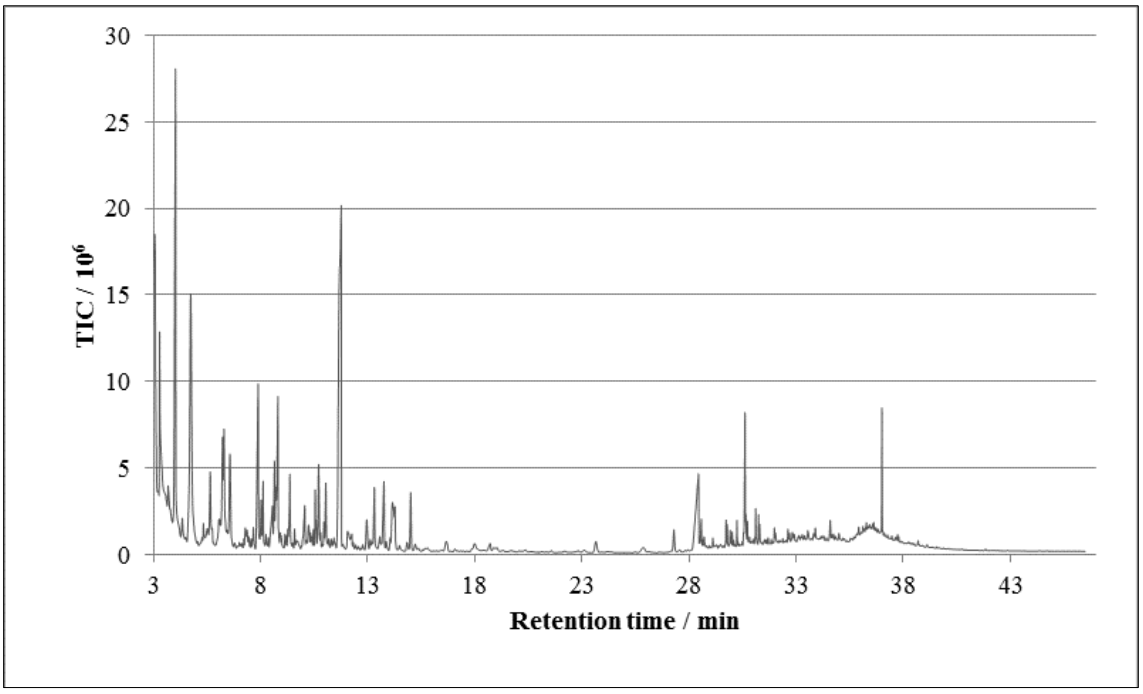

Fig. S 3-13 Total ion current chromatogram for a conditioned twister soaked in a 1:1 mixture of methanol and dichloromethane for 4 hours and dried afterwards for 4 hours

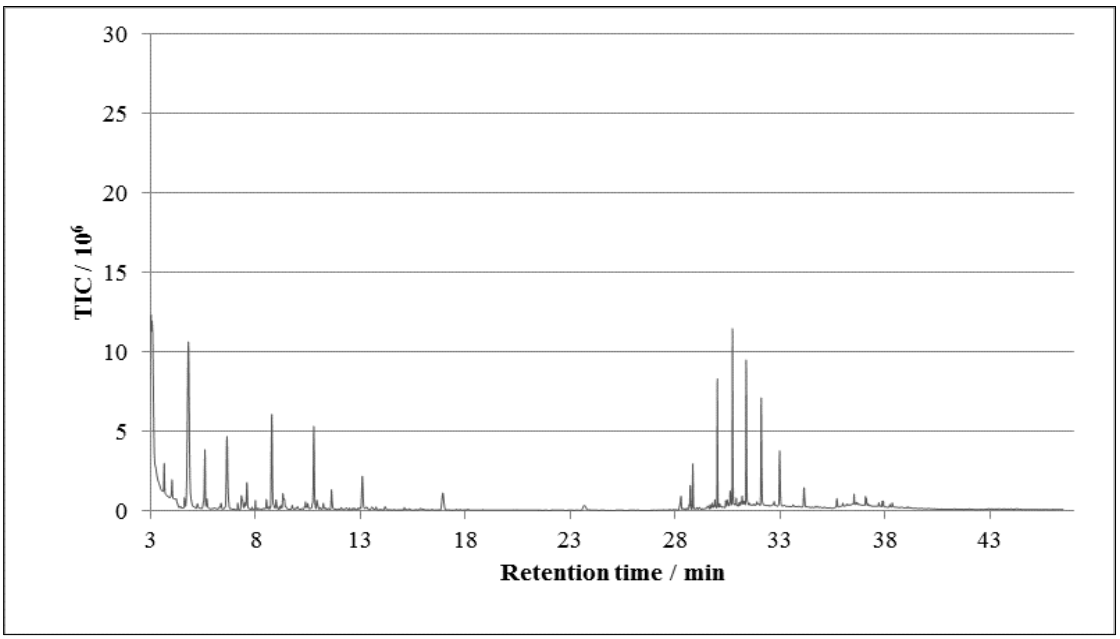

Fig. S 3-14 Total ion current chromatogram for a conditioned twister soaked in a 1:1 mixture of methanol and dichloromethane, sonicated for 15 min four times while exchanging the solution every time and dried afterwards for 4 hours

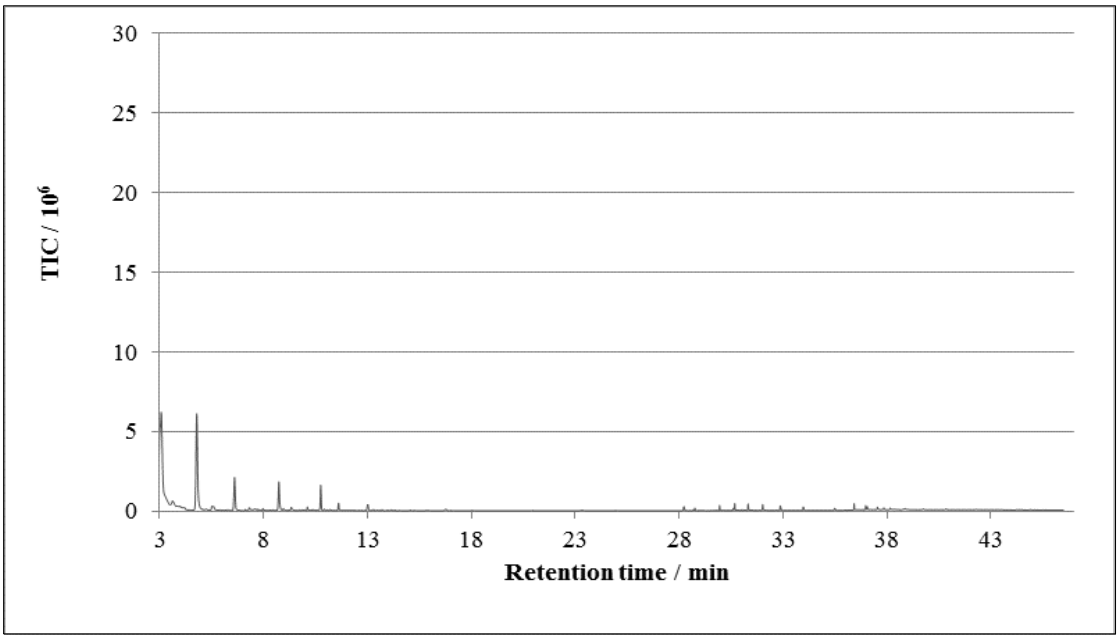

Fig. S 3-15 Total ion current chromatogram for a conditioned twister soaked in a 1:1 mixture of methanol and dichloromethane for 4 hours with an exchange of the solution after 2 hours and dried afterwards for two times 4 hours

3.6.4 Stir bar conditioning

Tab. S 3-7 Summarized target ion peak areas for the studied stir bar conditioning procedures.
4 hours in the washing solution with a subsequent 4 hour drying time (="4h"), 4 times 15 min sonication with a subsequent 4 hour drying time ("4x15 sonic") and 2 hour washing for two times with subsequent 2 times a 4 hour drying("2h+2h"). The values given are average values for N = 9.

Substance	4h	4x15 sonic	2h+2h
phenols			
phenol	2.6E+06	1.0E+06	3.8E+05
2'-hydroxyacetophenone	5.7E+04	1.0E+05	n.d.
2-*tert*-butylphenol	n.d.	n.d.	n.d.
4-*tert*-butylphenol	2.5E+04	n.d.	n.d.
4-*tert*-amylphenol	n.d.	n.d.	n.d.
2,6-di-*tert*-butylphenol	n.d.	n.d.	n.d.
2,4-di-*tert*-butylphenol	3.2E+05	2.6E+05	1.5E+05
bisphenol A	2.9E+05	8.0E+04	8.2E+04
butylhydroxytoluene	5.3E+04	1.4E+05	n.d.
alcohols			
2-ethylhexanol	7.1E+04	2.8E+05	4.3E+04
benzyl alcohol	n.d.	3.2E+05	n.d.
dodecanol	1.4E+06	2.9E+05	n.d.
octadecanol	1.0E+06	7.7E+05	n.d.
carboxylic acids			
2-ethyl hexanoic acid	n.d.	n.d.	n.d.
heptanoic acid	n.d.	n.d.	n.d.
octanoic acid	n.d.	n.d.	n.d.
decanoic acid	2.5E+05	8.5E+04	n.d.
dodecanoic acid	1.5E+06	n.d.	n.d.
tetradecanoic acid	5.4E+05	5.3E+04	n.d.
hexadecanoic acid	2.0E+06	2.3E+05	1.7E+05
octadecanoic acid	1.9E+05	1.6E+04	1.1E+04
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	n.d.	2.4E+04	n.d.

Tab. S 3-7: Continued

Substance	4h	4x15 sonic	2h+2h
carboxamides			
decanamide	n.d.	2.0E+04	n.d.
dodecanamide	9.1E+04	1.9E+04	n.d.
tetradecanamide	2.0E+05	7.1E+04	6.4E+03
hexadecanamide	5.5E+05	9.8E+04	5.6E+03
stearamide	2.6E+05	4.6E+04	3.5E+03
oleamide	1.3E+05	7.2E+04	n.d.
erucamide	9.0E+04	1.1E+04	n.d.
phthalates			
diethylphthalate	2.4E+06	2.0E+06	1.1E+06
di-butylphthalate	2.7E+04	1.1E+05	n.d.
dicyclohexylphthalate	n.d.	n.d.	n.d.
misc			
undecane	n.d.	n.d.	n.d.
toluene	7.0E+05	n.d.	n.d.
ethylbenzene	2.0E+04	n.d.	n.d.
styrene	7.7E+05	3.1E+05	1.9E+05
divinylbenzene	0.0E+00	3.7E+04	n.d.
benzaldehyde	3.8E+05	3.4E+05	1.5E+05
1,4-diacetylbenzene	n.d.	n.d.	n.d.
1,3-diacetylbenzene	n.d.	n.d.	n.d.
1,2-dicyanobenzene	n.d.	n.d.	n.d.
chlorobenzene	n.d.	7.1E+04	n.d.
ketones			
methyl-iso-butylketone	n.d.	n.d.	n.d.
4-methyl-2-heptanone	n.d.	n.d.	n.d.
cyclohexanone	6.7E+05	2.5E+05	n.d.
5,5-dimethyl-2,4-hexandione	1.1E+05	9.4E+04	n.d.

n.d. = not determinable

3.6.5 SBSE Optimization

Tab. S 3-8 Summarized target ion peak areas for the variation of the stirring time from 30 min to 120 min.
The values given are average values for N = 3.

Substance	30 min	60 min	120 min
phenols			
phenol	6.4E+05	7.3E+05	5.1E+05
2'-hydroxyacetophenone	2.4E+07	3.0E+07	3.9E+07
2-*tert*-butylphenol	8.5E+07	1.1E+08	1.4E+08
4-*tert*-butylphenol	6.4E+06	8.5E+06	1.2E+07
4-*tert*-amylphenol	n.d.	2.2E+07	2.9E+07
2,6-di-*tert*-butylphenol	3.7E+08	4.7E+08	5.3E+08
2,4-di-*tert*-butylphenol	4.7E+08	6.2E+08	7.0E+08
bisphenol A	1.9E+05	2.5E+05	2.9E+05
butylhydroxytoluene	7.7E+07	1.0E+08	1.2E+08
alcohols			
2-ethylhexanol	7.8E+06	1.1E+07	1.5E+07
benzyl alcohol	n.d.	n.d.	5.9E+05
dodecanol	5.5E+07	7.7E+07	9.1E+07
octadecanol	8.8E+06	1.0E+07	1.6E+07
carboxylic acids			
2-ethyl hexanoic acid	n.d.	n.d.	n.d.
heptanoic acid	6.0E+05	5.9E+05	2.8E+05
octanoic acid	2.9E+05	9.7E+05	2.9E+05
decanoic acid	n.d.	3.4E+06	5.1E+06
dodecanoic acid	7.3E+06	1.0E+07	1.3E+07
tetradecanoic acid	1.7E+07	2.1E+07	2.3E+07
hexadecanoic acid	5.0E+06	6.6E+06	9.2E+06
octadecanoic acid	1.0E+06	8.6E+05	1.4E+06
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	n.d.	n.d.	n.d.

Tab. S 3-8: Continued

Substance	30 min	60 min	120 min
carboxamides			
decanamide	1.4E+07	1.8E+07	2.8E+07
dodecanamide	3.4E+07	4.8E+07	7.5E+07
tetradecanamide	1.2E+08	1.9E+08	2.8E+08
hexadecanamide	5.6E+07	7.9E+07	1.3E+08
stearamide	2.9E+07	3.4E+07	5.0E+07
oleamide	4.7E+07	6.2E+07	1.0E+08
erucamide	5.1E+06	6.4E+06	7.3E+06
phthalates			
diethylphthalate	2.5E+07	3.3E+07	4.8E+07
di-butylphthalate	5.3E+07	6.9E+07	7.5E+07
dicyclohexylphthalate	1.7E+06	2.1E+06	3.7E+06
misc			
undecane	3.0E+07	3.6E+07	4.1E+07
toluene	5.9E+07	4.9E+07	4.8E+07
ethylbenzene	1.6E+08	1.9E+08	2.1E+08
styrene	1.3E+08	1.5E+08	1.5E+08
divinylbenzene	8.8E+07	1.1E+08	1.2E+08
benzaldehyde	1.8E+07	2.2E+07	2.9E+07
1,4-diacetylbenzene	1.4E+06	2.1E+06	2.7E+06
1,3-diacetylbenzene	2.0E+06	2.2E+06	3.6E+06
1,2-dicyanobenzene	5.1E+06	6.7E+06	9.0E+06
chlorobenzene	8.6E+07	9.6E+07	1.0E+08
ketones			
methyl-*iso*-butylketone	n.d.	8.4E+05	1.2E+06
4-methyl-2-heptanone	2.4E+07	3.1E+07	4.1E+07
cyclohexanone	n.d.	1.2E+06	1.6E+06
5,5-dimethyl-2,4-hexandione	3.0E+07	4.4E+07	6.4E+07

n.d. = not determinable

Tab. S 3-9 Summarized target ion peak areas for the variation of the stirring time from 240 min to 1440 min.
The values given are average values for N = 3.

Substance	240 min	360 min	960 min	1440 min
phenols				
phenol	6.5E+05	6.1E+05	5.5E+05	5.9E+05
2'-hydroxyacetophenone	3.5E+07	3.3E+07	3.4E+07	3.2E+07
2-tert-butylphenol	1.3E+08	1.3E+08	1.3E+08	1.2E+08
4-tert-butylphenol	1.2E+07	1.2E+07	1.2E+07	1.1E+07
4-tert-amylphenol	3.0E+07	2.8E+07	2.9E+07	2.8E+07
2,6-di-tert-butylphenol	5.2E+08	5.0E+08	5.0E+08	5.0E+08
2,4-di-tert-butylphenol	6.9E+08	6.8E+08	6.5E+08	6.6E+08
bisphenol A	3.7E+05	4.5E+05	4.9E+05	5.9E+05
butylhydroxytoluene	1.2E+08	1.1E+08	1.1E+08	1.1E+08
alcohols				
2-ethylhexanol	1.7E+07	1.5E+07	1.6E+07	1.6E+07
benzyl alcohol	6.3E+05	6.4E+05	6.3E+05	5.5E+05
dodecanol	8.7E+07	8.4E+07	6.3E+07	5.6E+07
octadecanol	1.3E+07	1.5E+07	2.8E+07	3.8E+07
carboxylic acids				
2-ethyl hexanoic acid	n.d.	n.d.	n.d.	1.2E+06
heptanoic acid	3.5E+05	3.5E+05	1.6E+05	n.d.
octanoic acid	3.4E+05	3.4E+05	1.0E+05	n.d.
decanoic acid	6.7E+06	6.3E+06	n.d.	n.d.
dodecanoic acid	1.6E+07	1.2E+07	3.0E+06	1.6E+06
tetradecanoic acid	2.3E+07	2.1E+07	9.9E+06	9.2E+06
hexadecanoic acid	9.6E+06	9.2E+06	5.4E+06	5.8E+06
octadecanoic acid	3.1E+06	3.4E+06	2.5E+06	3.0E+06
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	n.d.	n.d.	n.d.	2.4E+06

Tab. S 3-9: Continued

Substance	240 min	360 min	960 min	1440 min
carboxamides				
decanamide	4.2E+07	4.8E+07	7.4E+07	7.4E+07
dodecanamide	9.8E+07	1.0E+08	1.1E+08	1.1E+08
tetradecanamide	3.1E+08	3.1E+08	2.8E+08	2.9E+08
hexadecanamide	1.7E+08	1.7E+08	1.7E+08	1.9E+08
stearamide	6.2E+07	6.5E+07	9.4E+07	1.3E+08
oleamide	9.5E+07	8.6E+07	7.4E+07	7.3E+07
erucamide	9.3E+06	9.8E+06	1.2E+07	1.8E+07
phthalates				
diethylphthalate	5.9E+07	5.9E+07	6.5E+07	6.3E+07
di-butylphthalate	7.5E+07	7.3E+07	7.0E+07	7.2E+07
dicyclohexylphthalate	3.3E+06	3.7E+06	2.7E+06	3.5E+06
misc				
undecane	4.3E+07	3.9E+07	4.0E+07	4.3E+07
toluene	7.2E+07	6.6E+07	4.3E+07	4.2E+07
ethylbenzene	2.1E+08	1.9E+08	1.8E+08	1.8E+08
styrene	1.5E+08	1.4E+08	1.4E+08	1.4E+08
divinylbenzene	1.2E+08	1.1E+08	1.1E+08	1.1E+08
benzaldehyde	2.1E+07	1.6E+07	1.7E+06	7.0E+05
1,4-diacetylbenzene	3.3E+06	3.4E+06	4.3E+06	4.1E+06
1,3-diacetylbenzene	4.5E+06	4.6E+06	5.8E+06	5.4E+06
1,2-dicyanobenzene	1.1E+07	1.1E+07	1.3E+07	1.3E+07
chlorobenzene	1.0E+08	9.0E+07	9.0E+07	8.9E+07
ketones				
methyl-*iso*-butylketone	1.1E+06	1.5E+06	1.1E+06	1.1E+06
4-methyl-2-heptanone	4.2E+07	3.9E+07	4.2E+07	4.1E+07
cyclohexanone	1.7E+06	1.6E+06	1.6E+06	1.6E+06
5,5-dimethyl-2,4-hexandione	7.1E+07	6.5E+07	7.2E+07	6.7E+07

n.d. = not determinable

Tab. S 3-10 Summarized target ion peak areas for the variation of the sample volume from 5 mL to 29 mL.
The values given are average values for N = 3.

Substance	5 mL	10 mL	20 mL	29 mL
phenols				
phenol	1.8E+06	1.7E+06	2.0E+06	1.2E+06
2'-hydroxyacetophenone	5.5E+06	9.9E+06	1.6E+07	6.5E+07
2-*tert*-butylphenol	1.8E+07	3.2E+07	9.6E+07	2.1E+08
4-*tert*-butylphenol	1.0E+07	1.5E+07	3.7E+07	2.3E+07
4-*tert*-amylphenol	9.0E+06	1.3E+07	3.4E+07	4.8E+07
2,6-di-*tert*-butylphenol	9.7E+06	1.5E+07	4.7E+07	7.3E+08
2,4-di-*tert*-butylphenol	8.2E+07	9.7E+07	3.1E+08	9.6E+08
bisphenol A	5.3E+06	2.1E+06	3.7E+06	6.8E+05
butylhydroxytoluene	3.3E+06	4.9E+06	2.0E+07	1.8E+08
alcohols				
2-ethylhexanol	8.8E+06	1.6E+07	3.2E+07	1.9E+07
benzyl alcohol	8.8E+05	9.6E+05	9.9E+05	9.0E+05
dodecanol	1.0E+07	9.0E+06	4.1E+07	1.3E+08
octadecanol	1.2E+07	7.4E+06	3.3E+07	4.3E+07
carboxylic acids				
2-ethyl hexanoic acid	3.6E+05	5.6E+05	6.7E+05	8.2E+05
heptanoic acid	7.8E+05	2.6E+05	3.2E+05	4.1E+05
octanoic acid	3.1E+05	4.2E+05	3.6E+05	4.1E+05
decanoic acid	5.2E+06	3.3E+06	7.1E+06	1.1E+07
dodecanoic acid	1.0E+06	2.5E+06	3.8E+06	3.1E+07
tetradecanoic acid	4.4E+06	6.4E+05	4.9E+06	3.5E+07
hexadecanoic acid	5.7E+06	6.7E+06	1.9E+06	2.5E+07
octadecanoic acid	4.5E+05	8.7E+05	1.6E+06	3.5E+06
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	4.2E+06	2.4E+05	1.1E+06	1.9E+06

Tab. S 3-10: Continued

Substance	5 mL	10 mL	20 mL	29 mL
carboxamides				
decanamide	7.2E+07	6.5E+07	1.6E+08	7.0E+07
dodecanamide	2.5E+07	1.0E+07	5.2E+07	1.5E+08
tetradecanamide	5.3E+07	1.7E+07	9.2E+07	4.4E+08
hexadecanamide	3.9E+07	1.0E+07	6.8E+07	3.1E+08
stearamide	1.8E+07	4.5E+06	3.3E+07	1.3E+08
oleamide	1.4E+07	3.3E+06	2.3E+07	1.5E+08
erucamide	3.6E+06	9.2E+05	7.4E+06	2.2E+07
phthalates				
diethylphthalate	3.0E+07	3.2E+07	7.7E+07	9.8E+07
di-butylphthalate	1.6E+07	6.9E+06	3.4E+07	1.2E+08
dicyclohexylphthalate	6.9E+07	2.8E+07	1.4E+08	2.4E+08
misc				
undecane	5.0E+06	8.0E+06	1.1E+07	3.1E+07
toluene	5.6E+06	5.5E+06	1.1E+07	3.2E+07
ethylbenzene	6.4E+06	8.3E+06	1.8E+07	1.7E+08
styrene	5.5E+06	7.1E+06	1.5E+07	1.4E+08
divinylbenzene	3.3E+06	5.2E+06	1.1E+07	1.5E+08
benzaldehyde	4.6E+06	6.7E+06	8.8E+06	2.7E+07
1,4-diacetylbenzene	5.8E+06	6.2E+06	9.4E+06	8.7E+06
1,3-diacetylbenzene	5.0E+06	5.3E+06	7.9E+06	6.1E+06
1,2-dicyanobenzene	1.6E+07	1.6E+07	1.9E+07	2.4E+07
chlorobenzene	4.6E+06	5.5E+06	1.2E+07	9.4E+07
ketones				
methyl-iso-butylketone	8.7E+05	1.2E+06	1.8E+06	2.4E+06
4-methyl-2-heptanone	2.5E+06	4.3E+06	8.8E+06	4.5E+07
cyclohexanone	1.0E+06	1.2E+06	1.2E+06	2.2E+06
5,5-dimethyl-2,4-hexandione	1.3E+07	2.2E+07	4.2E+07	6.9E+07

n.d. = not determinable

Tab. S 3-11 Summarized target ion peak areas for different stir bar dimensions. 10 mm length with a 1 mm PDMS coating (=”10x1”), 20 mm length with a 0.5 mmm thick PDMS coating (“20x0.5”) and a 20 mm long stir bar with a 1 mm PDMS coating (“20x1”). These measurements were performed with a reduced set of analytes. The values given are average values for N = 3.

Substance	1 x 10	0.5 x 20	1 x 20
phenols			
2'-hydroxyacetophenone	6.3E+06	7.8E+06	9.3E+06
2-*tert*-butylphenol	1.7E+07	1.7E+07	1.9E+07
4-*tert*-butylphenol	1.8E+06	2.4E+06	3.0E+06
4-*tert*-amylphenol	2.4E+06	3.1E+06	3.2E+06
2,6-di-*tert*-butylphenol	3.8E+07	4.4E+07	3.2E+07
2,4-di-*tert*-butylphenol	7.6E+07	8.4E+07	6.3E+07
bisphenol A	9.9E+06	1.2E+07	1.1E+07
alcohols			
2-ethylhexanol	1.0E+07	1.6E+07	1.4E+07
benzyl alcohol	3.1E+05	4.5E+05	5.6E+05
dodecanol	9.6E+06	1.3E+07	1.1E+07
carboxamides			
decanamide	5.1E+06	1.0E+07	8.9E+06
dodecanamide	7.8E+06	1.0E+07	8.9E+06
hexadecanamide	3.3E+06	6.4E+06	4.9E+06
stearamide	7.9E+05	1.9E+06	1.0E+06
oleamide	1.8E+05	4.7E+05	3.3E+05
phthalates			
diethylphthalate	9.5E+06	3.6E+07	2.7E+07
di-butylphthalate	9.5E+07	7.7E+07	4.5E+07
dicyclohexylphthalate	1.0E+07	1.1E+07	7.7E+06
misc			
undecane	1.7E+06	5.1E+06	4.1E+06
ethylbenzene	6.3E+06	6.2E+06	8.2E+06
styrene	1.6E+07	1.6E+07	1.6E+07
divinylbenzene	1.3E+07	1.6E+07	1.6E+07
benzaldehyde	3.8E+06	4.7E+06	6.6E+06
1,4-diacetylbenzene	6.7E+05	1.3E+06	1.6E+06
1,3-diacetylbenzene	6.3E+05	1.2E+06	1.5E+06
1,2-dicyanobenzene	1.6E+06	2.6E+06	3.7E+06
chlorobenzene	1.1E+07	1.0E+07	1.5E+07

Tab. S 3-12 Summarized target ion peak areas for the effect of the addition of various amounts of salt (NaCl) from 3 g to 9 g and without salt ("w/o"). The values given are average values for N = 3.

Substance	w/o	3 g	6 g	8.5 g	9 g
phenols					
phenol	1.2E+06	1.9E+06	4.1E+06	4.5E+06	5.3E+06
2'-hydroxyacetophenone	6.5E+07	3.8E+07	6.5E+07	1.4E+08	1.4E+08
2-*tert*-butylphenol	2.1E+08	1.4E+08	2.2E+08	4.0E+08	4.1E+08
4-*tert*-butylphenol	2.3E+07	2.3E+07	5.4E+07	1.3E+08	1.2E+08
4-*tert*-amylphenol	4.8E+07	3.2E+07	4.8E+07	9.7E+07	1.0E+08
2,6-di-*tert*-butylphenol	7.3E+08	3.3E+08	3.5E+08	4.1E+08	4.6E+08
2,4-di-*tert*-butylphenol	9.6E+08	5.3E+08	5.6E+08	7.6E+08	8.4E+08
bisphenol A	6.8E+05	1.8E+06	6.5E+06	1.4E+07	2.0E+07
butylhydroxytoluene	1.8E+08	8.0E+07	7.8E+07	5.7E+07	6.8E+07
alcohols					
2-ethylhexanol	1.9E+07	1.9E+07	3.4E+07	5.6E+07	6.0E+07
benzyl alcohol	9.0E+05	1.1E+06	2.1E+06	3.4E+06	3.3E+06
dodecanol	1.3E+08	6.1E+07	7.1E+07	7.0E+07	6.8E+07
octadecanol	4.3E+07	1.9E+07	2.5E+07	2.4E+07	3.6E+07
carboxylic acids					
2-ethyl hexanoic acid	8.2E+05	2.3E+06	6.9E+06	3.5E+07	3.3E+07
heptanoic acid	4.1E+05	8.3E+05	3.0E+06	8.7E+06	7.9E+06
octanoic acid	4.1E+05	7.2E+05	3.0E+06	1.6E+07	1.7E+07
decanoic acid	1.1E+07	1.8E+07	4.5E+07	9.0E+07	8.5E+07
dodecanoic acid	3.1E+07	2.3E+07	2.9E+07	1.7E+07	3.9E+07
tetradecanoic acid	3.5E+07	1.4E+07	1.0E+07	6.2E+06	7.6E+06
hexadecanoic acid	2.5E+07	4.9E+06	3.6E+06	1.8E+06	2.9E+06
octadecanoic acid	3.5E+06	1.2E+06	9.5E+05	8.5E+05	9.1E+05
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	n.d.	1.7E+06	2.2E+07	2.5E+07	3.5E+07

Tab. S 3-12: Continued

Substance	w/o	3 g	6 g	8.5 g	9 g
carboxamides					
decanamide	7.0E+07	9.1E+07	1.9E+08	3.9E+08	4.5E+08
dodecanamide	1.5E+08	8.2E+07	9.6E+07	1.9E+08	2.0E+08
tetradecanamide	4.4E+08	2.0E+08	1.8E+08	1.3E+08	1.4E+08
hexadecanamide	3.1E+08	7.3E+07	6.8E+07	4.4E+07	5.7E+07
stearamide	1.3E+08	3.9E+07	4.7E+07	3.5E+07	4.5E+07
oleamide	1.5E+08	4.6E+07	3.5E+07	2.4E+07	3.0E+07
erucamide	2.2E+07	8.8E+06	1.1E+07	5.2E+06	7.5E+06
phthalates					
diethylphthalate	9.8E+07	6.3E+07	8.6E+07	1.9E+08	2.1E+08
di-butylphthalate	1.2E+08	4.2E+07	4.5E+07	9.5E+07	9.8E+07
dicyclohexylphthalate	2.4E+08	9.3E+07	9.3E+07	6.5E+07	7.0E+07
misc					
undecane	3.1E+07	1.4E+07	1.6E+07	3.7E+06	5.2E+06
toluene	3.2E+07	2.0E+07	8.1E+07	3.7E+07	4.1E+07
ethylbenzene	1.7E+08	1.1E+08	1.5E+08	1.7E+08	1.9E+08
styrene	1.4E+08	8.5E+07	1.2E+08	1.5E+08	1.7E+08
divinylbenzene	1.5E+08	6.2E+07	7.8E+07	1.2E+08	1.3E+08
benzaldehyde	2.7E+07	1.8E+07	3.3E+07	4.8E+07	4.9E+07
1,4-diacetylbenzene	8.7E+06	6.3E+06	9.9E+06	2.4E+07	2.5E+07
1,3-diacetylbenzene	6.1E+06	4.6E+06	7.7E+06	1.8E+07	1.9E+07
1,2-dicyanobenzene	2.4E+07	1.6E+07	2.4E+07	4.7E+07	5.2E+07
chlorobenzene	9.4E+07	5.8E+07	8.8E+07	1.2E+08	1.3E+08
ketones					
methyl-*iso*-butylketone	2.4E+06	9.6E+05	3.5E+06	4.5E+06	7.2E+06
4-methyl-2-heptanone	4.5E+07	3.2E+07	4.7E+07	6.1E+07	6.5E+07
cyclohexanone	2.2E+06	1.9E+06	3.8E+06	5.8E+06	6.4E+06
5,5-dimethyl-2,4-hexandione	6.9E+07	3.9E+07	1.7E+08	2.3E+08	2.3E+08

n.d. = not determinable

Tab. S 3-13 Summarized target ion peak areas for the effect of various stirring speeds from 200 rpm to 1100 rpm.
These measurements were performed on a HP Innowax column instead of the ZB 50 column. Thus four substances of the standard mixture were not determinable. The values given are average values for N = 3.

Substance	200 rpm	400 rpm	600 rpm	800 rpm	1100 rpm
phenols					
phenol	n.d.	n.d.	n.d.	n.d.	n.d.
2'-hydroxyacetophenone	3.1E+06	3.3E+06	3.4E+06	3.3E+06	3.5E+06
2-*tert*-butylphenol	5.1E+06	5.1E+06	5.5E+06	5.6E+06	5.6E+06
4-*tert*-butylphenol	1.7E+06	1.8E+06	2.1E+06	2.2E+06	4.0E+06
4-*tert*-amylphenol	1.8E+06	2.0E+06	2.3E+06	2.6E+06	3.0E+06
2,6-di-*tert*-butylphenol	2.0E+06	2.0E+06	2.1E+06	2.5E+06	5.7E+06
2,4-di-*tert*-butylphenol	6.7E+06	7.5E+06	8.7E+06	1.4E+07	3.3E+07
bisphenol A	n.d.	n.d.	n.d.	n.d.	n.d.
butylhydroxytoluene	3.8E+05	3.1E+05	3.7E+05	4.7E+05	1.3E+06
alcohols					
2-ethylhexanol	1.3E+06	1.3E+06	1.4E+06	1.4E+06	1.4E+06
benzyl alcohol	8.5E+05	5.3E+05	1.1E+06	9.5E+05	8.4E+05
dodecanol	3.2E+06	3.8E+06	3.9E+06	4.3E+06	4.2E+06
octadecanol	8.4E+06	6.3E+06	8.0E+06	9.7E+06	1.1E+07
carboxylic acids					
2-ethyl hexanoic acid	7.8E+05	6.6E+05	6.9E+05	8.7E+05	1.2E+06
heptanoic acid	3.0E+05	3.3E+05	3.2E+05	4.1E+05	2.4E+05
octanoic acid	3.1E+05	3.4E+05	3.2E+05	4.4E+05	3.7E+05
decanoic acid	2.6E+06	3.2E+06	3.2E+06	4.1E+06	3.7E+06
dodecanoic acid	3.0E+06	3.7E+06	3.4E+06	3.7E+06	3.8E+06
tetradecanoic acid	1.4E+06	1.9E+06	1.1E+06	2.8E+06	3.3E+06
hexadecanoic acid	7.6E+06	1.5E+06	6.4E+05	2.0E+06	1.7E+06
octadecanoic acid	1.5E+05	1.9E+05	8.9E+04	3.3E+05	2.5E+05
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	2.4E+06	3.9E+06	3.1E+06	4.2E+06	5.0E+06

Tab. S 3-13: Continued

Substance	200 rpm	400 rpm	600 rpm	800 rpm	1100 rpm
carboxamides					
decanamide	3.8E+07	4.0E+07	4.0E+07	4.1E+07	3.9E+07
dodecanamide	1.3E+07	1.5E+07	1.5E+07	1.5E+07	1.7E+07
tetradecanamide	1.3E+07	1.3E+07	5.0E+06	2.1E+07	3.3E+07
hexadecanamide	1.0E+07	1.1E+07	7.8E+06	1.5E+07	2.5E+07
stearamide	5.8E+06	6.5E+06	4.4E+06	8.4E+06	1.3E+07
oleamide	3.3E+06	3.5E+06	4.3E+06	6.6E+06	8.4E+06
erucamide	n.d.	n.d.	n.d.	n.d.	n.d.
phthalates					
diethylphthalate	6.7E+06	7.7E+06	8.8E+06	1.0E+07	1.2E+07
di-butylphthalate	6.8E+06	7.1E+06	7.0E+06	8.6E+06	1.3E+07
dicyclohexylphthalate	1.1E+07	1.2E+07	1.0E+07	1.8E+07	2.8E+07
misc					
undecane	2.0E+05	2.2E+05	2.7E+05	2.3E+05	5.4E+05
toluene	2.6E+06	2.7E+06	2.5E+06	2.7E+06	3.6E+06
ethylbenzene	3.5E+06	1.9E+06	3.3E+06	3.8E+06	4.7E+06
styrene	3.6E+06	3.8E+06	3.5E+06	4.0E+06	4.7E+06
divinylbenzene	1.8E+06	1.9E+06	1.8E+06	2.0E+06	2.4E+06
benzaldehyde	2.6E+06	2.6E+06	2.8E+06	2.8E+06	3.1E+06
1,4-diacetylbenzene	2.3E+06	2.4E+06	2.7E+06	2.9E+06	3.5E+06
1,3-diacetylbenzene	1.8E+06	1.9E+06	2.1E+06	2.3E+06	2.8E+06
1,2-dicyanobenzene	4.6E+06	4.8E+06	5.4E+06	5.5E+06	6.4E+06
chlorobenzene	3.1E+06	3.3E+06	2.9E+06	3.3E+06	4.0E+06
ketones					
methyl-iso-butylketone	n.d.	n.d.	n.d.	n.d.	n.d.
4-methyl-2-heptanone	1.6E+06	1.6E+06	1.8E+06	1.7E+06	1.9E+06
cyclohexanone	5.8E+05	6.2E+05	6.9E+05	7.5E+05	7.8E+05
5,5-dimethyl-2,4-hexandione	5.9E+06	6.2E+06	6.5E+06	6.5E+06	7.3E+06

n.d. = not determinable

Tab. S 3-14 Summarized target ion peak areas for the effect of desorption temperatures in the TDU of 250°C and 280°C.
The values given are average values for N = 3.

Substance	250°C	280°C
phenols		
phenol	1.6E+06	1.6E+06
2'-hydroxyacetophenone	1.4E+07	1.4E+07
2-*tert*-butylphenol	5.1E+07	5.1E+07
4-*tert*-butylphenol	2.2E+07	2.3E+07
4-*tert*-amylphenol	2.3E+07	2.4E+07
2,6-di-*tert*-butylphenol	4.3E+07	4.7E+07
2,4-di-*tert*-butylphenol	1.8E+08	1.9E+08
bisphenol A	1.3E+06	1.2E+06
butylhydroxytoluene	1.8E+07	2.0E+07
alcohols		
2-ethylhexanol	5.7E+06	5.5E+06
benzyl alcohol	6.9E+05	7.0E+05
dodecanol	2.1E+07	2.2E+07
octadecanol	1.7E+07	1.9E+07
carboxylic acids		
2-ethyl hexanoic acid	4.6E+05	1.4E+06
heptanoic acid	5.9E+05	7.5E+05
octanoic acid	1.5E+06	2.7E+06
decanoic acid	6.2E+06	1.8E+06
dodecanoic acid	4.0E+06	4.5E+06
tetradecanoic acid	7.1E+06	8.9E+06
hexadecanoic acid	3.1E+06	4.6E+06
octadecanoic acid	3.0E+05	9.4E+05
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	2.4E+06	5.0E+06

Tab. S 3-14: Continued

Substance	250°C	280°C
carboxamides		
decanamide	1.3E+08	1.2E+08
dodecanamide	4.5E+07	4.7E+07
tetradecanamide	7.6E+07	8.2E+07
hexadecanamide	6.1E+07	6.7E+07
stearamide	3.5E+07	3.6E+07
oleamide	2.6E+07	2.9E+07
erucamide	5.4E+06	7.9E+06
phthalates		
diethylphthalate	5.8E+07	5.9E+07
di-butylphthalate	2.0E+08	3.2E+07
dicyclohexylphthalate	1.5E+08	7.2E+07
misc		
undecane	1.1E+07	4.0E+06
toluene	1.4E+07	1.6E+07
ethylbenzene	1.9E+07	2.1E+07
styrene	1.8E+07	1.9E+07
divinylbenzene	1.3E+07	1.5E+07
benzaldehyde	9.6E+06	9.4E+06
1,4-diacetylbenzene	7.9E+06	7.7E+06
1,3-diacetylbenzene	6.4E+06	6.3E+06
1,2-dicyanobenzene	1.5E+07	1.5E+07
chlorobenzene	1.4E+07	1.5E+07
ketones		
methyl-*iso*-butylketone	1.5E+07	1.7E+06
4-methyl-2-heptanone	8.4E+06	8.7E+06
cyclohexanone	1.3E+06	1.2E+06
5,5-dimethyl-2,4-hexandione	3.3E+07	3.3E+07

n.d. = not determinable

Tab. S 3-15 Summarized target ion peak areas for the effect of desorption time from 2.5 min to 10 min in the TDU.
The values given are average values for N = 3.

Substance	2.5 min	5 min	7.5 min	10 min
phenols				
phenol	2.3E+06	2.4E+06	2.5E+06	2.3E+06
2'-hydroxyacetophenone	2.0E+07	2.1E+07	2.1E+07	2.2E+07
2-*tert*-butylphenol	5.5E+07	7.2E+07	7.7E+07	7.7E+07
4-*tert*-butylphenol	2.6E+07	3.4E+07	3.6E+07	3.5E+07
4-*tert*-amylphenol	2.8E+07	3.5E+07	3.7E+07	3.7E+07
2,6-di-*tert*-butylphenol	4.0E+07	5.5E+07	6.1E+07	7.1E+07
2,4-di-*tert*-butylphenol	2.1E+08	2.9E+08	3.1E+08	2.9E+08
bisphenol A	1.6E+06	2.2E+06	1.8E+06	1.8E+06
butylhydroxytoluene	1.3E+07	2.1E+07	2.6E+07	3.0E+07
alcohols				
2-ethylhexanol	8.0E+06	7.8E+06	8.2E+06	8.3E+06
benzyl alcohol	1.1E+06	1.2E+06	1.2E+06	1.0E+06
dodecanol	2.5E+07	3.7E+07	3.6E+07	3.3E+07
octadecanol	2.3E+07	2.7E+07	2.5E+07	2.8E+07
carboxylic acids				
2-ethyl hexanoic acid	6.8E+05	9.0E+05	9.9E+05	2.0E+06
heptanoic acid	5.6E+05	6.4E+05	7.5E+05	6.5E+05
octanoic acid	9.9E+05	1.4E+06	1.4E+06	1.5E+06
decanoic acid	8.3E+06	1.1E+07	1.1E+07	1.3E+07
dodecanoic acid	4.6E+06	6.0E+06	5.5E+06	6.7E+06
tetradecanoic acid	8.8E+06	8.9E+06	8.8E+06	1.3E+07
hexadecanoic acid	4.2E+06	3.1E+06	4.8E+06	6.8E+06
octadecanoic acid	1.4E+06	1.0E+06	6.8E+05	1.4E+06
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	2.0E+06	4.2E+06	3.2E+06	7.5E+06

Tab. S 3-15: Continued

Substance	2.5 min	5 min	7.5 min	10 min
carboxamides				
decanamide	1.7E+08	2.1E+08	1.9E+08	1.8E+08
dodecanamide	6.2E+07	7.3E+07	6.2E+07	7.0E+07
tetradecanamide	1.1E+08	1.2E+08	1.0E+08	1.2E+08
hexadecanamide	9.0E+07	9.2E+07	8.3E+07	1.0E+08
stearamide	5.3E+07	4.9E+07	4.7E+07	5.4E+07
oleamide	4.1E+07	3.8E+07	3.5E+07	4.3E+07
erucamide	9.5E+06	1.0E+07	8.9E+06	1.2E+07
phthalates				
diethylphthalate	7.4E+07	8.3E+07	8.8E+07	8.9E+07
di-butylphthalate	3.6E+07	4.3E+07	4.4E+07	4.7E+07
dicyclohexylphthalate	7.3E+07	8.7E+07	9.4E+07	1.1E+08
misc				
undecane	1.5E+07	1.6E+07	1.3E+07	5.9E+06
toluene	2.0E+07	2.1E+07	2.0E+07	2.4E+07
ethylbenzene	2.7E+07	2.8E+07	2.7E+07	3.2E+07
styrene	2.5E+07	2.6E+07	2.5E+07	2.9E+07
divinylbenzene	1.7E+07	1.8E+07	1.8E+07	2.1E+07
benzaldehyde	1.4E+07	1.3E+07	1.4E+07	1.4E+07
1,4-diacetylbenzene	1.1E+07	1.2E+07	1.3E+07	1.2E+07
1,3-diacetylbenzene	8.7E+06	9.9E+06	1.0E+07	9.4E+06
1,2-dicyanobenzene	2.0E+07	2.4E+07	2.5E+07	2.3E+07
chlorobenzene	1.9E+07	2.0E+07	2.0E+07	2.3E+07
ketones				
methyl-*iso*-butylketone	2.5E+06	2.5E+06	2.6E+06	2.5E+06
4-methyl-2-heptanone	1.1E+07	1.2E+07	1.2E+07	1.3E+07
cyclohexanone	1.9E+06	1.9E+06	1.9E+06	1.8E+06
5,5-dimethyl-2,4-hexandione	5.0E+07	5.0E+07	5.1E+07	5.0E+07

n.d. = not determinable

4

Quantification of leachables from packaging in pharmaceutical solutions in the low ppt range by 'stir bar sorptive extraction'-GC-MS
-
method validation and application to real samples

4.1 Introduction

Amongst others packaging made of plastics frequently protect pharmaceutical solutions such as dialysis solutions [1–4] from changes of their composition for example by outgassing of solution components on the one hand and from outside factors like humidity or oxygen on the other hand. Thus plastic packing materials improve the shelf life and stability of these products during storage. Furthermore, plastic packagings have for example the advantage of a lower weight and an easier handling compared to glass bottles. In addition, their properties such as stability, elasticity or permeability to gases can be matched to particular requirements within a wide range of possibilities. On the other hand plastics harbor the risk that low molecular weight substances, such as monomers, oligomers, plasticizers or catalyst components might migrate from the packaging into the pharmaceutical [5–8] and pose a safety risk to patients this way. These considerations are especially problematic for pharmaceutical liquids due to the direct contact of the packaging with the product and the likelihood that soluble substances derived from the packaging are rapidly diffusing into the solution. Since the end of 2005, the European Medicines Agency (EMEA) has consequently required the exact identification and quantification of migrating substances, the so called leachables, to comply with the Guideline on Plastic Immediate Packaging Materials (CPMP/QWP/4359/03). This guideline demands a toxicological assessment of all migrating substances regardless of their amount. Because it seems reasonable to assume that below a certain level a substance is of no risk to human safety, the product quality research institute (PQRI, Arlington, Virginia, USA) proposed a safety concern threshold (SCT) in response to this guideline. A SCT value of 150 ng/day was recommended basing on a scientific rationale [9] for orally inhaled and nasal drug products (OINDP) as a threshold below which no toxicological qualification for a leachable is necessary. The parenteral and ophthalmic drug products (PODP) leachables and extractables working group of the PQRI advised to adopt this value for pharmaceutical solutions [10], which includes dialysis solutions used for peritoneal dialysis (PD) and hemodialysis (HD) as well.

Solutions used for the PD place the highest demands on the quantification of leachables. Usually a volume of 2 liter dialysis solution is introduced in the abdomen of the patient, remains there for around 5 hours and is finally replaced with fresh solution [11,12]. This leads to a total volume of about 10 L per day, which doubles, when using automated solution changers [13]. Therefore the determination of the SCT of 150 ng/day requires an analytical method with a limit of detection (LOD) of 15 ng/kg (10 L dialysis solution per day) or even 7.5 ng/kg (20 L per day). Corresponding calculations lead to a required LOD for hemodialysis or more precisely hemofiltration solutions of 35 ng/kg. Hemofiltration is a hemodialysis treatment modality where in comparison to the standard hemodialysis

higher filtrate flow rates are utilized for the cleaning of the blood. The LOD was calculated by assuming three hemofiltration treatments per week with a 4 hour duration and a final infused volume of 10 L per treatment [14]. However, hemodialysis treatments, which involve solutions in plastic bags, get more and more replaced by treatments that use online prepared dialysis solutions [15]. Although acute hemodialysis therapies employ exchange volumes up to 75 liters per day, these treatments are mostly prescribed just for a short period of a few days or weeks until recovery of the renal functions. Thus the SCT concept that is meant for a lifetime daily intake does not apply here and acceptable amounts of leachables are presumably higher in this case.

Analytical methods for the determination of leachables in pharmaceutical solutions below the SCT have to fulfill at least two requirements. Firstly they must be able to quantify a possibly large number of compounds in a mixture simultaneously and secondly they have to be sensitive enough for trace analysis with regard to their limit of detection (LOD) and limit of quantification (LOQ), respectively. Trace analysis of aqueous samples usually requires a preconcentration technique that extracts and enriches the analytes. A novel technique that has proven its high potential [16–18] is the stir bar sorptive extraction (SBSE) which was first described by Baltussen et al. in 1999 [19]. They adopted stir bars coated with polydimethylsiloxane (PDMS) for the preconcentration of analytes followed by a gas chromatographic (GS) separation and a mass spectrometric (MS) detection. So far just Sun et al. investigated pharmaceutical solutions by SBSE. They determined four kinds of phthalate esters in polyvinyl chloride infusion bags [20].

The aim of the current study was to validate the SBSE-GC-MS method described in chapter 3 and to determine leachables in exemplary real samples. In this foregoing chapter the method was optimized for the quantification of a multi-component mixture in down to the low ng/kg (ppt) range. This limit is necessary to decide if leachables in dialysis solutions are below the SCT as reasoned above. The mixture of more than 40 analytes comprised phenols, alcohols, carboxylic acids, carboxylic acid amides, phthalates and hydrocarbons among others, although the method is not limited to these leachables. The real samples measured represent dialysis solutions currently available on the market produced by three different suppliers.

4.2 Experimental

The experimental details were described in detail in chapter 3. Therefore they are just briefly presented in the following.

4.2.1 Solvents, Chemicals

Two standard stock solutions were prepared. One of them was prepared by dissolving the following 39 components in ethanol with a final concentration of about 270 mg/kg for each: Phenol, 2'-hydroxyacetophenone, 2-*tert*-butylphenol, 4-*tert*-butylphenol, 2,6-di-*tert*-butylphenol, 2,4-di-*tert*-butylphenol, bisphenol A, butylhydroxytoluene, cyclohexanol, 2-ethylhexanol, benzyl alcohol, dodecanol, octadecanol, diethylphthalate, di-*iso*-butylphthalate, di-butylphthalate, dicyclohexylphthalate, undecane, 2-(2-butoxyethoxy)ethyl acetate, methyl-*iso*-butylketone, cyclohexanone, toluene, ethylbenzene, styrene, divinylbenzene, benzaldehyde, 1,2-dicyanobenzene and chlorobenzene were purchased from Merck KGaA (Darmstadt, Germany). 4-*tert*-amylphenol, oleamide, erucamide and 1,4-diacetylbenzene were purchased from Sigma-Aldrich Co. LLC (Steinheim, Germany). Decanamide, dodecanamide, hexadecanamide, stearamide and 4-methyl-2-heptanone were purchased from ABCR GmbH & Co. KG (Karlsruhe, Germany). Tetradecanamide, 5,5-dimethyl-2,4-hexandione and 1,3-diacetylbenzene were purchased from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany). The following ten carboxylic acids were used to prepare the second stock solution with a final concentration of 530 mg/kg for each acid: 2-Ethylhexanoic acid, heptanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid and docosanoic acid were purchased from Merck and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid was purchased from Alfa Aesar. All substances were purchased in the highest purity available. The stock solutions were stored in the darkness at room temperature.

Four of the analytes (cyclohexanol, docosanoic acid, di-*iso*-butylphthalate and 2-(2-butoxyethoxy)ethyl acetate) were excluded from the determination as already reasoned in chapter 3.

Additionally a solution of phenanthrene-D_{10} (Sigma Aldrich) at 2 mg/kg in ethanol (p.a., Merck KGa) was used to spike all samples to have a final concentration of 75 µg/kg. It served as a quality assurance marker to ensure a proper enrichment process.

Serial dilutions from a mixture of both stock solutions were prepared freshly every day using water purified with a Milli-Q Gradient system from Millipore. The concentrations ranged from 20 µg/kg to 0.001 µg/kg for the method validation. For studying matrix effects four matrix solutions were prepared and used for the dilution steps instead of water.

The matrices employed were two bicarbonate buffered solutions (hereinafter referred to as “BH” and “BL”) with pH 7 and two lactate buffered solutions (“LH” and “LL”) having a pH of 5.5. For the “BH” solution the molarity of the main components were: 134 mmol/L Na^+, 34 mmol/L HCO_3^- and 236 mmol/L glucose. The “BL” matrix was prepared with 140 mmol/L Na^+, 35 mmol/L HCO_3^- and 5.6 mmol/L glucose. The “LH” matrix was prepared similar to the “BH” solution, but instead HCO_3^- lactate was used with the same molarity. The “LL” solution exhibited a composition of 134 mmol/L Na^+, 38 mmol/L lactate and 5.6 mmol/L glucose.

The PDMS coated stir bars (Twister™) were purchased from Gerstel GmbH (Mülheim, Germany). Before every extraction they were conditioned by cleaning them two times in a 1:1 (v/v) mixture of methanol and dichloromethane for two hours and subsequently drying them two times at 300°C for four hours.
Methanol (p.a., Merck KGaA) and dichloromethane (GC-grade, VWR) were used to clean the stir bar. Sodium chloride in p.a quality was purchased from Bernd Kraft (Duisburg, Germany). All chemicals were used without any further purification.

4.2.2 Real samples

Five currently on the market available dialysis solutions from three different suppliers, herein afterwards referred to as 1, 2 and 3, were studied. Three of them based on a bicarbonate buffer, denoted as ‘Bic’ in the following, while the other two were lactate buffered, denoted as ‘Lac’. Four solutions from two different production lots referred to as ‘A’ and ‘B’ were analyzed from each supplier. The dialysis solutions came in dual-chamber 5 L bags and the contents of both chambers were thoroughly mixed directly before the measurement as described in the respective instructions of use. Afterwards four samples with 25 mL from each bag were extracted as described in the following.

4.2.3 Sample Extraction Procedure

After adding 8.5 g sodium chloride to 25 mL samples 2 cm long stir bars with a 1 mm thick PDMS coating were added and stirred at ambient temperatures for four hours at a speed of 1100 rpm. Afterwards the stir bars were gently rinsed with water, dried and placed in desorption glass tubes. These were finally placed on the auto sampler tray.
To avoid possible contamination by direct contact, either with skin or gloves, the stir bar handling was done with magnetic retrievers or tweezers.

4.2.4 TD-GC-MS analysis

A GC 7890 system from Agilent equipped with a thermal desorption unit “TDU” (Gerstel), a cold injection system “CIS” (Gerstel), a multipurpose autosampler “MPS” (Gerstel) and an Agilent 5973 quadrupole mass spectrometer was used for the GC-MS analysis. The analytes sorbed in the stir bars were desorbed in the TDU at 280°C for 10 min and were transferred into the CIS with helium 5.0 at a flow of 50 mL/min. After the cryo-focussing at -120°C the analytes were injected into the GC-column by heating up the CIS to 280°C. Helium was used as the carrier gas at a constant flow of 1 mL/min. After a waiting time of one minute at a column oven temperature of 50°C, the temperature was increased from 50°C to 150°C at a rate of 10°C/min, held for 5.5 min and was further raised at a rate of 50°C/min to 300°C. This temperature was kept constant for 10 min. The MS was equipped with an electron impact (EI) source and was used in scan-mode to record mass-to-charge ratios (m/z) between 25 and 700. The column used was a Zebron (ZB-50) capillary column (length 30 m, diameter 0.25 mm, film thickness 0.50 µm, stationary phase: 50% diphenyl polysiloxane, 50% dimethyl polysiloxane) purchased from Phenomenex(Aschaffenburg, Germany).

4.3 Results and Discussion

4.3.1 Method Validation

Following the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline [21] method validation involves testing the specificity, determining the linear range, the limits of detection and quantification, the accuracy and the robustness of the method. In the following these validation characteristics will be presented for the method under study here. Accuracy will be discussed in terms of precision (repeatability and intermediate precision) and trueness. Furthermore, matrix effects for 4 different matrixes and the stability of the standard stock solutions and the loaded stir bars will be discussed.

The concentration range for the validation ranges from 20 µg/kg to 0.001 µg/kg. Due to a non-homogenous variance of the values over the full range, which covered more than 4 decades, the calibration range was divided into two subparts ranging from 20 µg/kg to 0.1 µg/kg and from 0.1 µg/kg to 0.001 µg/kg.

Specificity

The IUPAC definition says that specificity is the ability of an analytical method to discriminate between two similar structures, which is important to ensure the correct quantification of a certain substance. Specificity of the method to be validated here is given by the fact that every compound is characterized by its retention time and its mass spectrum. The mass spectra of all compounds were recorded in single measurements via direct injection and were added to a database. Even if compounds from the same substance class, e.g. the amides or carboxylic acids have similar structures they have different retention times. Vice versa when two components under study here have quite similar retention times, their mass spectra will in general differ. Thus specificity was not checked by additional measurements, e.g. by spiking blanks.

Linearity

Linearity was studied for the two ranges from 20 µg/kg to 0.1 µg/kg and from 0.1 µg/kg to 0.001 µg/kg. The higher concentration range was validated with 6 calibration points (20 µg/kg, 15 µg/kg, 10 µg/kg, 5 µg/kg, 1 µg/kg and 0.1 µg/kg) while 5 points were used for the lower range (0.1 µg/kg, 0.05 µg/kg, 0.01 µg/kg, 0.005 µg/kg and 0.001 µg/kg). At least four replicates were measured. For some of the compounds the LOQ was above the lowest

concentration of 0.001 µg/kg. In this case the lowest concentration above the LOQ value was chosen as the limit for the validation range.

The residual plots of the linear fits showed a heteroscedasticity, despite the already splitted validation range. Thus the logarithm was taken of the x- (standard concentration) and y-values (peak area of the target ion), which finally results in a homogeneous variance within the validation ranges. The slope, the intercept and the coefficient of correlation were determined for a linear regression of the values. Furthermore, appropriateness of a linear fit was statistical evaluated by the lack-of-fit test. Hereby the pure error and the error due to a wrong fit are compared. Simply spoken, the second one should not be higher than the first one if a linear fit is appropriate. For a correct comparison the F-value is calculated by dividing the mean square lack of fit error by the mean square pure error. This F-value is compared with a tabulated F-value with a significance level of 5% and the corresponding degrees of freedom for the lack of fit and the pure error. As long as the calculated F-value is below the tabulated, the linear fit is statistically representative for the data. Finally the p (lack of fit)-value was calculated to have just one parameter to decide the appropriateness of the fit. This is the case for p-values above 0.05.

The p(lack of fit) values were above 0.05 for all linear fits for the higher as well as the lower concentration range that was validated. R^2 was 0.99 or above for most of the compounds in the higher concentration range and at least 0.95 for the lower concentration range. The parameters of the linear fit such as slope and axis intercept as well as R^2 and p(lack-of-fit) are summarized in supplement chapter 4.6. It was quite striking from the data that the carboxylic acids behave differently in the extraction process than most of the other compounds in the mixture. First of all, the LODs were quite high with 1 µg/kg, secondly the linear fits had an R^2 of just 0.95 or higher. The only exception was dodecanoic acid, which had an R^2 of 0.996 and was detectable down to 0.01 µg/kg. A similar result was reported in [22], where the extraction efficiency of octanoic, tetradecanoic and octadecanoic acid did not show a correlation to their $K_{O/W}$ values or molecular weights. Although both ($K_{O/W}$ values and molecular weight) increase from octanoic over tetradecanoic to octadecanoic acid, tetradecanoic acid showed the highest recovery at pH 7; octanoic and octadecanoic acid had partially much lower recoveries. The behavior of dodecanoic acid was not studied in [22] but it is reasonable to assume that this acid might give an even higher extraction yield as observed here. A reason for the unexpected extraction behavior of the carboxylic acids could possibly be the interplay between adsorption on the glass and the water surface, formation of micelles and dimers and sorption into the PDMS; processes which are related by the concentration of the acid monomers in solution and shortly discussed in chapter 3.

Limit of Quantification

The LOD, already determined and presented in chapter 3, is defined as the lowest analyte concentration that gives an unambiguous signal to detect but not necessarily quantify the analyte [21]. In contrast, the LOQ is the lowest amount of the analyte that can be quantified with a known calibration function and a known and suitable accuracy [21, 23]. The linear range and accuracy obtained within the validation process permit the calculation of the LOQ values basing on the already determined LOD values. LOQ was chosen to be 3.3 times LOD and is given in the supplement chaper 4.6.3 for all analytes.

Accuracy and Robustness

Precision was determined as the repeatability of the same sample measured at least four times immediately after each other in the same run. Repeatability for the lower limits of the two concentration ranges were below 16% (0.1 µg/kg or LOQ) and 23% (0.001 µg/kg or LOQ), respectively.

For the intermediate precision three samples were measured fourfold each day on three different days by at least two different analysts. The standard deviation was below 21% (0.1 µg/kg or LOQ) and 30% (0.001 µg/kg or LOQ) respectively.

It turns out that two parameters have a main influence on the repeatability. First, the stirring conditions during the extraction and second, the aging of the stir bars. To minimize the standard deviation of the data an undisturbed stirring at a constant speed is important. Furthermore, it is advantageous to use stir bars with a similar age and a similar number of conditioning steps. This ensures a reproducible extraction.

The trueness of the method was evaluated by determining the recovery. It was calculated as the ratio between the mean concentration found in the sample and the concentration of the compound added. The recoveries were between 90% and 110% for the high concentration range and between 80% and 120% for the low concentration range. Accuracy and trueness date are summarized in the supplement chaper 4.6.3.

Robustness of the method was not studied in detail in the sense of purposive small changes of important parameters and checking their impact on the result. Instead the intermediate precision was taken as an indication of the robustness. Slight variations in the parameters which are unavoidable when the measurements were performed on different days by different persons did not lead to a significant change in the results. This is proven by the intermediate precision, which was in all cases below 30%. Thus the method can be considered to be robust.

Stability of solutions and loaded stir bars

The ethanolic stock solution of the screening standards is stable for at least 6 months. The carboxylic acids stock solution has a stability of 3 months. In this context “stable” means that there are no significant changes in the target ion peak areas of the compounds. This was checked on a separate GC-MS, with a validated method by direct injection of the standard solution.

The diluted, aqueous solutions must be prepared freshly before every measurement, because especially the carboxylic acids tend to get lost, as discussed before.

The loaded stir bar is stable at room temperature for at least 48 h. This was tested by extracting a sample solution with 27 replicates in parallel and analyzing them one after the other with breaks in between in the TD-GC-MS. The analysis time for all samples together was about 48 h. During this time, no significant change in the target ion peak area was observed as shown exemplarily for phenol in figure 4-1.

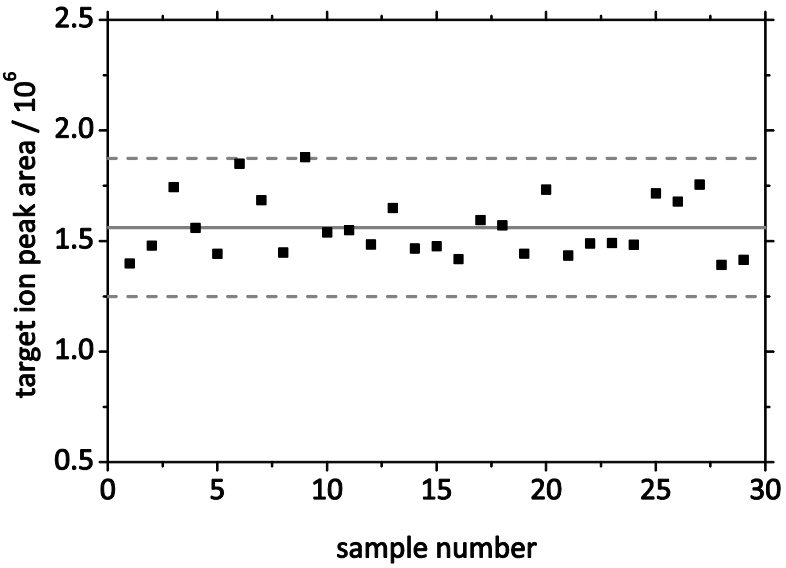

Figure 4-1 Variation in target ion peak areas for phenol for measurements conducted over a timeframe of 48 hours.
Individual peak areas (filled square), mean value (solid line) and +/- 20% (dashed line) values are shown.

Matrix effects

To study the influence of the matrix on the extraction of the standards by SBSE four different matrices were tested, which represent typical dialysis solutions. The main difference among the matrices is the buffer solution that is used to adjust the pH of the solutions. For two solutions a lactate buffer is used and the others are prepared with a bicarbonate buffer. In addition, the amount of glucose is varied for each of the two buffer solutions, between a high and a low value. The four matrices are called LL (lactate, low glucose), LH (lactate, high glucose), BL (bicarbonate, low glucose) and BH (bicarbonate, high glucose).
Matrix effects were studied for all compounds except the carboxylic acids at a concentration of 0.1 µg/kg. The influence of the matrix on the extraction of the acids was studied for a concentration of 1 µg/kg, as their LOD is higher than for the other substances. Additional pure water samples with the same concentrations were extracted after the addition of NaCl and taken as a reference. The matrix extraction results were normalized to the water extraction results and are shown in figures 4-2. The deviation is less than 30% for all compounds and less than 20% for most of them. Therefore, matrix effects are insignificant. This finding can be explained by the addition of salt to all samples that minimizes differences in sample composition relevant for extraction yields. In addition, Jahnke et al. [24] studied several quite different matrices with regard to the influence on the extraction and observed no significant influence on the sorption in PDMS in a SPME setup.

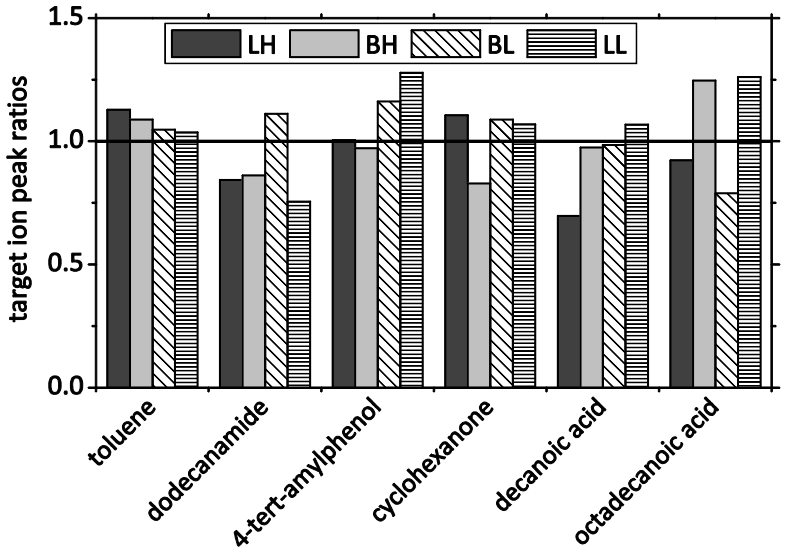

Figure 4-2 Effect of the matrix on the target ion peak areas.
Results are presented normalized to the target ion peak area of water that was set to 1.

4.3.2 Real Sample Measurements

The results for the real sample measurements are shown in figure 4-3, where averaged values are given for some exemplary leachables. For reasons of simplicity, these mean values were determined by averaging the results for all four solution bags from every supplier, despite the observed lot dependence for some substances, as discussed below. In addition lot averaged data are given in the supplement chaper 4.6.4.

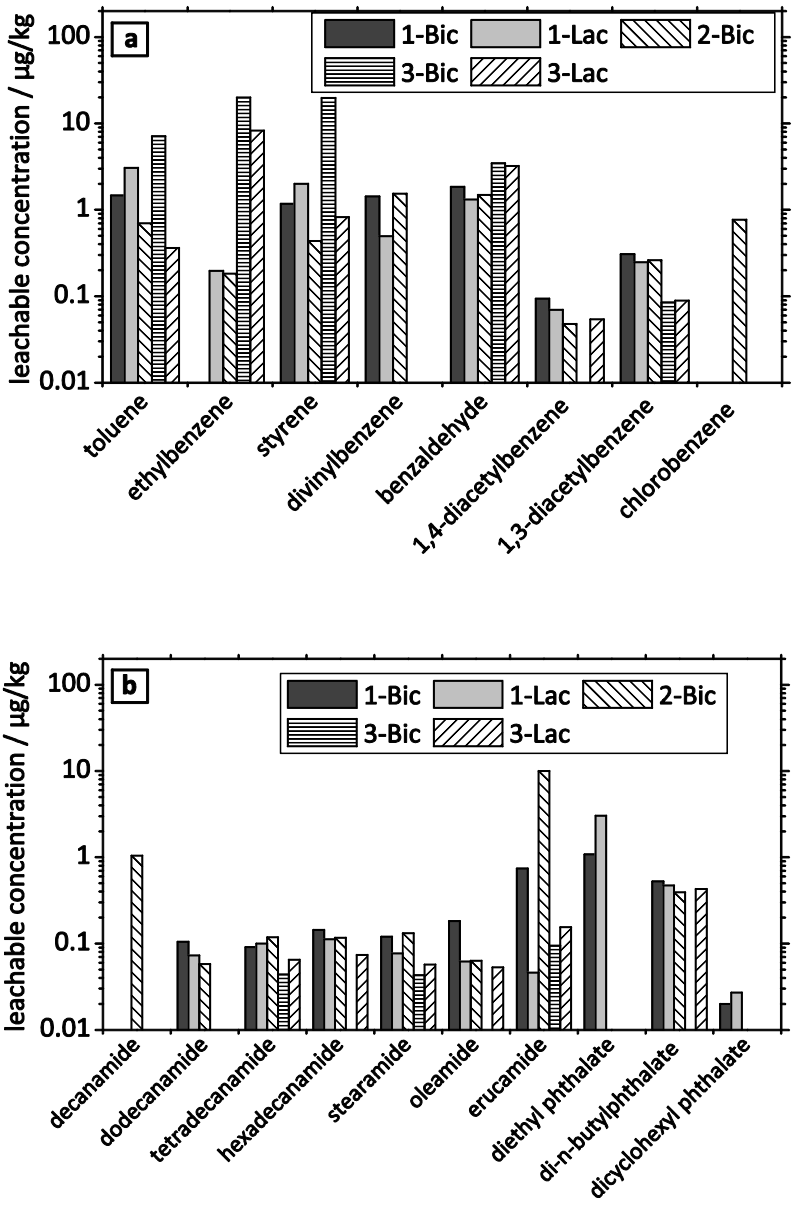

Figure 4-3 Concentrations of leachables found in the real samples.

All samples, independent of the supplier, exhibited a broad spectrum of leachables, which represented about 75% of the components used for the stand stock solutions. This finding proved the practical relevance of the standard solution composition. No unknown substances were detected in any of the solutions during the GC-MS analysis. Most of the leachables were found in concentrations below 1 µg/kg, with about one third of them between 0.1 µg/kg and 0.01 µg/kg.

The intra-lot comparison of the leachable concentrations in the two solutions from the same lot did not show differences with a few exceptions, independent of the supplier and type of buffer. In contrast, the inter-lot comparison showed in part considerable concentration differences between dialysis solutions from different lots for all suppliers. The most striking lot-to-lot variance was seen for divinylbenzene in the bicarbonate buffered solutions of supplier 2, where a concentration of 3.05 µg/kg was determined for lot A and 0.04 µg/kg for lot B. The divinylbenzene concentration in the lactate buffered solutions from supplier 1 showed a difference of factor five between Lot A and B (0.17 µg/kg and 0.83 µg/kg), while the bicarbonate buffered solution did not exhibit these large lot-to-lot variations (1.25 µg/kg and1.61 µg/kg). No divinylbenzene could be determined in solutions from supplier 3. Another example for the inter-lot variability is diethylphthalate in the bicarbonate buffered solutions of supplier 1. In lot A its concentration was about 1.09 µg/kg, whereas it was 0.23 µg/kg in lot B. Beside these extreme cases, there were some more examples for solutions of all three suppliers where inter-lot leachable concentrations differed by a factor of two or three for particular components. But for most of the substances, no pronounced lot-to-lot difference was determined.

A particular noticeable leachable was 2-ethylhexanol, which was determined in all solutions with high amounts above 20 µg/kg except for the bicarbonate buffered solution of supplier 3 (2.94 µg/kg lot average). This alcohol is known to be a residue from the production of di-2-ethylhexyl phthalate (DEHP), typically used as a plasticizer, and a hydrolysis product of DEHP as well [25]. Even if DEHP was not detected in any of the samples, DEHP and thus 2-ethyl-1-hexanol were probably involved in the production process of the packaging materials. Di-butyl phthalate was present in all dialysis solutions with similar concentrations around 0.40 µg/kg, whereas diethylphthalate was just determined in solutions of supplier 1 (1.8 µg/kg). In addition, all solutions of supplier 1 comprised dicyclohexylphthalate with amounts of about 0.025 µg/kg, which is below the SCT calculated for hemofiltration. This treatment modality is the least demanding as discussed in the beginning. Just one other leachable among the remaining was also below this SCT. 2-tert-butylphenol was determined in the LAC solution of supplier 1 at a concentration of 0.022 µg/kg. The presence of phthalates is not surprising because these components are typical additives

used as plasticizers or lubricants in the production process of several plastics. Phthalates can be found for example in polyethylene terephthalate (PET) [26], polypropylene (PP) [27], polyvinyl chloride (PVC) [20] and high density polyethylene (HDPE) [28]; all of them are plastics used for the production of packaging materials for pharmaceutical solutions. Furthermore, all dialysis solutions exhibited dodecanoic acid and tetradecanoic acid at similar concentrations for supplier 1 and 3 (0.6 µg/kg and3.3 µg/kg) but six times higher values for supplier 2 (3.5 µg/kg and 20 µg/kg). The concentrations of hexadecanoic acid and 3-(3,5-di-tert-butyl-4-hydroxyphenyl)-propionic acid (DBHPP) where supplier independent around 6.5 µg/kg. The aliphatic acids are known contaminations in calcium stearate that is used as a lubrication and stabilization agent in plastic production [29]. DBHPP is a hydrolysis product of the phenolic antioxidant Irganox 1010 ((pentaerythrityltetrakis[3-(3,5-di-1,1dimethylethyl-4-hydroxyphenyl)]-propionate) [30] that can be found in PVC, PP and HDPE among others [31]. Phenol, probably used as an additive in the production process, was just determined in the solutions of suppliers 1 and 2. Amides like dodecan-, tetradecan-, hexadecan- and stearamide with supplier independent varying amounts around 0.1 µg/kg are present in all solutions. Decanamide and erucamide was just determined in the bicarbonate buffered solution of supplier 2. In addition, chlorobenzene (0.77 µg/kg) and bisphenol A (0.16 µg/kg) were only found in this solution and could not be determined in the products of supplier 1 and 3. However, the presence of chlorobenzene and bisphenol A did not allow to identify a certain plastic used in the packaging. Instead both of them are used in the production process of several plastics [8, 32, 33], which renders an unambiguous identification due to their presence impossible. Supplier 3 had the highest toluene (about 7 µg/kg), ethylbenzene (> 20 µg/kg) and styrene (about 8 µg/kg) concentrations in its BIC solution. All point to the presence of polystyrene, where styrene is used as a monomer during the production and ethyl benzene in turn is employed in the synthesis of this monomer and is always present as a trace impurity in styrene as well as toluene [34].

In summary, all dialysis solutions exhibited similar concentrations for some of the leachables, which could possibly be related to having the same manufacturer for some of the raw materials, but on the other hand they also show distinct differences either in the leachable concentration or the chemical nature of the leachable. In combination with leachable data of raw materials, which have to be determined with the same method, a 'SBSE-fingerprinting' of materials seems promising. This can afterwards be used to determine material sources of packaging materials basing on the concentration and kind of leachables found in the packed solution.

Compared to the SCT concept discussed in the introduction, almost all of the leachable concentrations in the dialysis solutions examined here are at least a factor 10 higher than

the calculated safety concern thresholds for the more demanding PD treatments. These relatively high concentrations compared to the SCT do not indicate that the dialysis solutions under study here pose any danger to the patience's health. However, concentrations above the SCT reveal that toxicological assessments are necessary for the leachables found.

4.4 Conclusions

The validation and real sample application of a SBSE-GC-MS method for the determination of more than 40 potential leachables emerging from plastic packaging in a concentration from 20 µg/kg down to the low ng/kg range was described. Thus the method has clearly the potential to quantify leachables below the safety concern threshold (SCT) of 0.15 µg/day, as recommended by the product research quality institute (PQRI) for pharmaceutical solutions amongst others. Only the carboxylic acids had a higher LOD of 1 µg/kg due to their low partition coefficient. The method repeatability was found to be below 23%, recoveries were between 80% and 120% and intermediate precisions were below 30%. No significant matrix effects were observed in typical dialysis solutions.

The method was successfully applied in the determination of leachables in five different, currently available dialysis solutions from three different suppliers. Some distinct differences in regard to the concentrations or chemical identity of the migrating components were observed between the suppliers and their products, but on the other hand similarities between all the dialysis solutions were found as well. Most of the leachables were quantified with concentrations between 0.1 µg/kg and 10 µg/kg. These concentrations were much higher than the SCT and therefore toxicological assessments are necessary for these leachables.

4.5 References

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4.6 Supplement

4.6.1 Solutions

Tab. S 4-1 Exemplary preparation of the used stock solution "screening standard (SC)" by weighing and solving all standards without the carboxylic acids in 90.3828 g of ethanol

Substance	Weight / mg	Concentration / mg/kg
phenols		
phenol	27.0	295.1
2'-hydroxyacetophenone	26.9	294.0
2-*tert*-butylphenol	26.0	284.2
4-*tert*-butylphenol	28.1	307.2
4-*tert*-amylphenol	26.1	285.3
2,6-di-*tert*-butylphenol	28.7	313.7
2,4-di-*tert*-butylphenol	25.7	280.9
bisphenol A	27.2	297.4
butylhydroxytoluene	23.6	258.0
alcohols		
2-ethylhexanol	22.7	248.1
benzyl alcohol	25.0	273.3
dodecanol	23.6	258.3
octadecanol	23.9	261.3
carboxamides		
decanamide	25.2	275.5
dodecanamide	25.9	283.1
tetradecanamide	25.5	278.7
hexadecanamide	24.0	262.3
stearamide	26.4	288.7
oleamide	24.5	267.8
erucamide	23.9	261.3
phthalates		
diethylphthalate	26.4	288.6
di-butylphthalate	23.3	254.7
dicyclohexylphthalate	24.2	264.5

Tab. S 4-1: Continued

Substance	Weight / mg	Concentration / mg/kg
misc		
undecane	23.3	254.7
toluene	26.6	290.8
ethylbenzene	26.5	289.7
styrene	28.5	311.5
divinylbenzene	22.6	246.7
benzaldehyde	23.4	255.8
1,4-diacetylbenzene	25.7	280.9
1,3-diacetylbenzene	23.2	253.6
1,2-dicyanobenzene	26.1	285.3
chlorobenzene	24.5	267.8
ketones		
methyl-*iso*-butylketone	23.4	255.8
4-methyl-2-heptanone	25.3	276.6
2,4-pentandione	22.4	244.9
5,5-dimethyl-2,4-hexandione	25.8	282.0

Tab. S 4-2 Exemplary preparation of the used stock solution for the carboxylic acids "carboxylic acids standard (CS)" by weighing and solving the acids in 43.6299 g of ethanol

Substance	Weight / mg	Concentration / mg/kg
2-ethyl hexanoic acid	23.6	538.3
heptanoic acid	23.5	535.4
octanoic acid	23.5	535.4
decanoic acid	23.6	537.7
dodecanoic acid	23.1	526.3
tetradecanoic acid	22.9	521.8
hexadecanoic acid	23.5	535.4
octadecanoic acid	23.8	542.3
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	23.6	537.7

Tab. S 4-3 Exemplary preparation of the 20 µg/kg mixed standard "standard I" and the 1 µg/kg mixed standard "standard II" including the SC and CS.

SC weight / mg	SC average concentration / mg/kg	CS weight / mg	CS average concentration / mg/kg	solvent	solvent weight / g	SC final concentration / µg/kg	CS final concentration / µg/kg	name
50.0	275.3	28.4	535.0	water	700.25	19.65	21.70	standard I
1,000.0	275.3			ethanol	9.00	27,530		SC II
		1,000.0	535.0	ethanol	19.00		26,750	CS II
18.8	27.53 (SC II)	18.5	26.75 (CS II)	water	500.36	1.03	0.99	standard II

Tab. S 4-4 Exemplary preparation of the diluted standards with concentrations from 15 µg/kg to 1µg/kg

standard I weight / g	water weight / g	SC final concentration / µg/kg	CS final concentration / µg/kg
150.00	50.00	14.7	16.3
100.00	100.00	9.8	10.8
50.00	150.00	4.9	5.4
10.00	191.00	1.0	1.1

Tab. S 4-5 Exemplary preparation of the diluted standards with concentrations from 100 ng/kg to 1ng/kg

standard II weight / g	water weight / g	SC final concentration / µg/kg	CS final concentration / µg/kg
20.00	180.00	0.1030	0.0989
10.00	190.00	0.0517	0.0494
2.00	198.00	0.0103	0.0099
1.00	199.00	0.0052	0.0049
0.20	200.50	0.0010	0.0010

4.6.2 Chromatograms

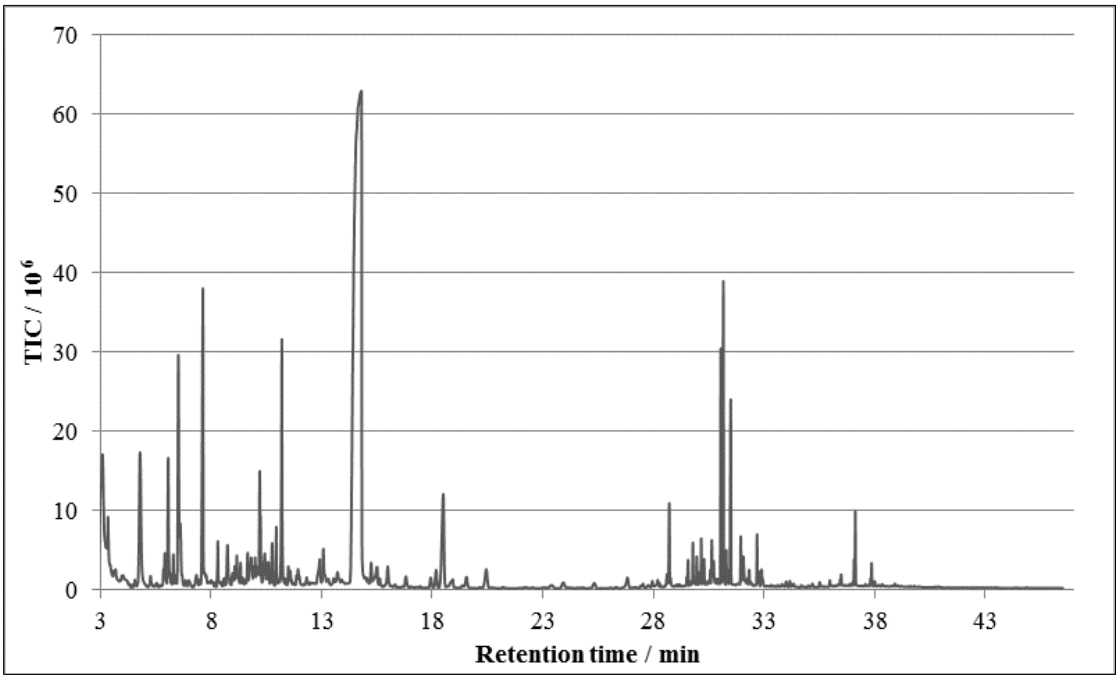

Fig. S 4-1 Total ion current chromatogram in scan mode for the dialysis solution "1-BIC"

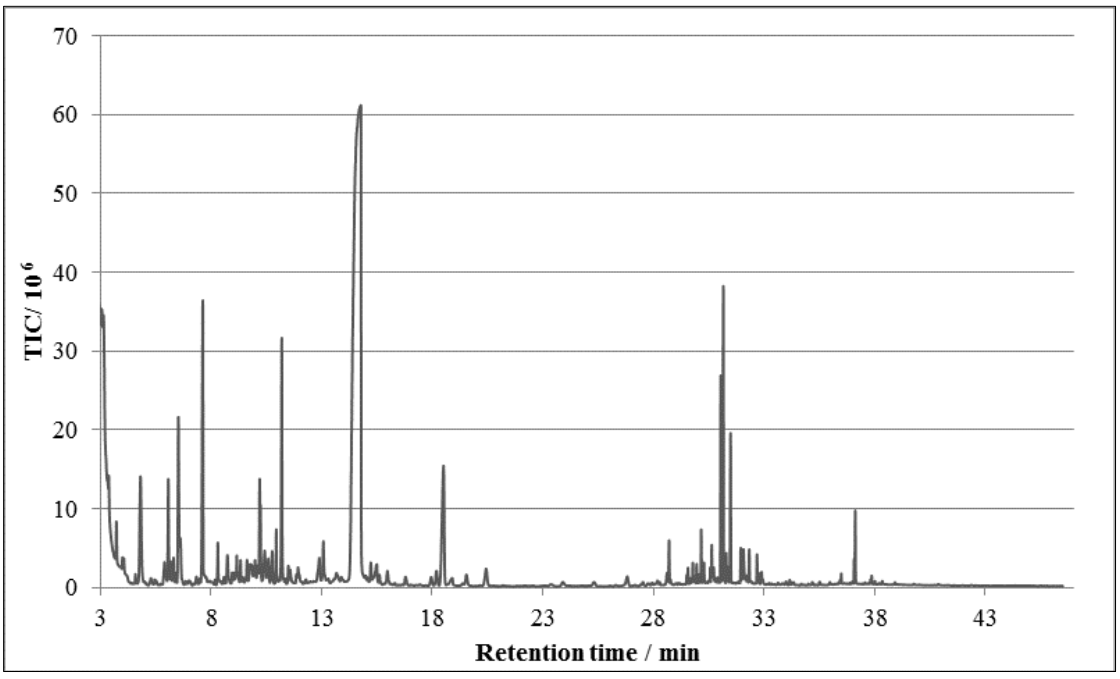

Fig. S 4-2 Total ion current chromatogram in scan mode for the dialysis solution "1-LAC"

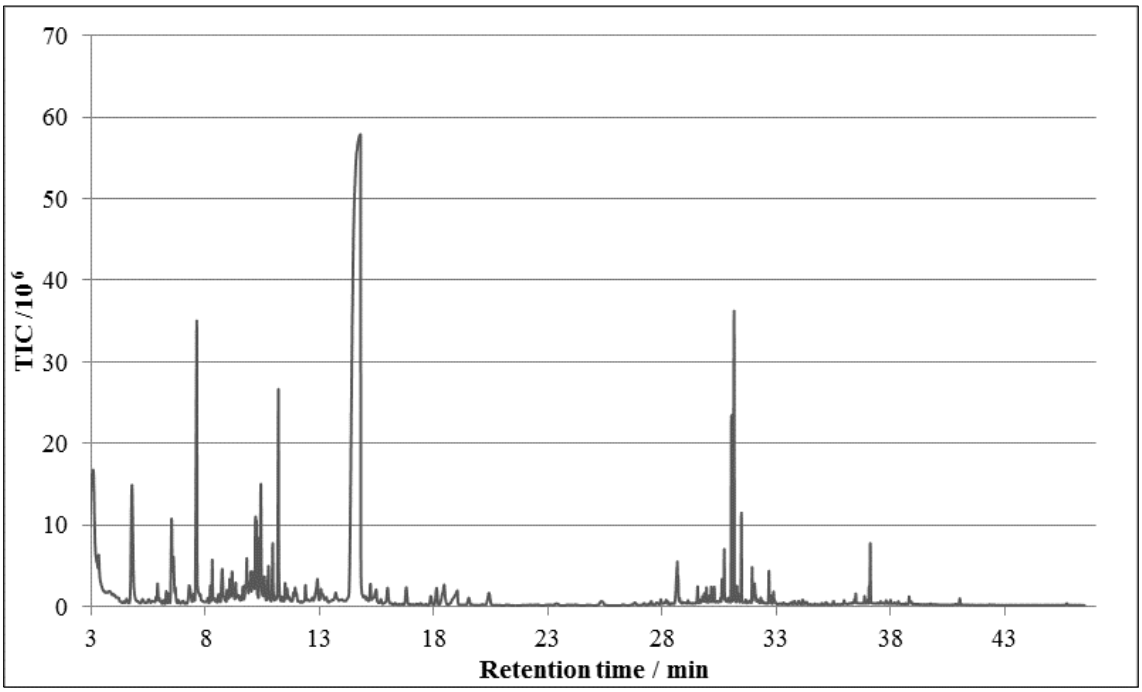

Fig. S 4-3 Total ion current chromatogram in scan mode for the dialysis solution "2-BIC"

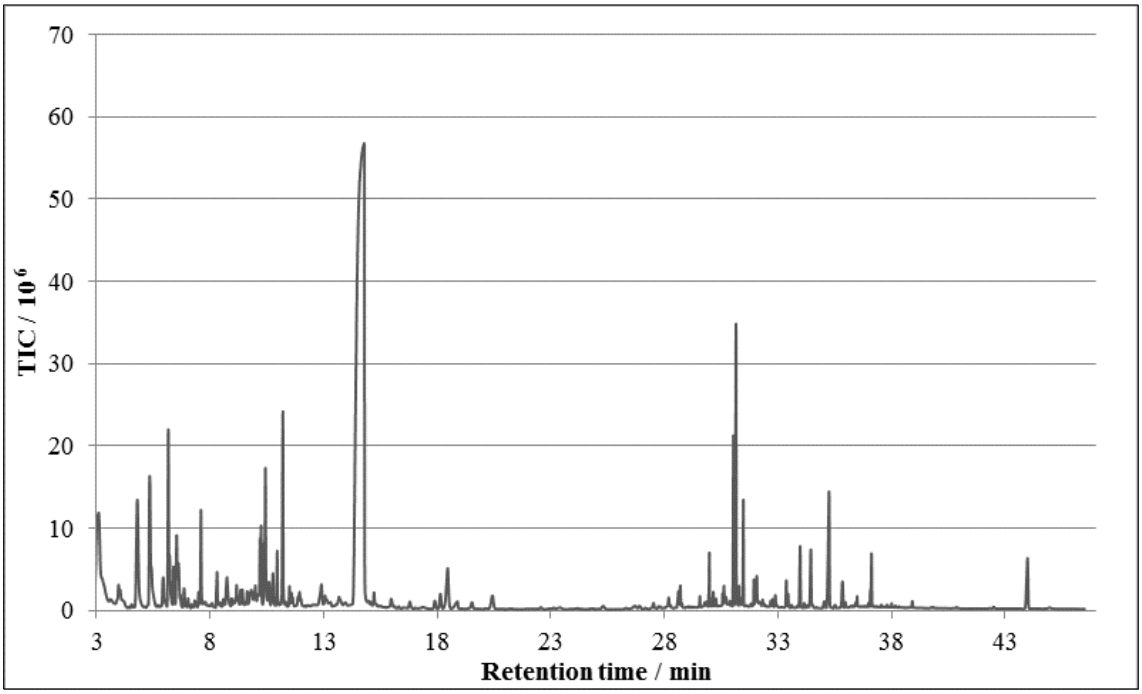

Fig. S 4-4 Total ion current chromatogram in scan mode for the dialysis solution "3-BIC"

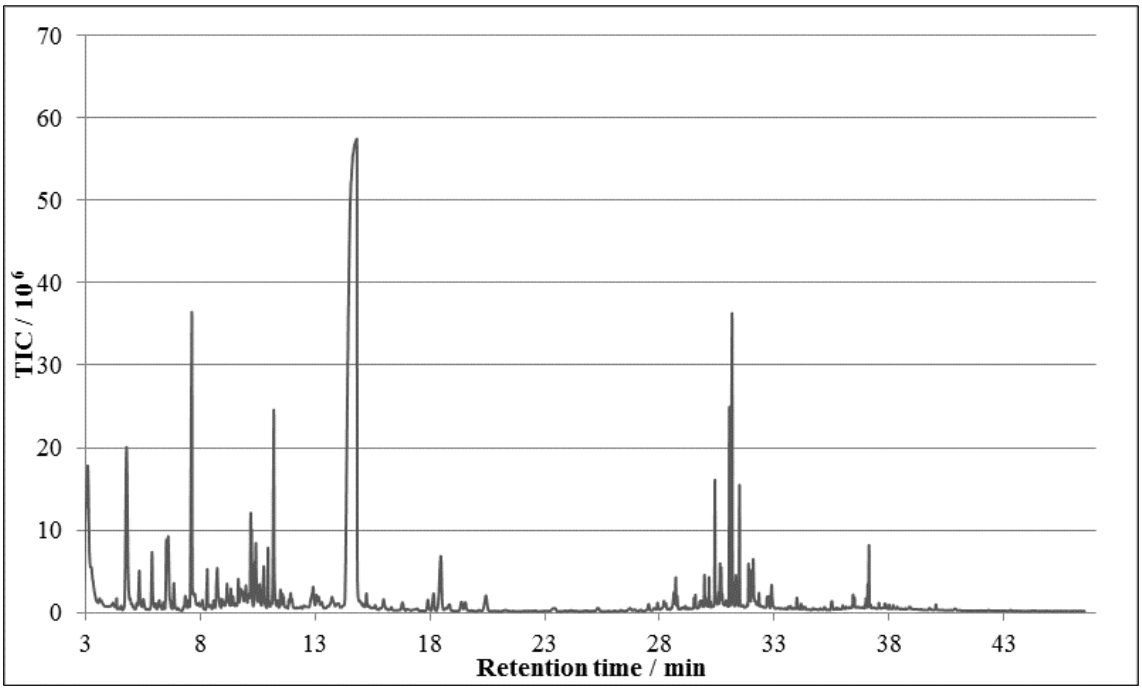

Fig. S 4-5 Total ion current chromatogram in scan mode for the dialysis solution "3-LAC"

4.6.3 Method validation

Tab. S 4-6 Parameters of the linear fit for the validation range from 20 µg/kg to 0.1 µg/kg.
m and n are the slope and axis intercept of the linear fit y = m x + n, R^2 is the coefficient of determination and p(lof) is the lack of fit probability, where values above 0.05 represent an appropriate fit.

Substance	m	n	R^2	p(lof)
phenols				
phenol	1.046	6.513	0.997	0.440
2'-hydroxyacetophenone	0.990	6.780	0.999	0.638
2-*tert*-butylphenol	1.018	7.228	0.999	0.275
4-*tert*-butylphenol	0.997	6.703	1.000	0.327
4-*tert*-amylphenol	0.995	6.567	1.000	0.546
2,6-di-*tert*-butylphenol	1.091	7.152	0.999	0.062
2,4-di-tert-butylphenol	0.938	7.587	0.998	0.231
bisohenol A	0.971	6.432	0.996	0.065
butylhydroxytoluene	1.106	6.652	0.998	0.164
alcohols				
2-ethylhexanol	0.630	6.971	0.998	0.162
benzyl alcohol	0.704	5.588	0.994	0.386
dodecanol	0.752	6.887	0.993	0.282
octadecanol	0.422	6.870	0.990	0.970
carboxylic acids				
2-ethyl hexanoic acid	1.046	6.088	0.988	0.185
heptanoic acid	1.016	5.556	0.971	0.303
octanoic acid	0.673	6.024	0.979	0.240
decanoic acid	0.900	6.665	0.992	0.393
dodecanoic acid	0.719	6.581	0.996	0.064
tetradecanoic acid	0.569	6.105	0.949	0.621
hexadecanoic acid	0.857	5.789	0.967	0.833
octadecanoic acid	0.731	5.694	0.966	0.893
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	0.956	6.673	0.997	0.183

Tab. S 4-6: Continued

Substance	m	n	R^2	p(lof)
carboxamides				
decanamide	1.210	7.041	0.999	0.112
dodecanamide	0.895	7.126	0.999	0.123
tetradecanamide	1.014	6.848	0.995	0.081
hexadecanamide	1.016	6.461	0.995	0.073
stearamide	0.893	6.547	0.995	0.076
oleamide	0.913	6.330	0.995	0.094
erucamide	0.815	5.885	0.995	0.222
phthalates				
diethylphthalate	0.671	7.270	0.997	0.389
di-butylphthalate	0.722	6.859	0.997	0.166
dicyclohexylphthalate	0.967	6.609	0.997	0.334
misc				
undecane	1.057	5.481	0.975	0.079
toluene	0.967	6.962	0.997	0.065
ethylbenzene	1.079	6.966	0.998	0.237
styrene	1.057	6.923	0.998	0.148
divinylbenzene	1.116	6.649	0.998	0.150
benzaldehyde	0.894	6.552	0.997	0.172
1,4-diacetylbenzene	0.776	6.221	0.994	0.098
1,3-diacetylbenzene	0.810	6.057	0.998	0.109
1,2-dicyanobenzene	0.998	6.301	0.999	0.386
chlorobenzene	1.152	6.686	0.998	0.241
ketones				
methyl-*iso*-butylketone	0.971	5.819	0.998	0.228
4-methyl-2-heptanone	1.086	6.768	0.999	0.237
cyclohexanone	0.781	6.082	0.994	0.155
5,5-dimethyl-2,4-hexandione	1.056	7.088	0.998	0.346

Tab. S 4-7 Parameters of the linear fit for the validation range from 0.1 µg/kg to 0.001 µg/kg (or LOQ, depending on which is the higher value). m and n are the slope and axis intercept of the linear fit $y = m\,x + n$, R^2 is the coefficient of determination and p(lof) is the lack of fit probability, where values above 0.05 represent an appropriate fit.

Substance	m	n	R^2	p(lof)
phenols				
phenol	0.606	6.089	0.930	1.000
2'-hydroxyacetophenone	0.967	6.747	0.982	0.110
2-*tert*-butylphenol	0.764	6.878	0.975	0.073
4-*tert*-butylphenol	0.611	6.346	0.997	0.070
4-*tert*-amylphenol	0.460	6.033	0.993	0.651
2,6-di-*tert*-butylphenol	0.550	6.621	0.978	0.213
2,4-di-*tert*-butylphenol	0.392	7.082	0.963	0.379
bisohenol A	1.065	6.558	0.976	0.968
butylhydroxytoluene	0.855	6.459	0.965	0.153
alcohols				
2-ethylhexanol	1.187	7.517	0.998	0.743
benzyl alcohol	0.298	5.189	0.963	0.469
dodecanol	0.886	6.950	0.955	0.062
octadecanol	0.557	7.013	0.952	0.549
carboxylic acids				
2-ethyl hexanoic acid	n.d.	n.d.	n.d.	n.d.
heptanoic acid	n.d.	n.d.	n.d.	n.d.
octanoic acid	n.d.	n.d.	n.d.	n.d.
decanoic acid	n.d.	n.d.	n.d.	n.d.
dodecanoic acid	0.481	6.378	0.972	0.107
tetradecanoic acid	n.d.	n.d.	n.d.	n.d.
hexadecanoic acid	n.d.	n.d.	n.d.	n.d.
octadecanoic acid	n.d.	n.d.	n.d.	n.d.
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	0.962	6.671	0.989	0.249

Tab. S 4-7: Continued

Substance	m	n	R^2	p(lof)
carboxamides				
decanamide	0.475	6.278	0.972	0.585
dodecanamide	0.462	6.699	0.993	0.356
tetradecanamide	0.741	6.526	0.957	0.515
hexadecanamide	0.762	6.273	0.962	0.064
stearamide	0.852	6.550	0.951	0.592
oleamide	0.975	6.428	0.967	0.983
erucamide	0.730	5.829	0.965	0.129
phthalates				
diethylphthalate	n.d.	n.d.	n.d.	n.d.
di-butylphthalate	n.d.	n.d.	n.d.	n.d.
dicyclohexylphthalate	0.539	6.155	0.948	0.310
misc				
undecane	n.d.	n.d.	n.d.	n.d.
toluene	0.554	6.555	0.954	0.129
ethylbenzene	0.465	6.336	0.953	0.656
styrene	0.757	6.684	0.980	0.405
divinylbenzene	1.009	6.609	0.986	0.154
benzaldehyde	n.d.	n.d.	n.d.	n.d.
1,4-diacetylbenzene	0.811	6.281	0.989	0.603
1,3-diacetylbenzene	0.659	5.938	0.966	0.104
1,2-dicyanobenzene	n.d.	n.d.	n.d.	n.d.
chlorobenzene	1.055	6.654	0.972	0.069
ketones				
methyl-*iso*-butylketone	0.816	5.634	0.950	0.308
4-methyl-2-heptanone	1.184	6.922	0.986	0.067
cyclohexanone	0.673	5.998	0.955	0.961
5,5-dimethyl-2,4-hexandione	0.914	6.969	0.994	0.088

n.d. = not determinable

Tab. S 4-8 LOD and LOQ values determined within the method validation for the standards (LOD values are taken from chapter 3 for the sake of clarity

Substance	LOD	LOQ
phenols		
phenol	0.014	0.045
2'-hydroxyacetophenone	0.004	0.013
2-*tert*-butylphenol	0.001	0.002
4-*tert*-butylphenol	0.001	0.002
4-*tert*-amylphenol	0.001	0.003
2,6-di-*tert*-butylphenol	0.001	0.003
2,4-di-*tert*-butylphenol	0.003	0.008
bisohenol A	0.006	0.021
butylhydroxytoluene	0.005	0.016
alcohols		
2-ethylhexanol	0.004	0.015
benzyl alcohol	0.011	0.036
dodecanol	0.007	0.023
octadecanol	0.006	0.018
carboxylic acids		
2-ethyl hexanoic acid	1.000	3.300
heptanoic acid	1.000	3.300
octanoic acid	1.000	3.300
decanoic acid	1.000	3.300
dodecanoic acid	0.002	0.005
tetradecanoic acid	1.000	3.300
hexadecanoic acid	1.000	3.300
octadecanoic acid	1.000	3.300
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	0.014	0.046

Tab. S 4-8: Continued

Substance	LOD	LOQ
carboxamides		
decanamide	0.001	0.003
dodecanamide	0.003	0.011
tetradecanamide	0.009	0.028
hexadecanamide	0.008	0.025
stearamide	0.010	0.032
oleamide	0.011	0.035
erucamide	0.005	0.016
phthalates		
diethylphthalate	0.256	0.845
di-butylphthalate	0.100	0.330
dicyclohexylphthalate	0.004	0.012
misc		
undecane	1.000	3.300
toluene	0.035	0.116
ethylbenzene	0.048	0.158
styrene	0.004	0.013
divinylbenzene	0.001	0.003
benzaldehyde	0.100	0.330
1,4-diacetylbenzene	0.013	0.043
1,3-diacetylbenzene	0.009	0.030
1,2-dicyanobenzene	1.000	3.300
chlorobenzene	0.013	0.043
ketones		
methyl-*iso*-butylketone	0.008	0.028
4-methyl-2-heptanone	0.005	0.017
cyclohexanone	0.006	0.020
5,5-dimethyl-2,4-hexandione	0.016	0.053

n.d. = not determinable

Tab. S 4-9 Accuracy and trueness at the lower limits of the two validation ranges. LOQ was chosen as the limit instead, if LOQ was higher than the respective lower limit of the range

Substance	0.1 µg/kg (or LOQ)		LOQ	
	repeatability / %	trueness / %	repeatability / %	trueness / %
phenols				
phenol	14	94	17	101
2'-hydroxyacetophenone	8	105	22	93
2-*tert*-butylphenol	11	108	24	85
4-*tert*-butylphenol	8	94	8	91
4-*tert*-amylphenol	4	101	4	108
2,6-di-*tert*-butylphenol	6	103	10	112
2,4-di-*tert*-butylphenol	15	108	16	94
bisohenol A	12	101	16	101
butylhydroxytoluene	5	90	19	113
alcohols				
2-ethylhexanol	4	99	7	100
benzyl alcohol	12	106	14	91
dodecanol	17	110	15	86
octadecanol	8	103	13	98
carboxylic acids				
2-ethyl hexanoic acid	13	100	n.d.	n.d.
heptanoic acid	15	106	n.d.	n.d.
octanoic acid	14	100	n.d.	n.d.
decanoic acid	15	98	n.d.	n.d.
dodecanoic acid	7	93	13	113
tetradecanoic acid	16	94	n.d.	n.d.
hexadecanoic acid	16	110	n.d.	n.d.
octadecanoic acid	16	92	n.d.	n.d.
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	9	97	20	106

Tab. S 4-9: Continued

Substance	0.1 µg/kg		LOQ	
	repeatability / %	trueness / %	repeatability / %	trueness / %
carboxamides				
decanamide	7	108	19	86
dodecanamide	4	103	12	97
tetradecanamide	12	106	16	95
hexadecanamide	14	91	19	117
stearamide	12	97	18	109
oleamide	12	101	23	102
erucamide	14	91	10	113
phthalates				
diethylphthalate	10	106	n.d.	n.d.
di-butylphthalate	9	97	n.d.	n.d.
dicyclohexylphthalate	9	109	n.d.	n.d.
misc				
undecane	11	107	n.d.	n.d.
toluene	10	111	12	87
ethylbenzene	11	110	16	98
styrene	10	89	19	113
divinylbenzene	15	92	21	116
benzaldehyde	14	97	n.d.	n.d.
1,4-diacetylbenzene	14	103	19	95
1,3-diacetylbenzene	5	94	18	119
1,2-dicyanobenzene	3	98	n.d.	n.d.
chlorobenzene	14	92	19	120
ketones				
methyl-*iso*-butylketone	9	105	23	109
4-methyl-2-heptanone	6	91	18	109
cyclohexanone	15	105	17	102
5,5-dimethyl-2,4-hexandione	14	94	15	105

n.d. = not determinable

Tab. S 4-10 Summarized target ion peak areas for the effect of different matrices and water.
LH and LL denote lactate buffered matrices with a high (H) and low (L) glucose values. BH and BL are the corresponding the values given are average values for N = 3.

Substance	LH	BH	BL	LL	water
phenols					
phenol	5.3E+05	6.3E+05	5.8E+05	5.6E+05	7.4E+05
2'-hydroxyacetophenone	4.1E+05	4.1E+05	4.4E+05	4.7E+05	3.7E+05
2-*tert*-butylphenol	3.4E+05	3.5E+05	3.6E+05	5.1E+05	3.9E+05
4-*tert*-butylphenol	1.8E+05	1.5E+05	1.8E+05	2.1E+05	1.6E+05
4-*tert*-amylphenol	1.5E+05	1.5E+05	1.7E+05	1.9E+05	1.5E+05
2,6-di-*tert*-butylphenol	4.7E+05	3.4E+05	4.4E+05	4.4E+05	3.7E+05
2,4-di-*tert*-butylphenol	4.9E+06	5.9E+06	7.1E+06	4.1E+06	5.9E+06
bisphenol A	2.3E+05	2.8E+05	3.2E+05	2.8E+05	3.1E+05
butylhydroxytoluene	1.6E+05	1.9E+05	1.6E+05	1.8E+05	1.4E+05
alcohols					
2-ethylhexanol	4.6E+05	4.8E+05	5.6E+05	4.9E+05	4.2E+05
benzyl alcohol	2.8E+05	3.4E+05	2.6E+05	3.2E+05	2.5E+05
dodecanol	6.9E+05	6.1E+05	8.0E+05	1.2E+06	9.2E+05
octadecanol	7.3E+06	6.3E+06	8.2E+06	6.3E+06	6.2E+06
carboxylic acids					
2-ethyl hexanoic acid	1.5E+05	1.5E+05	1.9E+05	2.7E+05	1.9E+05
heptanoic acid	1.8E+05	1.4E+05	1.6E+05	2.5E+05	1.6E+05
octanoic acid	1.4E+05	1.0E+05	7.4E+04	8.1E+04	9.8E+04
decanoic acid	1.2E+05	8.5E+04	8.4E+04	7.8E+04	8.3E+04
dodecanoic acid	9.0E+05	7.9E+05	8.9E+05	9.0E+05	8.5E+05
tetradecanoic acid	5.8E+05	6.3E+05	5.6E+05	6.1E+05	5.3E+05
hexadecanoic acid	8.7E+05	1.1E+06	8.3E+05	7.5E+05	9.2E+05
octadecanoic acid	7.3E+04	5.4E+04	8.5E+04	5.3E+04	6.7E+04
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	1.8E+06	1.5E+06	1.5E+06	1.9E+06	1.7E+06
carboxamides					
decanamide	1.2E+06	9.6E+05	1.6E+06	1.4E+06	1.2E+06
dodecanamide	1.0E+06	1.1E+06	1.4E+06	9.2E+05	1.2E+06
tetradecanamide	1.3E+06	1.2E+06	2.1E+06	2.0E+06	1.6E+06
hexadecanamide	3.7E+06	3.4E+06	5.7E+06	3.0E+06	4.3E+06
stearamide	1.4E+06	1.9E+06	1.8E+06	2.2E+06	2.0E+06
oleamide	1.1E+06	8.8E+05	1.1E+06	1.5E+06	3.4E+05
erucamide	5.3E+05	7.3E+05	6.9E+05	7.8E+05	6.5E+05

Tab.S 4-10: Continued

Substance	LH	BH	BL	LL	water
phthalates					
diethylphthalate	4.5E+07	4.4E+07	4.8E+07	4.6E+07	5.3E+07
di-butylphthalate	2.2E+07	2.9E+07	2.3E+07	2.0E+07	2.4E+07
dicyclohexylphthalate	1.5E+06	1.2E+06	1.3E+06	1.4E+06	1.4E+06
misc					
undecane	2.0E+06	2.2E+06	2.2E+06	2.1E+06	1.7E+06
toluene	1.2E+06	1.1E+06	1.1E+06	1.1E+06	1.0E+06
ethylbenzene	3.4E+05	3.4E+05	4.2E+05	3.5E+05	3.6E+05
styrene	1.3E+05	1.3E+05	1.6E+05	1.6E+05	1.6E+05
divinylbenzene	1.1E+05	9.4E+04	9.2E+04	1.1E+05	1.0E+05
benzaldehyde	1.8E+07	1.6E+07	1.7E+07	1.8E+07	1.5E+07
1,4-diacetylbenzene	2.4E+05	3.1E+05	3.4E+05	3.6E+05	3.1E+05
1,3-diacetylbenzene	3.6E+05	4.1E+05	4.5E+05	5.9E+05	4.5E+05
1,2-dicyanobenzene	7.6E+06	8.5E+06	8.8E+06	8.6E+06	9.7E+06
chlorobenzene	4.0E+05	3.4E+05	4.0E+05	4.1E+05	3.6E+05
ketones					
methyl-iso-butylketone	7.9E+04	8.5E+04	9.2E+04	9.0E+04	7.4E+04
4-methyl-2-heptanone	2.1E+05	1.9E+05	1.7E+05	1.6E+05	1.7E+05
cyclohexanone	3.1E+06	2.3E+06	3.1E+06	3.0E+06	2.8E+06
5,5-dimethyl-2,4-hexandione	6.1E+05	4.7E+05	5.9E+05	6.8E+05	5.2E+05

n.d. = not determinable

4.6.4 Real Samples

Tab. S 4-11 Leachables and their concentrations found in the real samples; in bicarbonate (BIC) buffered solutions of suppliers 1 and 2 and in the lactate (LAC) buffered solution of supplier 1
Concentrations given are the average of a fourfold measurement of samples taken from two solution bags per lot.

Substance	1-BIC / µg/kg		1-LAC / µg/kg		2-BIC / µg/kg	
	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B
phenols						
phenol	0.725	0.705	0.560	0.575	0.615	0.615
2'-hydroxyacetophenone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-*tert*-butylphenol	n.d.	n.d.	0.025	0.019	n.d.	n.d.
4-*tert*-butylphenol	0.217	0.220	0.209	0.165	n.d.	n.d.
4-*tert*-amylphenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,6-di-*tert*-butylphenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,4-di-*tert*-butylphenol	6.608	6.623	10.231	7.849	1.314	1.349
bisohenol A	n.d.	n.d.	n.d.	n.d.	0.155	0.158
butylhydroxytoluene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
alcohols						
2-ethylhexanol	20.000	20.000	20.000	20.000	20.000	20.000
benzyl alcohol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
dodecanol	1.173	1.320	1.071	0.841	1.001	0.990
octadecanol	0.738	0.436	0.295	0.557	0.305	0.383
carboxylic acids						
2-ethyl hexanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
heptanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
octanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
decanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
dodecanoic acid	0.777	0.759	0.613	0.460	3.150	3.982
tetradecanoic acid	2.326	2.270	1.824	n.d.	20.000	20.000
hexadecanoic acid	6.327	6.589	7.724	5.355	6.998	8.540
octadecanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	4.849	4.285	6.962	7.535	3.678	4.422

Tab. S 4-11: Continued

Substance	1-BIC / µg/kg		1-LAC / µg/kg		2-BIC / µg/kg	
	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B
carboxamides						
decanamide	n.d.	n.d.	n.d.	n.d.	1.035	1.048
dodecanamide	0.140	0.070	0.111	0.034	0.051	0.065
tetradecanamide	0.077	0.106	0.104	0.096	0.096	0.140
hexadecanamide	0.156	0.131	0.115	0.108	0.065	0.169
stearamide	0.137	0.102	0.050	0.104	0.074	0.189
oleamide	0.224	0.140	0.070	0.054	0.061	0.065
erucamide	0.835	0.646	0.057	0.035	9.210	10.829
phthalates						
diethylphthalate	1.085	0.233	2.875	3.204	n.d.	n.d.
di-butylphthalate	0.504	0.548	0.440	0.500	0.394	0.393
dicyclohexylphthalate	0.025	0.016	0.020	0.035	n.d.	n.d.
misc						
undecane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
toluene	1.453	1.506	3.795	2.341	0.707	0.702
ethylbenzene	n.q.	n.q.	0.198	n.q.	n.q.	0.183
styrene	1.138	1.227	2.208	1.823	0.413	0.461
divinylbenzene	1.250	1.610	0.167	0.830	3.057	0.044
benzaldehyde	1.800	1.917	1.268	1.380	1.522	1.473
1,4-diacetylbenzene	0.113	0.075	n.q.	0.070	n.q.	0.048
1,3-diacetylbenzene	0.250	0.366	0.161	0.337	0.256	0.269
1,2-dicyanobenzene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
chlorobenzene	n.d.	n.d.	n.d.	n.d.	0.616	0.925
ketones						
methyl-*iso*-butylketone	6.886	8.423	14.531	16.166	0.373	0.483
4-methyl-2-heptanone	0.512	0.515	0.436	0.443	0.425	0.404
cyclohexanone	0.601	0.631	n.d.	n.d.	n.d.	n.d.
5,5-dimethyl-2,4-hexandione	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Tab. S 4-12 Leachables and their concentrations found in the real samples; in bicarbonate (BIC) and lactate (LAC) buffered solutions of suppliers 3 Concentrations given are the average of a fourfold measurement of samples taken from two solution bags per lot.

Substance	3-BIC / µg/kg		3-LAC / µg/kg	
	Lot A	Lot B	Lot A	Lot B
phenols				
phenol	n.d.	n.d.	n.d.	n.d.
2'-hydroxyacetophenone	n.d.	n.d.	n.d.	n.d.
2-*tert*-butylphenol	n.d.	n.d.	n.d.	n.d.
4-*tert*-butylphenol	0.071	0.082	0.240	0.272
4-*tert*-amylphenol	n.d.	n.d.	n.d.	n.d.
2,6-di-*tert*-butylphenol	n.d.	n.d.	n.d.	n.d.
2,4-di-*tert*-butylphenol	3.020	3.036	4.151	4.327
bisohenol A	n.d.	n.d.	n.d.	n.d.
butylhydroxytoluene	n.d.	n.d.	n.d.	n.d.
alcohols				
2-ethylhexanol	3.207	2.678	20.000	20.000
benzyl alcohol	n.d.	n.d.	n.d.	n.d.
dodecanol	0.913	0.752	0.767	0.816
octadecanol	0.166	0.261	0.344	0.164
carboxylic acids				
2-ethyl hexanoic acid	n.d.	n.d.	n.d.	n.d.
heptanoic acid	n.d.	n.d.	n.d.	n.d.
octanoic acid	n.d.	n.d.	n.d.	n.d.
decanoic acid	n.d.	n.d.	n.d.	n.d.
dodecanoic acid	0.805	0.838	0.411	0.381
tetradecanoic acid	4.992	5.962	4.039	2.239
hexadecanoic acid	3.309	4.953	9.977	8.352
octadecanoic acid	n.d.	n.d.	n.d.	n.d.
3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid	7.284	7.066	10.031	9.772

Tab. S 4-12: Continued

Substance	3-BIC / µg/kg		3-LAC / µg/kg	
	Lot A	Lot B	Lot A	Lot B
carboxamides				
decanamide	n.d.	n.d.	n.d.	n.d.
dodecanamide	n.d.	n.d.	n.d.	n.d.
tetradecanamide	0.050	0.039	0.064	0.065
hexadecanamide	n.d.	n.d.	0.075	0.074
stearamide	0.041	0.045	0.069	0.045
oleamide	n.q.	n.q.	0.072	n.q.
erucamide	0.078	0.112	0.199	0.112
phthalates				
diethylphthalate	n.d.	n.d.	n.d.	n.d.
di-butylphthalate	n.q.	n.q.	0.429	n.q.
dicyclohexylphthalate	n.d.	n.d.	n.d.	n.d.
misc				
undecane	n.d.	n.d.	n.d.	n.d.
toluene	7.543	6.681	0.374	0.350
ethylbenzene	20.000	20.000	7.966	8.636
styrene	20.000	19.384	0.895	0.753
divinylbenzene	n.d.	n.d.	n.d.	n.d.
benzaldehyde	3.940	2.997	3.471	2.980
1,4-diacetylbenzene	n.d.	n.d.	0.060	0.049
1,3-diacetylbenzene	0.071	0.100	0.100	0.078
1,2-dicyanobenzene	n.d.	n.d.	n.d.	n.d.
chlorobenzene	n.d.	n.d.	n.d.	n.d.
ketones				
methyl-*iso*-butylketone	2.361	1.914	0.433	0.379
4-methyl-2-heptanone	1.988	1.850	0.386	0.365
cyclohexanone	n.d.	n.d.	n.d.	n.d.
5,5-dimethyl-2,4-hexandione	n.d.	n.d.	n.d.	n.d.

n.d. = not determinable, n.q.=not quantifiable.

5

Automated dynamic headspace GC-MS for leachables from packaging materials in pharmaceutical solutions

5.1 Introduction

Compounds migrating out of plastic packaging materials are an issue of concern for the manufacturers that use those to package their goods. Frequently plastics are used for the package of food or pharmaceutical products [1–4], due to their low production costs, their low weight and the fact that their properties can be matched to a particular application within a wide range of possibilities. Some of these properties such as elasticity, light or UV resistance or antioxidant effects are usually achieved by the addition of low molecular weight components. In addition, small amounts of monomers, catalysts or organic solvents probably remain in the plastic after the production process. Finally, degradation products may form during the shelf life of the packed good. All in all there is a variety of low molecular weight substances in the plastic packaging, which may leach out and contaminate the packed product [5–8]. This risk of contamination especially for pharmaceutical products is addressed in the Guideline on Plastic Immediate Packaging Materials (CPMP/QWP/4359/03) that was published in 2005 by the European Medicines Agency (EMEA). Because these so called leachables may pose a safety risk to the patient, this guideline consequently requires the identification and quantification of migrating substances and demands a toxicological assessment. These considerations are especially important for pharmaceutical liquids due to the direct contact of the packaging with the product and the likelihood that leachables from the packaging are rapidly diffusing into the bulk solution.

For the evaluation of new plastic packaging materials but in particular for routine quality controls with a high number of samples to be analyzed, a simple, fast and cost effective analytical method for a multi-component mixture is desirable. Gas chromatography (GC) is the method of choice to fulfill this requirement. Historically, GC was the dominating method for the determination of residual solvents [9] and it still is the method of choice. It is therefore recommended by the United States Pharmacopoeia (USP) [10] but also in European Pharmacopoeia (EP) [11] and in the ICH guidelines topic Q3C as well. Both pharmacopoeias describe headspace analysis for samples where a direct injection onto the GC column is not feasible. Headspace analysis can be performed under static or dynamic conditions. In static headspace (SHS) the analytes in an aqueous sample equilibrate with the gas phase above and finally a part of the gas phase is taken for the GC analysis. In dynamic headspace (DHS) a continuous gas flow is either bubbled through (the so called purge and trap approach) or swept over an aqueous sample [12] and is then passed along an adsorbent to accumulate and enrich the analytes. A subsequent thermal desorption step transfers the analytes to the GC column. The advantages of DHS are on the one hand that it does not base on equilibrium conditions and thus there is no need to wait till the

equilibrium is reached. On the other hand it has higher sensitivity due to the enrichment step. Limits of detection (LOD) for DHS are usually at least ten times lower than for SHS [12]. Therefore, DSH is the preferred method for trace analysis even if it is technically more demanding than the static method.

There are some studies, which employ DHS to determine volatile components in solid pharmaceutical products [13–15] or in plastic packaging materials for pharmaceutical products [16,17], but there are no DHS studies that aim at the determination of leachables directly in pharmaceutical solutions and especially not in dialysis solutions used for peritoneal dialysis (PD) or hemodialysis (HD).

In the following the optimization and validation of a fully automated DHS method is described, which is perfectly suited for a high sample throughput in daily quality control due to its solventless and very cost and time effective character. At the same time it offers a high sensitivity to detect leachables in the sub µg/kg range, which is important for a high-level quality assurance required nowadays for existing plastic packaging materials but also for the qualification of new materials. In order to minimize water vapor interferences and to avoid a time-consuming drying step of the adsorbent the gas flow is swept over the surface of the aqueous sample instead of purging it through the sample. Furthermore, this procedure also prevents effectively the risk of cross contaminations.

5.2 Experimental

5.2.1 Solvents, Chemicals

For the standard stock solution all standards given below were dissolved in ethanol to give a final concentration of about 330 mg/kg for each of the 23 substances. Phenol, 2'-hydroxyacetophenone, 2-*tert*-butylphenol, 4-*tert*-butylphenol, 2,6-di-*tert*-butylphenol, 2,4-di-*tert*-butylphenol, butylhydroxytoluen, cyclohexanol, 2-ethylhexanol, benzyl alcohol, dodecanol, diethyl phthalate, di-*iso*-butyl phthalate, di-butyl phthalate, undecane, 2-(2-butoxyethoxy)ethyl acetate, ethylbenzene, styrene, divinylbenzene, benzaldehyde, 1,2-dicyanobenzene, chlorobenzene were purchased from Merck KGaA (Darmstadt, Germany). 4-tert-amylphenol was purchased from Sigma-Aldrich Co. LLC (Steinheim, Germany). All substances were purchased in the highest purity available.

In comparison to chapters 3 and 4 a reduced set of analytes representing typical leachables from plastic packaging materials was used.

Di-*iso*-butylphthalate was excluded from this study as it turned out that it was not reasonable quantifiable, which was reasoned with contaminations. Phthalate analysis is known to be troublesome due to their background level in every laboratory [18, 19].

The ethanolic stock solution of the screening standards is stable for at least 6 months, where “stable” means that there are no significant changes in the target ion peak areas of the compounds. This was checked on a separate GC-MS, with a validated method by direct injection of the standard solution.

The water used for all experiments was purified with a Milli-Q Gradient system from Millipore.

The matrices employed were a bicarbonate buffered solution (called “BIC”) at pH 7.2, a lactate buffered solution (called “LAC”) at pH 7.0 and two phosphate buffered solutions at pH 2 and pH 10. For the BIC solution the molarity of the main components are: 140 mmol/L Na^+, 35 mmol/L HCO_3^- and 5.6 mmol/L glucose. The composition of the LAC solutions was 134 mmol/L Na^+, 35 mmol/L lactate and 83.2 mmol/L glucose. The phosphate buffer with pH 2 was prepared by dissolving 8.95 g of disodium hydrogen phosphate (dodecahydrate) and 3.4 g of potassium dihydrogen phosphate in 1 L water and adding about 20 mL phosphoric acid to adjust pH to 2. The basic phosphate buffer solution was prepared by dissolving 17.4 g potassium dihydrogen phosphate in 1 L water and adding about 130 mL 1 M potassium hydroxide solution to adjust pH to 10.

The water used for all experiments was purified with a Milli-Q Gradient system from Millipore. Methanol (p.a., Merck KGaA) and sodium chloride in p.a. quality were purchased

from Bernd Kraft (Duisburg, Germany). All chemicals were used without further purification.

The standard stock solution was used to prepare samples with concentrations from 100 µg/kg down to 0.1 µg/kg freshly every day by diluting it with water. For studying matrix effects samples were prepared in the same way but instead of water the respective matrix solution was used for the dilution steps.

Tenax TA adsorption tubes (length 60 mm, inner diameter 4 mm, 60 mg Tenax TA (60/80 mesh) were purchased from Gerstel GmbH (Mülheim a.d. Ruhr, Germany). New Tenax tubes were conditioned at 280°C for 10 min before the first use. No special cleaning or conditioning was applied to the used Tenax tubes.

5.2.2 Sample extraction procedure

In the optimized procedure a sample volume of 10 mL was placed in 20 mL glass headspace vials. Afterwards the vials were closed with a septum screw cap (septum: Silicone / PTFE, 45° Shore A, 1.3 mm) and placed on the auto sampler tray. The samples were automatically agitated at 250 rpm at a temperature of 35°C while being extracted under a helium flow of 60 mL/min for about 13 min after a 5 min incubation time.

All DHS optimization experiments were done in a way that the parameter under study was varied, while all the other parameters were kept constant.

5.2.3 TD-GC-MS analyses

The GC-MS measurements were performed using a GC 7890 system from Agilent equipped with a DHS module (Gerstel, Mülheim a.d. Ruhr, Germany) for the agitation and heating of the sample, a thermal desorption unit “TDU” (Gerstel) and a cold injection system “CIS” (Gerstel). For the adsorption of volatile components Tenax TA in adsorption tubes made of glass were used. A multipurpose auto sampler “MPS” (Gerstel) was used to introduce these tubes into the TDU. The desorption took place in solvent vent mode at 250°C for 5 min. Helium 5.0 at a flow of 50 mL/min was used to transfer the analytes into the CIS where they were focused at 20°C. Finally the CIS was heated up to 260°C at a speed of 12°C/s and the analytes were injected into the GC column. Helium 5.0 was used as the carrier gas at a constant flow of 1 mL/min. The temperature program used for chromatographic separation involved increasing the temperature from 50°C for 1 min to 150°C at a rate of 20°C/min, maintaining it at this level for 1.5 min before increasing the temperature further at the same rate to 300°C, and then maintaining the temperature at this level for 6 min. The transfer line to the mass spectrometer (MS) was heated to a temperature 150°C and

the ion source was held at a temperature of 230°C. The detector was an Agilent 5973 quadrupole mass spectrometer with an electron impact (EI) source. Mass-to-charge ratios (m/z) were recorded at values between 16 and 550. The column used was a HP-5MS capillary column (length 30 m, diameter 0.25 mm, film thickness 0.25 µm, stationary phase: 5% phenylmethylsiloxane, 95% dimethylpolysiloxane).

5.3 Results and discussion

5.3.1 DHS Optimization

Drying of the adsorbent

Despite the low water adsorption properties of Tenax it is usually dried after a dynamic headspace extraction by purging it with some hundred mL of an inert gas [20]. Assuming a typical flow of a few 10 mL/min the drying step takes easily about 30 min. Therefore, the necessity of the drying step was studied with respect to a shorter analysis time and to decide if drying can be omitted without a loss of sensitivity. For one sample extraction the Tenax adsorbent was kept at a temperature of 35°C and was subsequently dried with 300 mL helium at a flow of 15 mL/min. For the comparative measurement the Tenax was at a higher temperature of 60°C to avoid excessive water adsorption but the drying step was left out. No significant differences were observed between both approaches as summarized in the supplement chapter 5.6.2. However, the analysis time was 20 min longer for the drying procedure. Since a short analysis time increases the sample throughput the higher Tenax temperature without an additional drying step was favored and subsequently used.

Adsorbent Temperature

As dynamic headspace offers the option to have different temperatures for the sample and the adsorbent, the effect of temperature of the latter at a constant sample temperature of 35°C was studied. Even if Tenax TA is known for its low water adsorption [21] it still adsorbs sufficient water in a high humidity environment that this amount will disturb the chromatographic separation afterwards. Therefore, the adsorbent was kept at a higher temperature than the sample and the optimal adsorbent temperature was studied between 50°C and 70°C. In figure 5-1 relative extraction yields are given, normalized to target ion peak areas at 50°C. All date are presented in the supplement chapter. With the exception of cyclohexanol all data have a common characteristic, because all of them show a maximum value at 60°C. For a higher adsorbent temperature especially the highly volatile substances like for example chlorobenzene, styrene or ethylbenzene show a decrease of the extracted amounts even below the 50°C value because they already desorb in non-negligible amounts. The lower extraction yield at 50°C compared to 60°C can presumably be explained by the adsorption of water molecules, which occupy free places on the adsorbent surface. It is well known that low molecular substances easily adsorb on Tenax TA

and similar sorbents and occupy adsorption places. Finally 60°C was taken as the optimal Tenax temperature.

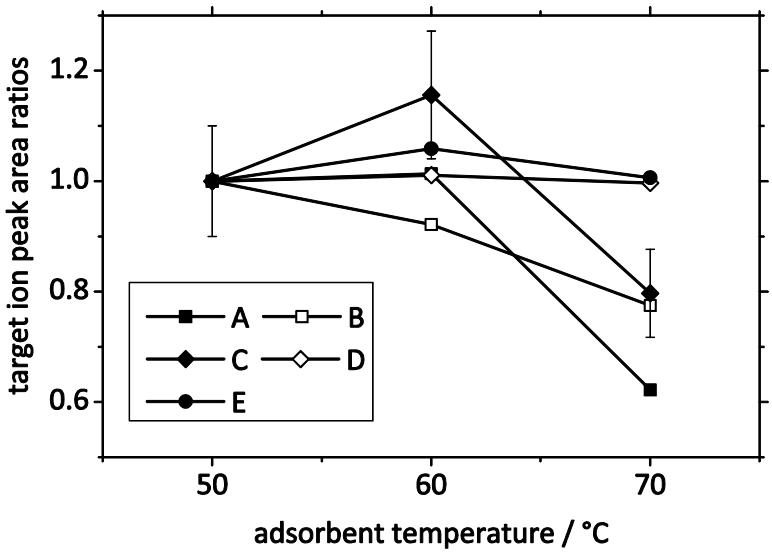

Figure 5-1 Influence of the adsorbent temperature on the detected target ion peak areas.
Peak areas for chlorobenzene (A), cyclohexanol (B), ethylbenzene (C), 2-*tert*-butylphenol (D) and 2-ethylhexanol (E) are normalized to the respective ion peak areas at 50°C. Standard deviations (N=3) were below 10% for the analytes.

Purge volume and purge flow

Both parameters directly influence the extraction efficiency and mainly determine the runtime for an analysis. Combinations of these two parameters are conceivable: a large purge volume at a high purge flow to minimize the time needed for an intensive purging of the headspace or a large purge volume at a low flow to have a higher saturation of the purging gas at the cost of a longer analysis time. To study the influence of every parameter alone, one parameter was varied while keeping the other one constant.

To study the influence of the purge volume a sample was purged at a constant flow of 60 mL/min with varying total volumes from 200 mL up to 800 mL. The resulting target ion peak areas normalized to the peak area at a volume of 200 mL are shown in figure 5-2 for some of the components. It is obvious that higher purge volumes give an approximately linear increase in the extraction yield. The optimal purge volume is clearly the highest applied volume of 800 mL. This behavior fits perfectly to the theoretical recoveries *R* for a DHS experiment calculated with equation (5-1) [19, 22].

$$R = 1 - exp\left(-\frac{F \cdot t}{\frac{V_w}{K_{aw}} + V_g}\right) \quad (5\text{-}1)$$

where F is the purge flow, t is the purge time, K_{aw} is the air-water partition coefficient that is also called Henry's law constant, and V_w and V_g are the sample and headspace volume, respectively.

For substances with a high K_{aw} a smaller purge volume is sufficient to significantly reduce the amount of analyte in the sample. Thus higher purge flow just lead to a low additional gain in extraction efficiency. Conversely, the extraction efficiency for substances with a K_{aw} below 10^{-3} linearly increases with the purge volume up to a factor of 4 when increasing the purge volume from 200 mL to 800 mL.

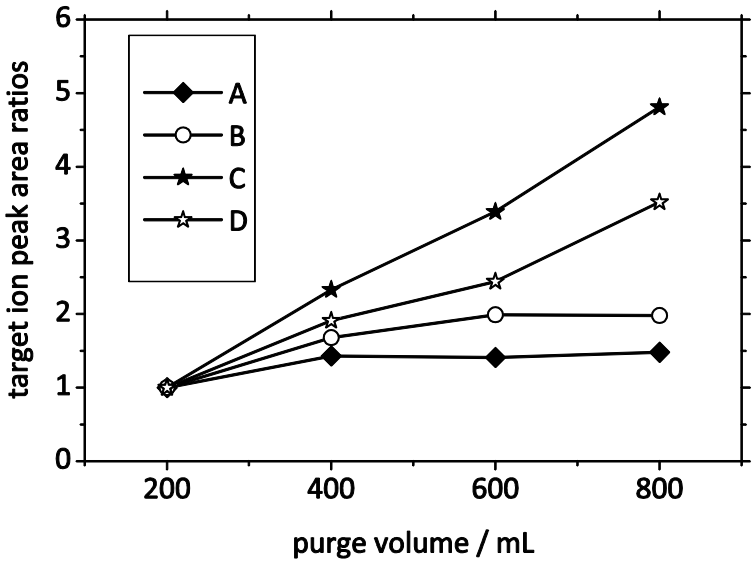

Figure 5-2 Influence of the purge volume on the detected target ion peak areas. Peak areas for ethylbenzene (A), divinylbenzene (B), 2'-hydroxyacetophenone (C) and 2,4-di-*tert*-butylphenol (D) are normalized to the respective ion peak areas at 200 mL. Standard deviations (N=3) were below 10% for the analytes.

The influence of the purge flow was studied at a purge volume of 800 mL by varying the purge flow from 20 mL/min to 80 mL/min and thereby reducing the purge time from 40 min to 10 min. Regarding equation (5-1) the recovery is constant for a constant total purge volume, independent of how it was achieved; either at a low purge flow and a long purging time or a high flow at a short purging time. This behavior was observed for the

highly volatile substance having a high K_{aw} as shown in figure 5-3a. Here the target ion peak areas were independent of the purge flow. But for the components with a low K_{aw} a significant decrease in the peak areas was observed for the highest purge flow of 80 mL/min, which is illustrated in figure 5-3b. A possible explanation is that the air-water-transfer velocities decrease for components having low K_{aw} values [23]. Thus their extraction becomes less efficient at too high purge flows. In additon, measurements were performed with a purge volume of 400 mL at flows of 40 mL/min, 60 mL/min and 80 mL/min. Surprisingly, a reduced extraction efficiency was now observed for the highly-volatile components as well at the highest purge flow. This finding can not be explained by air-water-transfer velocities. Thus, another possibility is that the flow conditions for the purge gas through the headspace volume get worse for higher flow and thus the sample is extracted less efficient. This impairment is not apparent with large purge volumes for components with a high K_{aw}, because these highly volatile substances exhibit a very high recovery - even at worsened flow conditions.

Based on these findings a purge volume of 800 mL at a purge flow of 60 mL/min was determined to be optimal. The results presented here exemplary are summarized in the supplement chapter 5.6.2.

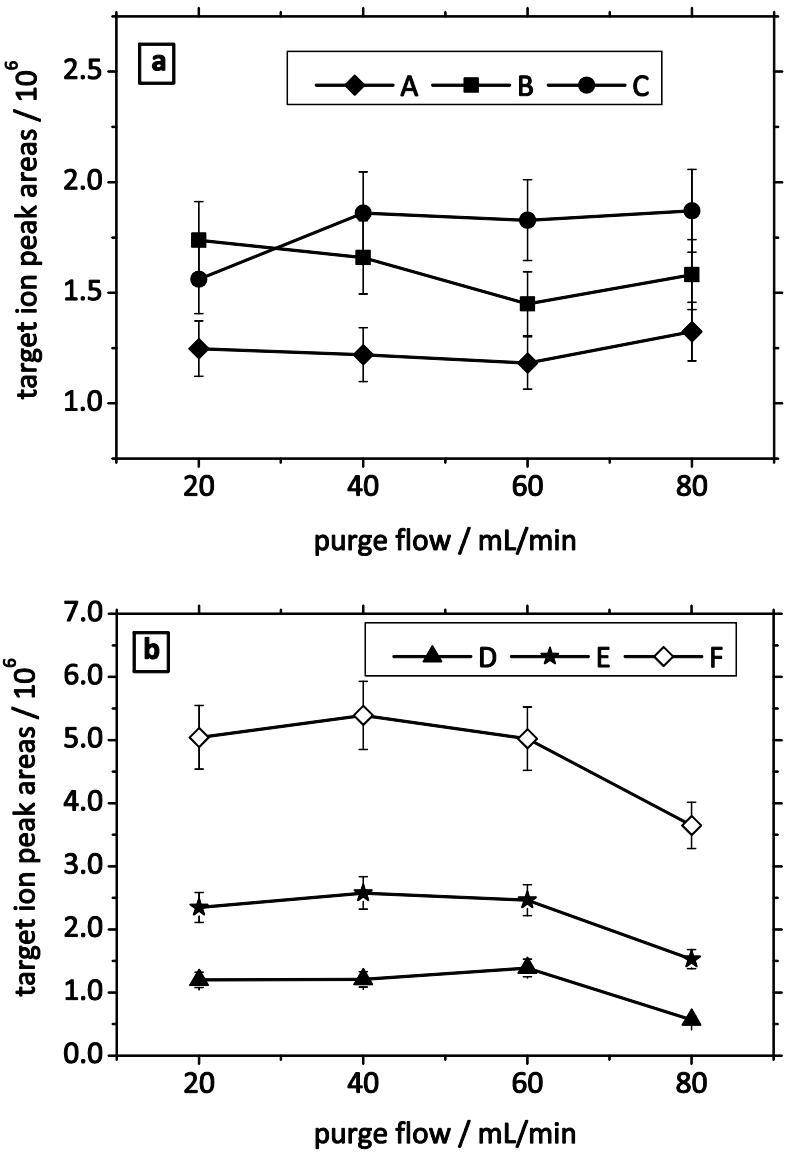

Figure 5-3 Influence of the purge flow on the extraction efficiency for highly volatile (a) and semi-volatile (b) components at a purge volume of 800 mL
Data are presented for ethylbenzene (A), chlorobenzene (B), 2-ethylhexanol (C), benzaldehyde (D), 2-hydroxyacetophenon (E) and 2-*tert*-butylphenol (F). Error bars represent standard deviations (N=3) of 10%

Incubation time

Incubation time is the time to equilibrate the sample before the extraction. During this time the sample is agitated and heated but not purged. The incubation time ensures that the sample has a homogenous temperature and thus is important for the reproducibility of the measurement. For a constant temperature of 35°C the incubation time was varied

from 3 min to 10 min. In doing so the target ion peak areas increased for a few substances, like for example chlorobenzene, styrene or ethylbenzene, at a longer incubation time of 5 min instead of 3 min, but even longer times did not lead to a further increase. Therefore, an incubation time of 5 min was chosen as the optimal time.

Incubation temperature

To optimize the incubation temperature at which the sample was kept during the extraction process the temperature was varied in 5°C steps between 20°C and 40°C. Figure 5-4 shows that for all substances the detected ion signal increased at higher temperatures as expected. A higher temperature T increases the air-water-partition coefficient $K_{aw,0}$ at the temperature T_0 [24,25] as shown by equation (5-2)

$$K_{aw,T} = K_{aw,0} \cdot exp\left(-\frac{\Delta_{aw}H}{R_g}\left(\frac{1}{T}-\frac{1}{T_0}\right)\right) \qquad (5\text{-}2)$$

where $\Delta_{aw}H$ is the enthalpy the phase transfer and R_g is the gas constant.

It turns out that at temperatures above 35°C the amount of water that gets adsorbed on the Tenax was so high that it disturbs the gas chromatographic separation. A clear shift in the retention times, a tailing of the peaks and a quite high background noise for the early retention times up to the 5th minute were observed at an incubation time of 40°C [26–28]. The increase in extraction efficiency for a temperature increase from 20°C to 35°C was not the same for all analytes, as expected due to the substance specific term $\Delta_{aw}H$ /R_g in equation (5-2). Data for this term of some of the components used here were given in [25]. The increase in recoveries R due to the increase in $K_{aw,T}$ was calculated for some exemplary substances: for phenol the calculated R increases 3.2 times vs. 4.7 times observed in the experiment; for benzaldehyde the theoretical R increases 2.3 times vs. 3.1 times in the experiment and for chlorobenzene the calculated R kept constant, whereas in the experiment a slight increase of a factor of 1.2 was observed. Comparing the theoretical and experimental values an at least qualitative correlation becomes obvious, which indicates that the temperature dependence of K_{aw} mainly provokes the observed increasing extraction yields.

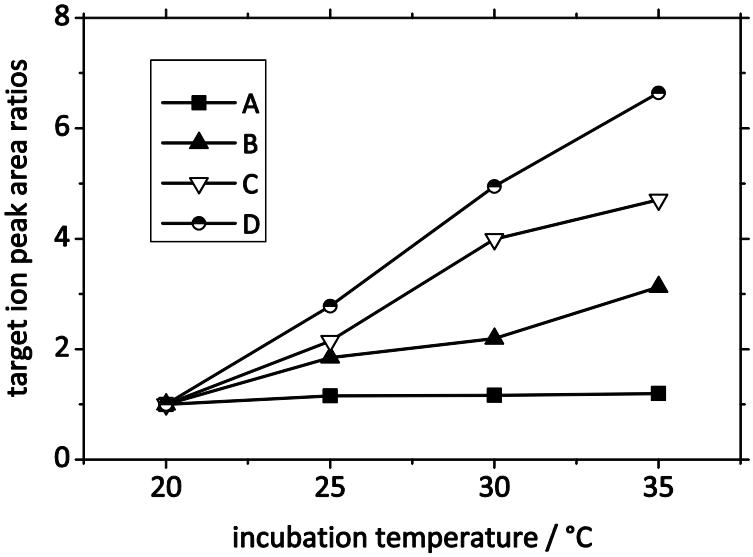

Figure 5-4 Influence of the incubation temperature on the detected target ion peak areas.
Peak areas for chlorobenzene (A), benzaldehyde (B), phenol (C) and 4-tert-amylphenol (D) are normalized to the respective ion peak areas at 20°C.
Standard deviations (N=3) were below 10% for the analytes.

Sample Volume

To study the influence of the sample volume five volumes 2 mL and 10 mL were tested. Larger volumes were not suitable as the headspace vials had a total volume of 20 mL and a sample volume of more than 50% of the total volume poses the risk that water droplets could form while purging the headspace which in turn can lead to an increased transport of water onto the adsorbent. For this comparison a purge flow of 60 mL/min, a total purge volume of 800 mL and an incubation temperature of 35°C were chosen. Regarding equation (5-1) an increasing volume should lead to a proportional increase in the target ion peak area for a highly volatile component, whereas for the semi-volatile substance a less pronounced or even no increase should be observed. This theoretically expected increase in extraction yield was just seen for the highly volatile substances, but instead a linear, a nonlinear increase was observed (shown in figure 5-5a) where the increase got smaller for higher sample volumes. Surprisingly a linear increase in target ion peak area was seen for the semi-volatile components as depicted in figure 5-5b. These findings, summarized also in the supplement chapter, cannot be explained by the theory. Presumably the purging efficiency of the headspace depends on the volume to be purged. A small sample volume of for example 2 mL results in a headspace volume in the vial of 20 mL. As the inlet for the

purge flow as well as the outlet are placed next to each other in the upper part of the vial, the incoming purge flow probably did not purge this big volume properly. The improved flushing for higher sample volumes and thus smaller headspace volumes resulted in the signal increase for the semi-volatile components.

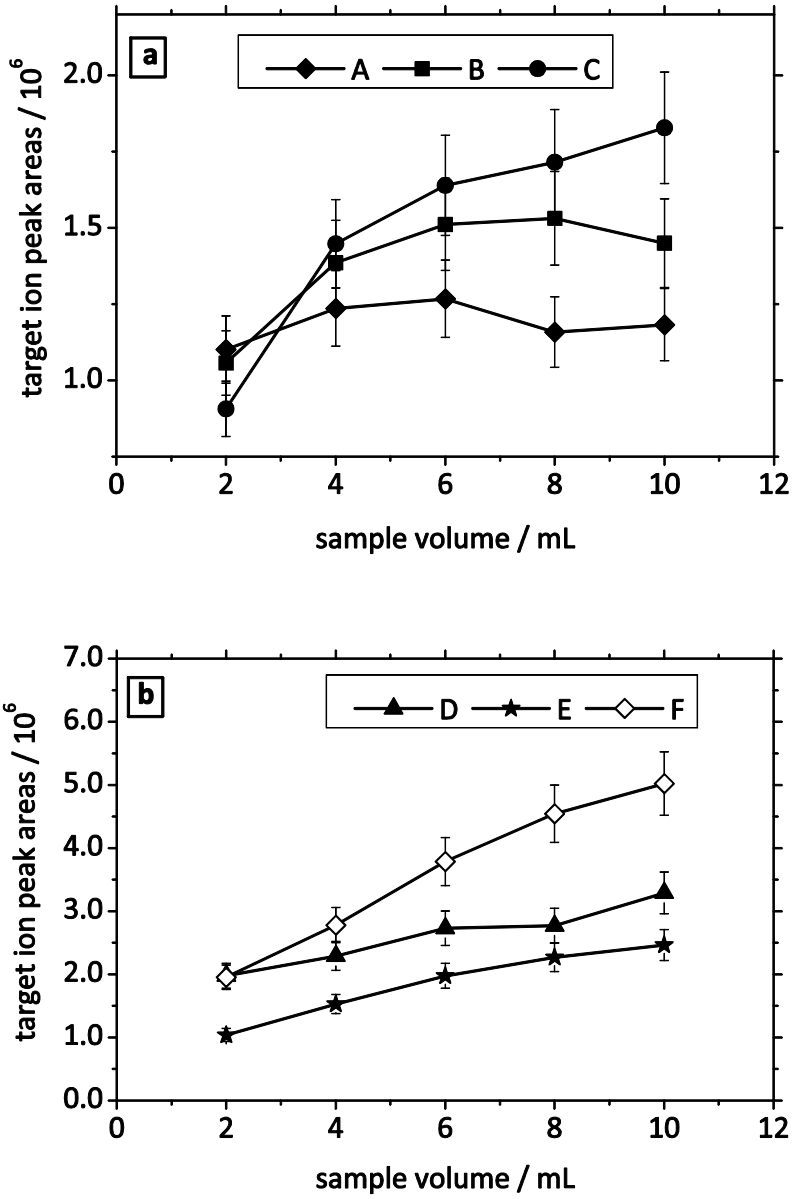

Figure 5-5 Influence of the sample volume on the extraction efficiency for highly volatile (a) and semi-volatile (b) components
Data are presented for ethylbenzene (A), chlorobenzene (B), 2-ethylhexanol (C), benzaldehyd (D), 2-hydroxyacetophenon (E) and 2-tert-butylphenol (F). Error bars represent standard deviations (N=3) of 10%

Addition of Salt and Methanol

It is known that the addition of salt and the associated increase of the ionic strength influences the solubility of organic components in water [29–31]. This so called salting out effect depends on the polarity of the substance and can lead to an increased or decreased solubility. As the substances within this study comprise a wide range of polarities the salting out effect was studied here by adding 1 g, 2 g and 3 g sodium chloride to the 10 mL samples. In addition, the effect of methanol on the extraction was studied. Especially for non-polar analytes the addition of alcohol in the range of 5% to 10% (v/v) might reduce the adsorption of these substances on the vial surface [32]. Methanol does not disturb the DHS process, as it possesses a low breakthrough volume on Tenax [33].

For the addition of salt the expected behavior was found. For polar substances such as benzaldehyde, 2'-hydroxyacetophenone or phenol a 50% increase in the target ion peak areas was observed for 3 g NaCl. In contrast nonpolar components, for example butylhydroxytoluene or undecane, were 50% less efficiently extracted. All in all the positive effect of salt for some analytes was balanced by the negative effect for others. Therefore, the addition of salt was waived. Adding methanol also did not lead to be a general enhancement. For some components an increase in extraction yield was observed, for others a decrease occurred. In contrast to the results for NaCl, no correlation was seen to the polarity in form of the octanol-water partition coefficient $K_{O/W}$ nor to the volatility, given by K_{aw}. Therefore, methanol was not further employed in this method.

Multiple Extractions

Multiple extractions were performed with the same sample to study the extraction efficiency with the optimal conditions determined above. Eight consecutive extractions were performed at a sample temperature of 35°C with a purge flow of 60 mL/min and a total purge gas volume of 800 mL/min. The adsorbent was kept at 60°C.

In figure 5-6 depletion curves for some highly volatile and some less volatile analytes are exemplified. All curves exhibit the typical exponential decrease in signal intensity as expected for a multiple extraction. This behavior is more pronounced for the substances with high K_{aw} values. Due to their high tendency to leave the aqueous phase and to favor the gaseous phase, these compounds got totally depleted from the solution. In contrast, semi-volatile components showed a linear-like decrease in their target ion peak areas for the repeated extractions. This is of course still an exponential decay, but due to their high solubility they possess just a low concentration in the gas phase. Therefore, just small amounts get removed in every subsequent extraction and in contrast to a total depletion

the signal drops just about 30% or less. Thus the exponential decrease becomes less obvious.

To calculate the extraction efficiencies for the one step extraction, the peak area of the whole analyte amount before the extraction must be known. This can either be determined by a direct injection of the sample in the GC – which was not possible here – or by employing a mathematical fit to the multi extraction curves. This can be accomplished as described in [34] by plotting $\ln(A_i)$ vs. (i-1), where i is the number of the extraction step and A_i is the corresponding target ion peak area. The slope b of this line allows in combination with the peak area of the first extraction A_1 the calculation of the total peak area A_t as given in equation (5-3).

$$\mathrm{A_t} = \frac{\mathrm{A_1}}{1-\mathrm{b}} \tag{5-3}$$

The ratio of A_t and A_1 gives the efficiency of the extraction procedure and is summarized in table 5-1. As expected, for increasing K_{aw} values the extraction becomes more efficient. For example the amount of ethylbenzene (K_{aw} = 0.3) in the sample was reduced by 70% in the first extraction, whereas just 4% of 4-tert-butylphenol (K_{aw} = $2.5 \cdot 10^{-5}$) got extracted under the same conditions. Similar results were obtained by equation (5-1) assuming that the i^{th} extraction corresponds to a single extraction with a duration i times longer.

The concentration of high K_{aw} substances such as chlorobenzene, ethylbenzene or styrene dropped to below 10% already after the 2nd extraction and below 1% after the 4th extraction. For semi-volatile components even after the 8th extraction 50% or more of the initial concentration were found in the sample.

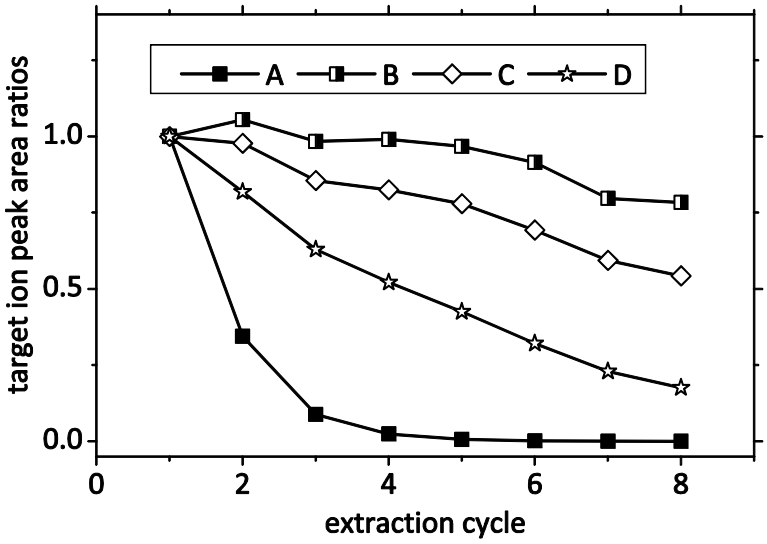

Figure 5-6 Decrease in detected ion peak areas for a multiple extraction of the same sample.
Peak areas for chlorobenzene (A), 4-tert-butylphenol (B), 2-tert-butylphenol (C) and 2,4-di-tert-butylphenol (D) are normalized to the respective ion peak for the 1st extraction.
Standard deviations (N=3) were below 10% for the analytes.

5.3.2 Method validation

Based on the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline [35] method validation involves testing the specificity of the particular method, determining its linear range, its limits of detection (LOD) and quantification (LOQ) and its accuracy, discussed in terms of repeatability and trueness. In the context of this work, the validation was carried out for pure water, a bicarbonate buffered and a lactate buffered matrix at pH 7 and two phosphate-buffered solutions at pH 2 and pH 10. All results are summarized in the supplement chapter 5.6.2.

The optimized experimental parameters given in the experimental section were used. The concentrations for the validation ranged from 100 µg/kg to 0.1 µg/kg.

Matrix Effects

Beside water four matrices were tested with regard to their influence on the dynamic headspace extraction of the compounds under study. Two of these matrices represent typical dialysis solutions, of which one is buffered with bicarbonate (called “BIC”) and the other one is buffered with lactate (called “LAC”). The remaining two matrices are phosphate buffers at pH 2 and 10 and were chosen to study the influence of pH.

None of the four matrices show a strong effect at high analyte concentrations above 10 µg/kg. Here the target ion peak areas for most of the substances differ less than 20% from the pure water values and all peak areas are within a range below 30% difference. But at concentrations below 1 µg/kg, influences of the matrix became obvious. Deviations of 50% or more were observed for several analytes. These deviations are not acceptable, especially not in regard to the aimed application of this method: trace analysis of leachables in pharmaceutical solutions. Thus a method validation for every single matrix was performed as described in the following.

Specificity

The ICH [35] definition says that specificity is the ability to unambiguously determine a particular analyte in the presence of other components. Within this study two parameters are used for the analyte identification: its retention time and its mass spectrum. Both were determined with pure substances in a single analyte measurement. Based on these data an unequivocally identification of all compounds is ensured and thus the specificity of the method is given.

Linearity

Linearity was studied for the range from 100 µg/kg to 0.1 µg/kg with 7 calibration points (100 µg/kg, 50 µg/kg, 10 µg/kg, 5 µg/kg, 1 µg/kg, 0.5 µg/kg and 0.1 µg/kg). Three replicates were measured for every concentration. For some of the compounds the LOQ was above the lowest concentration of 0.1 µg/kg. In this case the lowest concentration above the LOQ value was chosen as the limit for the validation range.

The residual plots of the linear fits showed a heteroscedasticity. Therefore, the logarithm was taken of the x (standard concentration) and y-values (peak area of the target ion), which finally results in a homogeneous variance within the validation ranges. The slope, the intercept and the coefficient of correlation R^2 were determined for a linear regression of the values. The calculated R^2 values were higher than 0.99 for nearly all components in

all five matrices. The exceptions had slightly lower R^2 values, which were still above 0.98. The R^2 values for the pure water matrix are listed in table 5-1, whereas the R^2 values for the other matrices are given in the supplement chapter.

Limit of Detection and Quantification

The LOD is defined as the analyte concentration at which the analytical method gives an unambiguous signal that is at least three times the S/N ratio of a blank [35], whereas the LOQ is defined to be the tenfold S/N ratio. Blanks were prepared as usual by performing all analytical steps, which are part of the method, but without adding the analyte. For most of the components the lowest concentration of 0.1 µg/kg was already 10 times higher than the S/N ratio and thus no LOD but LOQ values were determined instead. Exceptions are 1,2-dicyanobenzene and 2-(2-butoxyethoxy)ethyl acetate. The first one could only be determined at a concentration of 50 µg/kg and above, the second exhibited an even higher of LOD of 100 µg/kg. The remaining components could be quantified at concentrations of 0.5 µg/kg or below. The LOQ values for the water matrix validation are given in table 5-1. The values for the remaining matrices are summarized in the supplement chapter 5.6.2.

Accuracy

Precision was determined as the repeatability of the same sample measured three times immediately after each other in the same run. Repeatability at the limit of quantification was below 10% for most of the components independent of the matrix. For the remaining analytes, the repeatability was determined to be below 20%. Accuracy data in water are presented in table 5-1. The trueness of the method was evaluated by determining the recovery. It was calculated as the ratio between the mean concentration found in the sample and the concentration of the analyte added. The recoveries were between 80% and 120%. Data for accuracy and trueness for all matrices are given in the supplement chapter.

Table 5-1 Summarized K_{aw}-data, validation results for the water matrix and efficiencies for the extraction under optimized conditions

Substance	K_{aw} [#]	R^2	LOQ / µg/kg	repeatability / %	extraction efficiency / %
phenols					
phenol	1.40E-05	0.998	0.1	8	2
2'-hydroxyacetophenone	4.10E-04	0.999	0.1	4	11
2-*tert*-butylphenol	1.04E-03	0.997	0.1	13	8
4-*tert*-butylphenol	2.50E-05	0.998	1.0	7	4
4-*tert*-amylphenol	8.18E-05	0.991	5.0	3	4
2,6-di-*tert*-butylphenol	1.29E-04	0.997	0.1	9	63
2,4-di-*tert*-butylphenol	1.29E-04	0.999	0.1	8	22
butylhydroxytoluene	3.65E-03	0.997	0.1	11	60
alcohols					
cyclohexanol	1.40E-04	0.998	0.1	9	3
2-ethylhexanol	1.10E-03	0.996	0.1	6	22
benzyl alcohol	1.40E-05	0.998	0.1	7	3
dodecanol	5.00E-03	0.999	0.1	9	47
phthalates					
diethylphthalate	1.70E-06	0.997	0.5	7	5
di-butylphthalate	4.99E-05	0.998	0.1	11	7
misc					
undecane	2.53E-02	0.998	0.1	17	53
2-(2-butoxyethoxy)ethyl acetate	1.43E-05	0.997	n.q.	n.d.	4
ethylbenzene	3.00E-01	0.997	0.1	14	74
styrene	8.00E-02	0.997	0.1	12	67
divinylbenzene	5.70E-02	0.998	0.5	14	65
benzaldehyde	1.09E-03	0.997	0.1	4	13
1,2-dicyanobenzene	2.04E-05	0.960	50.0	6	4
chlorobenzene	1.27E-01	0.997	0.1	5	69

[#] Henry's law constants from http://toxnet.nlm.nih.gov/index.html and referenced there

5.4 Conclusions

The optimization and validation of a fully automated dynamic headspace method for the determination of 23 analytes is presented. These analytes are known to be typical leachables from plastic packaging materials. With the exception of three analytes the limits of quantification were 0.5 µg/kg or less with repeatabilities below 10% for most of the components. The linear fits mainly R^2 values above 0.990. Hence this study has shown that this new method for the multi leachable study in pharmaceutical solutions is most suitable for a daily laboratory routine due to its simple and fully automated sample preparation and that it allows trace analysis in the sub µg/kg range. The method is not limited to the analytes used to prepare the standard solution, because the MS was operated in scan-mode and thus a screening for even unknown leachables is possible.

Further work will demonstrate the reliability of the described method by quantifying leachables in dialysis solutions currently available on the market.

5.5 References

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5.6 Supplement

5.6.1 Solutions

Tab. S 5-1 Exemplary preparation of the used stock solution "screening standard (SC)" by weighing and solving all standards in 88.1236 g of ethanol

Substance	Weight mg	Concentration mg/L
phenols		
phenol	28.9	325.2
2'-hydroxyacetophenone	34.3	386.0
2-*tert*-butylphenol	31.2	348.9
4-*tert*-butylphenol	34.1	383.7
4-*tert*-amylphenol	30.7	345.5
2,6-di-*tert*-butylphenol	29.7	334.2
2,4-di-*tert*-butylphenol	26.8	301.6
butylhydroxytoluene	28.7	323.0
alcohols		
cyclohexanol	25.3	284.7
2-ethylhexanol	26.9	302.7
benzyl alcohol	26.6	299.3
dodecanol	26.0	292.6
phthalates		
diethylphthalate	31.9	359.0
di-butylphthalate	31.1	350.0
di-iso-butylphthalate	28.3	318.5
misc		
undecane	26.4	297.1
2-(2-butoxyethoxy)ethyl acetate	28.7	323.0
ethylbenzene	25.9	291.5
styrene	28.3	318.5
divinylbenzene	30.0	337.8
benzaldehyde	31.2	351.1
1,2-dicyanobenzene	28.0	315.1
chlorobenzene	32.4	364.6

Tab. S 5-2 Exemplary preparation of the 100 µg/kg mixed standard

SC weight / mg	SC average concentration / mg/kg	solvent	solvent weight / g	SC final concentration / µg/kg	name
177.8	328.4	water	580	100.6	standard I

Tab. S 5-3 Exemplary preparation of the diluted standards with concentrations from 50 µg/kg to 0.1µg/kg

standard I weight	water weight	SC final concentration
/ g	/ g	/ µg/kg
50.00	50.00	50.3
20.00	180.00	10.1
10.00	190.00	5.0
2.00	198.00	1.0
1.00	199.00	0.5
0.20	199.80	0.1

5.6.2 Optimization and validation data

Tab. S 5-4 Summarized target ion peak areas for the variation of the incubation temperature.
The values given are average values for N = 3.

Substance	20°C	25°C	30°C	35°C
phenols				
phenol	1.3E+05	2.8E+05	2.9E+05	6.1E+05
2'-hydroxyacetophenone	1.4E+06	3.2E+06	3.0E+06	6.5E+06
2-*tert*-butylphenol	3.2E+06	7.1E+06	7.2E+06	1.5E+07
4-*tert*-butylphenol	1.5E+05	3.5E+05	3.9E+05	8.1E+05
4-*tert*-amylphenol	8.1E+04	2.2E+05	2.3E+05	5.3E+05
2,6-di-*tert*-butylphenol	6.6E+07	8.7E+07	7.3E+07	1.0E+08
2,4-di-*tert*-butylphenol	1.2E+07	3.3E+07	3.3E+07	6.2E+07
butylhydroxytoluene	1.5E+07	2.1E+07	1.7E+07	2.5E+07
alcohols				
cyclohexanol	2.6E+05	6.2E+05	7.0E+05	1.6E+06
2-ethylhexanol	5.0E+06	1.1E+07	1.1E+07	2.3E+07
benzyl alcohol	4.7E+04	9.9E+04	1.1E+05	2.2E+05
dodecanol	1.0E+06	5.3E+06	4.8E+06	1.0E+07
phthalates				
diethylphthalate	5.9E+04	3.0E+05	2.1E+05	4.5E+05
di-butylphthalate	6.5E+04	1.5E+06	2.4E+06	2.6E+06
misc				
undecane	1.0E+07	1.6E+07	1.8E+07	1.3E+07
2-(2-butoxyethoxy)ethyl acetate	n.d.	n.d.	n.d.	n.d.
ethylbenzene	2.7E+07	3.1E+07	2.4E+07	3.4E+07
styrene	6.7E+07	7.5E+07	6.0E+07	8.3E+07
divinylbenzene	4.2E+07	5.0E+07	3.8E+07	5.6E+07
benzaldehyde	5.6E+06	1.0E+07	7.1E+06	1.8E+07
1,2-dicyanobenzene	6.5E+04	1.3E+05	1.5E+05	2.8E+05
chlorobenzene	7.1E+07	7.1E+07	6.3E+07	8.5E+07

n.d. = not determinable

Tab. S 5-5 Summarized target ion peak areas for the variation of the incubation time. The values given are average values for N = 3.

Substance	3 min	5 min	8 min	10 min
phenols				
phenol	8.9E+05	8.7E+05	8.5E+05	9.0E+05
2'-hydroxyacetophenone	4.2E+06	4.3E+06	4.2E+06	4.3E+06
2-*tert*-butylphenol	1.1E+07	1.1E+07	1.1E+07	1.1E+07
4-*tert*-butylphenol	8.7E+05	9.2E+05	9.2E+05	1.0E+06
4-*tert*-amylphenol	1.1E+06	1.1E+06	1.1E+06	1.2E+06
2,6-di-*tert*-butylphenol	1.7E+07	1.8E+07	1.8E+07	1.7E+07
2,4-di-*tert*-butylphenol	9.2E+07	9.2E+07	9.0E+07	9.7E+07
butylhydroxytoluene	7.2E+06	7.8E+06	7.7E+06	7.3E+06
alcohols				
cyclohexanol	n.d.	n.d.	n.d.	n.d.
2-ethylhexanol	1.4E+06	1.5E+06	1.5E+06	1.5E+06
benzyl alcohol	2.2E+05	2.2E+05	2.1E+05	2.2E+05
dodecanol	2.0E+07	1.9E+07	1.9E+07	1.8E+07
phthalates				
diethylphthalate	4.8E+05	4.8E+05	5.3E+05	6.6E+05
di-butylphthalate	1.3E+06	1.2E+06	1.0E+06	1.4E+06
misc				
undecane	1.3E+06	1.2E+06	1.1E+06	1.1E+06
2-(2-butoxyethoxy)ethyl acetate	n.d.	n.d.	n.d.	n.d.
ethylbenzene	1.2E+06	1.3E+06	1.1E+06	1.3E+06
styrene	3.2E+06	3.3E+06	3.1E+06	3.4E+06
divinylbenzene	1.1E+07	1.2E+07	1.1E+07	1.1E+07
benzaldehyde	3.9E+06	3.9E+06	3.9E+06	3.8E+06
1,2-dicyanobenzene	1.7E+05	1.7E+05	1.6E+05	2.0E+05
chlorobenzene	1.4E+06	1.5E+06	1.3E+06	1.4E+06

n.d. = not determinable

Tab. S 5-6 Summarized target ion peak areas for a drying step and without a drying step of the Tenax sorbent after the DHS extraction.
The values given are average values for N = 3.

Substance	drying	without dry-
phenols		
phenol	2.5E+05	2.5E+05
2'-hydroxyacetophenone	1.5E+06	1.9E+06
2-*tert*-butylphenol	3.3E+06	5.2E+06
4-*tert*-butylphenol	1.9E+05	2.8E+05
4-*tert*-amylphenol	1.4E+05	2.0E+05
2,6-di-*tert*-butylphenol	6.1E+07	5.9E+07
2,4-di-*tert*-butylphenol	2.4E+07	3.1E+07
butylhydroxytoluene	1.5E+07	1.5E+07
alcohols		
cyclohexanol	4.3E+05	5.3E+05
2-ethylhexanol	5.4E+06	8.7E+06
benzyl alcohol	1.2E+05	1.0E+05
dodecanol	2.3E+06	3.7E+06
phthalates		
diethylphthalate	n.d.	n.d.
di-butylphthalate	n.d.	n.d.
misc		
undecane	1.3E+07	1.1E+07
2-(2-butoxyethoxy)ethyl acetate	n.d.	n.d.
ethylbenzene	1.8E+07	1.6E+07
styrene	4.6E+07	4.2E+07
divinylbenzene	2.9E+07	2.7E+07
benzaldehyde	5.5E+06	6.8E+06
1,2-dicyanobenzene	1.4E+05	1.2E+05
chlorobenzene	5.1E+07	4.3E+07

n.d. = not determinable

Tab. S 5-7 Summarized target ion peak areas for the influence of the sample volume. The values given are average values for N = 3.

Substance	2 mL	4 mL	6 mL	8 mL	10 mL
phenols					
phenol	4.9E+05	4.7E+05	4.8E+05	6.6E+05	7.3E+05
2'-hydroxyacetophenone	1.0E+06	1.5E+06	2.0E+06	2.3E+06	2.5E+06
2-*tert*-butylphenol	2.0E+06	2.8E+06	3.8E+06	4.5E+06	5.0E+06
4-*tert*-butylphenol	1.2E+05	1.5E+05	1.9E+05	2.8E+05	3.1E+05
4-*tert*-amylphenol	n.d.	n.d.	n.d.	n.d.	n.d.
2,6-di-*tert*-butylphenol	1.7E+07	2.4E+07	2.8E+07	2.7E+07	2.4E+07
2,4-di-*tert*-butylphenol	2.1E+07	1.8E+07	2.2E+07	3.3E+07	3.0E+07
butylhydroxytoluene	5.8E+06	9.8E+06	1.1E+07	1.2E+07	1.0E+07
alcohols					
cyclohexanol	n.d.	n.d.	n.d.	n.d.	n.d.
2-ethylhexanol	9.1E+05	1.4E+06	1.6E+06	1.7E+06	1.8E+06
benzyl alcohol	6.8E+04	7.7E+04	9.7E+04	9.5E+04	1.1E+05
dodecanol	3.4E+06	5.3E+06	4.3E+06	3.2E+06	1.7E+06
phthalates					
diethylphthalate	2.6E+05	2.3E+05	2.5E+05	2.4E+05	4.4E+05
di-butylphthalate	5.6E+04	5.7E+04	1.3E+05	1.6E+05	1.9E+05
misc					
undecane	1.6E+06	2.0E+06	2.3E+06	2.2E+06	1.1E+06
2-(2-butoxyethoxy)ethyl acetate	5.0E+05	3.3E+05	2.8E+05	2.4E+05	2.0E+05
ethylbenzene	1.1E+06	1.2E+06	1.3E+06	9.6E+05	1.2E+06
styrene	3.0E+06	3.4E+06	3.7E+06	3.0E+06	3.1E+06
divinylbenzene	8.8E+06	1.2E+07	1.5E+07	1.3E+07	1.4E+07
benzaldehyde	2.0E+06	2.3E+06	2.7E+06	2.8E+06	1.4E+06
1,2-dicyanobenzene	5.2E+04	6.8E+04	8.4E+04	9.6E+04	1.1E+05
chlorobenzene	1.1E+06	1.4E+06	1.5E+06	1.7E+06	1.4E+06

n.d. = not determinable

Tab. S 5-8 Summarized target ion peak areas for the influence of the purge volume. The values given are average values for N = 3.

Substance	200 mL	400 mL	600 mL	800 mL
phenols				
phenol	7.1E+05	5.4E+05	4.1E+05	7.3E+05
2'-hydroxyacetophenone	5.1E+05	1.2E+06	1.3E+06	2.5E+06
2-*tert*-butylphenol	1.2E+06	2.5E+06	3.2E+06	5.0E+06
4-*tert*-butylphenol	7.1E+04	1.3E+05	1.5E+05	3.1E+05
4-*tert*-amylphenol	n.d.	n.d.	n.d.	n.d.
2,6-di-*tert*-butylphenol	1.4E+07	2.4E+07	3.1E+07	3.4E+07
2,4-di-*tert*-butylphenol	8.5E+06	1.6E+07	2.0E+07	3.0E+07
butylhydroxytoluene	5.1E+06	8.8E+06	1.0E+07	1.0E+07
alcohols				
cyclohexanol	n.d.	n.d.	n.d.	n.d.
2-ethylhexanol	5.1E+05	1.3E+06	1.7E+06	1.8E+06
benzyl alcohol	n.d.	6.5E+04	6.5E+04	1.1E+05
dodecanol	1.2E+06	1.5E+06	1.7E+06	1.7E+06
phthalates				
diethylphthalate	3.6E+05	3.1E+05	3.3E+05	4.4E+05
di-butylphthalate	9.1E+04	1.0E+05	1.3E+05	1.9E+05
misc				
undecane	2.0E+06	2.0E+06	2.4E+06	1.1E+06
2-(2-butoxyethoxy)ethyl acetate	2.0E+05	1.4E+05	8.5E+04	2.0E+05
ethylbenzene	9.3E+05	1.3E+06	1.3E+06	1.2E+06
styrene	2.3E+06	3.5E+06	3.6E+06	3.1E+06
divinylbenzene	7.0E+06	1.2E+07	1.4E+07	1.4E+07
benzaldehyde	6.8E+05	1.1E+06	4.2E+05	1.4E+06
1,2-dicyanobenzene	n.d	n.d.	n.d.	1.1E+05
chlorobenzene	9.9E+05	1.6E+06	1.6E+06	1.4E+06

n.d. = not determinable

Tab. S 5-9 Summarized target ion peak areas for the influence of the purge flow. The values given are average values for N = 3.

Substance	20 mL/min	40 mL/min	60 mL/min	80 mL/min
phenols				
phenol	6.6E+05	6.3E+05	7.3E+05	4.0E+05
2'-hydroxyacetophenone	2.3E+06	2.6E+06	2.5E+06	1.5E+06
2-*tert*-butylphenol	5.0E+06	5.4E+06	5.0E+06	3.6E+06
4-*tert*-butylphenol	2.8E+05	3.0E+05	3.1E+05	1.6E+05
4-*tert*-amylphenol	n.d.	n.d.	n.d.	n.d.
2,6-di-*tert*-butylphenol	3.3E+07	3.0E+07	3.4E+07	3.3E+07
2,4-di-*tert*-butylphenol	3.7E+07	3.9E+07	3.0E+07	2.2E+07
butylhydroxytoluene	1.6E+07	1.4E+07	1.0E+07	1.1E+07
alcohols				
cyclohexanol	n.d.	n.d.	n.d.	n.d.
2-ethylhexanol	1.6E+06	1.9E+06	1.8E+06	1.9E+06
benzyl alcohol	1.2E+05	1.1E+05	1.1E+05	5.1E+04
dodecanol	4.2E+05	6.2E+04	1.7E+06	0.0E+00
phthalates				
diethylphthalate	3.8E+05	3.6E+05	4.4E+05	3.7E+05
di-butylphthalate	1.3E+05	2.0E+05	1.9E+05	1.9E+05
misc				
undecane	1.1E+06	1.5E+06	1.1E+06	2.2E+06
2-(2-butoxyethoxy)ethyl acetate	1.5E+05	1.3E+05	2.0E+05	1.3E+05
ethylbenzene	1.2E+06	1.2E+06	1.2E+06	1.3E+06
styrene	3.6E+06	3.6E+06	3.1E+06	3.7E+06
divinylbenzene	1.7E+07	1.6E+07	1.4E+07	1.4E+07
benzaldehyde	1.2E+06	1.2E+06	1.4E+06	5.7E+05
1,2-dicyanobenzene	1.3E+05	9.9E+04	1.1E+05	n.d.
chlorobenzene	1.7E+06	1.7E+06	1.4E+06	1.6E+06

n.d. = not determinable

Tab. S 5-10 Summarized target ion peak areas for the influence of the sorbent temperature during the DHS extraction process.
The values given are average values for N = 3.

Substance	50°C	60°C	70°C
phenols			
phenol	2.8E+05	3.3E+05	2.9E+05
2'-hydroxyacetophenone	2.1E+06	2.9E+06	3.0E+06
2-*tert*-butylphenol	7.9E+06	8.0E+06	7.9E+06
4-*tert*-butylphenol	3.7E+05	3.9E+05	3.8E+05
4-*tert*-amylphenol	2.0E+05	2.3E+05	2.6E+05
2,6-di-*tert*-butylphenol	7.8E+07	7.6E+07	7.8E+07
2,4-di-*tert*-butylphenol	3.5E+07	3.5E+07	3.7E+07
butylhydroxytoluene	2.0E+07	1.9E+07	2.0E+07
alcohols			
cyclohexanol	7.6E+05	7.0E+05	5.9E+05
2-ethylhexanol	1.2E+07	1.2E+07	1.2E+07
benzyl alcohol	1.1E+05	1.2E+05	1.1E+05
dodecanol	5.7E+06	5.9E+06	6.3E+06
phthalates			
diethylphthalate	2.3E+05	3.1E+05	2.6E+05
di-butylphthalate	1.3E+06	1.4E+06	1.4E+06
misc			
undecane	1.7E+07	1.7E+07	1.7E+07
2-(2-butoxyethoxy)ethyl acetate	n.d.	n.d.	n.d.
ethylbenzene	2.4E+07	2.8E+07	1.9E+07
styrene	6.0E+07	6.4E+07	5.6E+07
divinylbenzene	4.1E+07	4.2E+07	4.0E+07
benzaldehyde	1.0E+07	1.1E+07	1.0E+07
1,2-dicyanobenzene	1.4E+05	1.6E+05	1.4E+05
chlorobenzene	6.5E+07	6.6E+07	4.0E+07

n.d. = not determinable

Tab. S 5-11 Summarized target ion peak areas for the effect of addition of none, 5% and 10% methanol (% = v/v).
The values given are average values for N = 3.

Substance	without	5% MeOH	10% MeOH
phenols			
phenol	1.0E+06	7.7E+05	7.3E+05
2'-hydroxyacetophenone	2.2E+06	2.9E+06	2.5E+06
2-*tert*-butylphenol	5.0E+06	6.2E+06	4.9E+06
4-*tert*-butylphenol	5.1E+05	4.1E+05	3.2E+05
4-*tert*-amylphenol	6.4E+05	6.2E+05	5.3E+05
2,6-di-*tert*-butylphenol	8.4E+06	1.7E+07	1.8E+07
2,4-di-*tert*-butylphenol	3.7E+07	4.8E+07	3.8E+07
butylhydroxytoluene	3.0E+06	6.9E+06	7.0E+06
alcohols			
cyclohexanol	n.d.	n.d.	n.d.
2-ethylhexanol	9.0E+05	1.4E+06	1.4E+06
benzyl alcohol	1.5E+05	1.5E+05	1.4E+05
dodecanol	7.6E+06	1.2E+07	9.5E+06
phthalates			
diethylphthalate	6.3E+05	3.2E+05	2.7E+05
di-butylphthalate	1.4E+06	5.9E+05	4.0E+05
misc			
undecane	6.1E+05	8.9E+05	8.4E+05
2-(2-butoxyethoxy)ethyl acetate	2.8E+05	4.1E+05	8.6E+05
ethylbenzene	5.2E+05	6.6E+05	6.2E+05
styrene	1.6E+06	2.0E+06	1.8E+06
divinylbenzene	4.6E+06	9.5E+06	9.6E+06
benzaldehyde	2.5E+06	3.2E+06	3.0E+06
1,2-dicyanobenzene	1.6E+05	1.3E+05	1.2E+05
chlorobenzene	1.2E+06	7.6E+05	7.3E+05

n.d. = not determinable

Tab. S 5-12 Summarized target ion peak areas for the effect of addition of no, 1 g, 2 g and 3 g NaCl.
The values given are average values for N = 3.

Substance	0 g	1 g	2 g	3 g
phenols				
phenol	6.6E+05	7.8E+05	8.2E+05	8.8E+05
2'-hydroxyacetophenone	2.8E+06	3.4E+06	3.6E+06	4.0E+06
2-*tert*-butylphenol	5.6E+06	7.3E+06	7.8E+06	8.5E+06
4-*tert*-butylphenol	3.5E+05	5.4E+05	6.4E+05	8.6E+05
4-*tert*-amylphenol	0.0E+00	7.6E+05	8.1E+05	1.0E+06
2,6-di-*tert*-butylphenol	2.0E+07	1.8E+07	1.5E+07	1.4E+07
2,4-di-*tert*-butylphenol	3.8E+07	6.0E+07	7.6E+07	1.1E+08
butylhydroxytoluene	8.7E+06	7.9E+06	6.5E+06	6.3E+06
alcohols				
cyclohexanol	n.d.	n.d.	n.d.	n.d.
2-ethylhexanol	1.2E+06	1.2E+06	1.1E+06	1.0E+06
benzyl alcohol	1.2E+05	1.7E+05	1.9E+05	2.3E+05
dodecanol	9.1E+06	1.6E+07	1.9E+07	2.6E+07
phthalates				
diethylphthalate	2.7E+05	3.8E+05	3.7E+05	5.1E+05
di-butylphthalate	2.0E+05	4.9E+05	9.8E+05	2.2E+06
misc				
undecane	1.5E+06	1.4E+06	9.1E+05	8.8E+05
2-(2-butoxyethoxy)ethyl acetate	4.3E+05	9.4E+05	1.8E+06	3.9E+06
ethylbenzene	1.1E+06	1.1E+06	9.7E+05	1.1E+06
styrene	2.9E+06	2.7E+06	2.6E+06	2.9E+06
divinylbenzene	7.3E+06	6.8E+06	5.9E+06	6.0E+06
benzaldehyde	3.2E+06	3.8E+06	4.0E+06	4.4E+06
1,2-dicyanobenzene	1.3E+05	1.6E+05	1.6E+05	2.0E+05
chlorobenzene	1.3E+06	1.2E+06	1.2E+06	1.3E+06

n.d. = not determinable

Tab. S 5-13 Summarized target ion peak areas for multiple extractions of the same sample.
The values given are average values for N = 3.

Substance	#1	#2	#3	#4	#5	#6	#7	#8
phenols								
phenol	5.8E+5	5.9E+5	5.7E+5	5.9E+5	5.9E+5	5.6E+5	5.1E+5	5.2E+5
2'-hydroxyacetophenone	4.0E+6	3.9E+6	3.5E+6	3.3E+6	3.2E+6	2.8E+6	2.4E+6	2.1E+6
2-*tert*-butylphenol	8.3E+6	8.1E+6	7.1E+6	6.8E+6	6.5E+6	5.8E+6	4.9E+6	4.5E+6
4-*tert*-butylphenol	4.7E+5	4.9E+5	4.6E+5	4.6E+5	4.5E+5	4.3E+5	3.7E+5	3.7E+5
4-*tert*-amylphenol	3.4E+5	3.5E+5	3.2E+5	3.1E+5	3.2E+5	2.9E+5	2.6E+5	2.5E+5
2,6-di-*tert*-butylphenol	8.3E+7	4.2E+7	1.6E+7	6.5E+6	2.4E+6	7.5E+5	2.4E+5	9.6E+4
2,4-di-*tert*-butylphenol	5.1E+7	4.2E+7	3.2E+7	2.7E+7	2.2E+7	1.7E+7	1.2E+7	9.0E+6
butylhydroxytoluene	2.5E+7	1.2E+7	4.7E+6	2.0E+6	8.2E+5	2.9E+5	1.0E+5	4.3E+4
alcohols								
cyclohexanol	9.6E+5	1.0E+6	1.0E+6	9.7E+5	9.4E+5	9.2E+5	8.4E+5	8.1E+5
2-ethylhexanol	1.6E+7	1.2E+7	9.3E+6	7.8E+6	6.1E+6	4.7E+6	3.3E+6	2.9E+6
benzyl alcohol	2.1E+5	2.2E+5	2.0E+5	2.1E+5	1.9E+5	1.9E+5	1.7E+5	1.7E+5
dodecanol	8.7E+6	5.0E+6	2.7E+6	1.6E+6	8.7E+5	4.2E+5	1.9E+5	1.0E+5
phthalates								
diethylphthalate	2.8E+5	2.8E+5	2.5E+5	2.6E+5	2.5E+5	2.3E+5	2.1E+5	2.0E+5
di-butylphthalate	2.9E+6	3.8E+6	4.1E+6	4.3E+6	4.2E+6	4.0E+6	3.4E+6	3.1E+6
misc								
undecane	1.9E+7	4.7E+6	2.3E+6	1.3E+6	6.9E+5	3.2E+5	1.5E+5	1.1E+5
2-(2-butoxyethoxy)ethyl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ethylbenzene	2.4E+7	6.8E+6	1.5E+6	4.0E+5	1.0E+5	3.2E+4	n.d.	n.d.
styrene	5.8E+7	2.1E+7	5.6E+6	1.6E+6	4.4E+5	1.3E+5	5.2E+4	3.0E+4
divinylbenzene	4.0E+7	1.5E+7	4.3E+6	1.4E+6	4.0E+5	1.3E+5	5.6E+4	3.4E+4
benzaldehyde	1.0E+7	9.5E+6	7.8E+6	7.1E+6	6.2E+6	5.2E+6	4.1E+6	2.2E+6
1,2-dicyanobenzene	1.9E+5	1.7E+5	1.6E+5	1.6E+5	1.7E+5	1.5E+5	1.5E+5	1.4E+5
chlorobenzene	4.6E+7	1.6E+7	4.1E+6	1.1E+6	3.0E+5	8.7E+4	3.4E+4	1.2E+4

n.d. = not determinable

Tab. S 5-14 Summarized data of the method validation for water.
m and n are the slope and axis intercept of the linear fit y = m x + n, R^2 is the coefficient of determination, LOQ is the limit of quantification, repeatability and trueness are given as +/- 100% values
The values given are average values for N = 3.

Substance	m	n	R^2	LOQ µg/kg	repeatability %	trueness %
phenols						
phenol	0.91	3.90	0.998	0.1	8	22
2'-hydroxyacetophenone	1.01	4.48	0.999	0.1	4	16
2-*tert*-butylphenol	1.05	4.71	0.997	0.1	13	22
4-*tert*-butylphenol	1.27	3.08	0.998	1.0	7	11
4-*tert*-amylphenol	1.40	2.80	0.991	5.0	3	18
2,6-di-*tert*-butylphenol	1.13	5.65	0.997	0.1	9	22
2,4-di-*tert*-butylphenol	1.04	5.61	0.999	0.1	8	17
butylhydroxytoluene	1.10	5.16	0.997	0.1	11	23
alcohols						
cyclohexanol	0.98	4.01	0.998	0.1	9	16
2-ethylhexanol	0.98	5.16	0.996	0.1	6	26
benzyl alcohol	0.76	3.77	0.998	0.1	7	21
dodecanol	1.17	4.76	0.999	0.1	9	15
phthalates						
diethylphthalate	0.84	3.57	0.997	0.5	7	20
di-butylphthalate	1.02	4.64	0.998	0.1	11	16
misc						
undecane	1.45	4.71	0.998	0.1	17	19
2-(2-butoxyethoxy)ethyl acetate	1.94	0.94	0.997	50 (LOD)	14	12
ethylbenzene	1.09	5.31	0.997	0.1	14	21
styrene	1.05	5.71	0.997	0.1	12	20
divinylbenzene	1.14	5.34	0.998	0.5	14	17
benzaldehyde	0.97	5.04	0.997	0.1	4	22
1,2-dicyanobenzene	1.76	1.76	0.960	50 (LOD)	6	72
chlorobenzene	1.03	5.65	0.997	0.1	5	22

n.d. = not determinable

Tab. S 5-15 Summarized data of the method validation for the "BIC" solution.
m and n are the slope and axis intercept of the linear fit y = m x + n, R^2 is the coefficient of determination, LOQ is the limit of quantification, repeatability and trueness are given as +/- 100% values
The values given are average values for N = 3.

Substance	m	n	R^2	LOQ µg/kg	repeatability %	trueness %
phenols						
phenol	1.01	3.74	0.998	0.1	9	17
2'-hydroxyacetophenone	1.32	4.02	0.998	0.5	6	19
2-*tert*-butylphenol	1.21	4.52	0.996	0.1	5	21
4-*tert*-butylphenol	1.34	3.05	0.996	5.0	4	17
4-*tert*-amylphenol	1.23	2.96	0.996	5.0	3	10
2,6-di-*tert*-butylphenol	1.27	5.42	0.998	0.1	7	19
2,4-di-*tert*-butylphenol	1.08	5.61	0.998	0.5	7	15
butylhydroxytoluene	1.19	5.00	0.998	0.1	7	20
alcohols						
cyclohexanol	1.11	3.85	0.998	0.5	6	17
2-ethylhexanol	1.14	5.00	0.997	0.5	5	18
benzyl alcohol	0.64	3.99	0.997	0.1	6	23
dodecanol	1.14	4.77	0.999	0.5	7	16
phthalates						
diethylphthalate	0.85	3.74	0.999	5.0	3	6
di-butylphthalate	0.79	4.78	0.998	0.1	5	19
misc						
undecane	1.18	4.98	0.998	0.5	12	15
2-(2-butoxyethoxy)ethyl acetate	1.25	2.36	0.965	50 (LOD)	11	12
ethylbenzene	1.16	5.18	0.998	0.1	6	20
styrene	1.17	5.52	0.997	0.1	4	23
divinylbenzene	1.24	5.16	0.998	0.1	6	19
benzaldehyde	1.08	4.89	0.998	0.5	6	16
1,2-dicyanobenzene	1.18	2.88	0.958	50 (LOD)	11	12
chlorobenzene	1.16	5.44	0.997	0.1	4	19

n.d. = not determinable

Tab. S 5-16 Summarized data of the method validation for the "LAC" solution.
m and n are the slope and axis intercept of the linear fit y = m x + n, R^2 is the coefficient of determination, LOQ is the limit of quantification, repeatability and trueness are given as +/- 100% values
The values given are average values for N = 3.

Substance	m	n	R^2	LOQ µg/kg	repeatability %	trueness %
phenols						
phenol	1.09	3.56	0.992	5.0	9	16
2'-hydroxyacetophenone	1.19	4.15	0.998	0.5	5	17
2-*tert*-butylphenol	1.17	4.52	0.998	0.1	8	17
4-*tert*-butylphenol	1.21	3.21	0.995	1.0	5	19
4-*tert*-amylphenol	1.37	2.79	0.999	5.0	5	8
2,6-di-*tert*-butylphenol	1.24	5.40	0.998	0.1	9	18
2,4-di-*tert*-butylphenol	1.05	5.56	0.996	0.5	7	20
butylhydroxytoluene	1.15	5.01	0.998	0.1	5	17
alcohols						
cyclohexanol	0.86	4.17	0.998	0.1	6	19
2-ethylhexanol	1.07	5.00	0.997	0.5	4	18
benzyl alcohol	0.87	3.55	0.992	5.0	3	17
dodecanol	1.19	4.55	0.991	5.0	15	20
phthalates						
diethylphthalate	0.94	3.47	0.995	0.5	12	22
di-butylphthalate	1.06	4.70	0.998	0.1	10	17
misc						
undecane	1.19	4.80	0.998	0.5	10	12
2-(2-butoxyethoxy)ethyl acetate	1.30	2.23	0.995	50 (LOD)	4	4
ethylbenzene	1.09	5.22	0.999	0.1	8	13
styrene	1.10	5.55	0.999	0.1	7	14
divinylbenzene	1.16	5.20	0.998	0.1	15	19
benzaldehyde	1.03	4.92	0.998	0.5	141	15
1,2-dicyanobenzene	1.21	2.82	0.964	50 (LOD)	11	13
chlorobenzene	1.09	5.48	0.999	0.1	6	12

n.d. = not determinable

Tab. S 5-17 Summarized data of the method validation for the pH 2 solution.
m and n are the slope and axis intercept of the linear fit y = m x + n, R^2 is the coefficient of determination, LOQ is the limit of quantification, repeatability and trueness are given as +/- 100% values
The values given are average values for N = 3.

Substance	m	n	R^2	LOQ µg/kg	repeatability %	trueness %
phenols						
phenol	0.90	3.91	0.997	0.1	8	20
2'-hydroxyacetophenone	1.22	4.14	0.998	0.5	2	17
2-*tert*-butylphenol	1.35	4.28	0.996	1.0	3	14
4-*tert*-butylphenol	1.34	2.99	1.000	5.0	3	4
4-*tert*-amylphenol	1.37	2.76	0.999	5.0	2	8
2,6-di-*tert*-butylphenol	1.20	5.49	0.998	0.1	6	17
2,4-di-*tert*-butylphenol	1.08	5.51	0.997	0.5	7	19
butylhydroxytoluene	1.26	5.08	0.998	0.1	5	19
alcohols						
cyclohexanol	1.05	3.88	0.999	0.1	5	21
2-ethylhexanol	1.07	5.03	0.996	0.5	7	20
benzyl alcohol	0.69	3.93	0.996	0.5	4	21
dodecanol	1.05	4.75	0.996	0.1	11	19
phthalates						
diethylphthalate	0.94	3.50	0.999	0.5	6	14
di-butylphthalate	1.12	4.62	0.998	0.1	6	17
misc						
undecane	1.12	4.95	0.998	0.5	5	18
2-(2-butoxyethoxy)ethyl acetate	n.d.	n.d.	n.d.	100 (LOD)	3	n.d.
ethylbenzene	1.10	5.24	0.999	0.1	6	15
styrene	1.10	5.60	0.998	0.1	5	19
divinylbenzene	1.15	5.28	0.999	0.1	7	15
benzaldehyde	1.04	4.95	0.998	0.5	4	16
1,2-dicyanobenzene	1.01	3.24	0.997	50 (LOD)	2	3
chlorobenzene	1.08	5.54	0.998	0.1	7	20

n.d. = not determinable

Tab. S 5-18 Summarized data of the method validation for the pH 10 solution. m and n are the slope and axis intercept of the linear fit y = m x + n, R^2 is the coefficient of determination, LOQ is the limit of quantification, repeatability and trueness are given as +/- 100% values. The values given are average values for N = 3.

Substance	m	n	R^2	LOQ µg/kg	repeatability %	trueness %
phenols						
phenol	0.73	3.98	0.998	0.1	5	17
2'-hydroxyacetophenone	1.11	4.20	0.998	0.5	3	13
2-*tert*-butylphenol	1.08	4.68	0.999	0.1	5	14
4-*tert*-butylphenol	1.15	3.17	0.998	1.0	7	13
4-*tert*-amylphenol	1.32	2.75	0.997	5.0	6	11
2,6-di-*tert*-butylphenol	1.13	5.70	0.999	0.1	15	19
2,4-di-*tert*-butylphenol	1.01	5.66	0.996	0.5	29	20
butylhydroxytoluene	1.10	5.19	0.999	0.1	12	19
alcohols						
cyclohexanol	0.96	4.03	0.998	0.5	4	16
2-ethylhexanol	1.01	5.18	0.999	0.5	23	13
benzyl alcohol	0.92	3.51	0.995	0.5	14	25
dodecanol	1.00	4.99	0.995	1.0	27	18
phthalates						
diethylphthalate	0.96	3.42	0.999	0.5	41	14
di-butylphthalate	1.03	4.40	0.999	0.1	7	14
misc						
undecane	1.03	5.26	0.999	0.1	6	19
2-(2-butoxyethoxy)ethyl acetate	n.d.	n.d.	n.d.	100 (LOD)	n.d.	n.d.
ethylbenzene	1.09	5.28	0.998	0.1	6	22
styrene	1.03	5.78	0.997	0.5	22	24
divinylbenzene	1.11	5.43	0.998	0.1	6	19
benzaldehyde	0.86	5.22	0.997	0.1	5	19
1,2-dicyanobenzene	1.05	3.12	0.956	50 (LOD)	10	13
chlorobenzene	1.10	5.52	0.997	0.1	8	21

n.d. = not determinable

5.6.3 Chromatograms

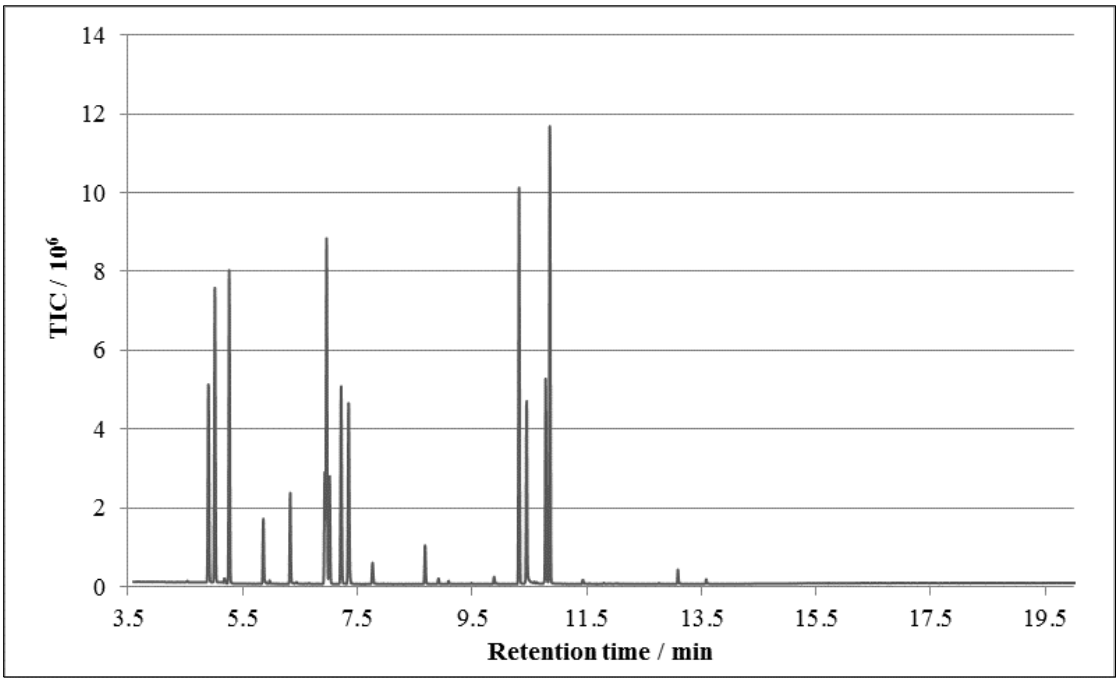

Fig. S 5-1 Total ion current chromatogram in scan mode for the standard solution

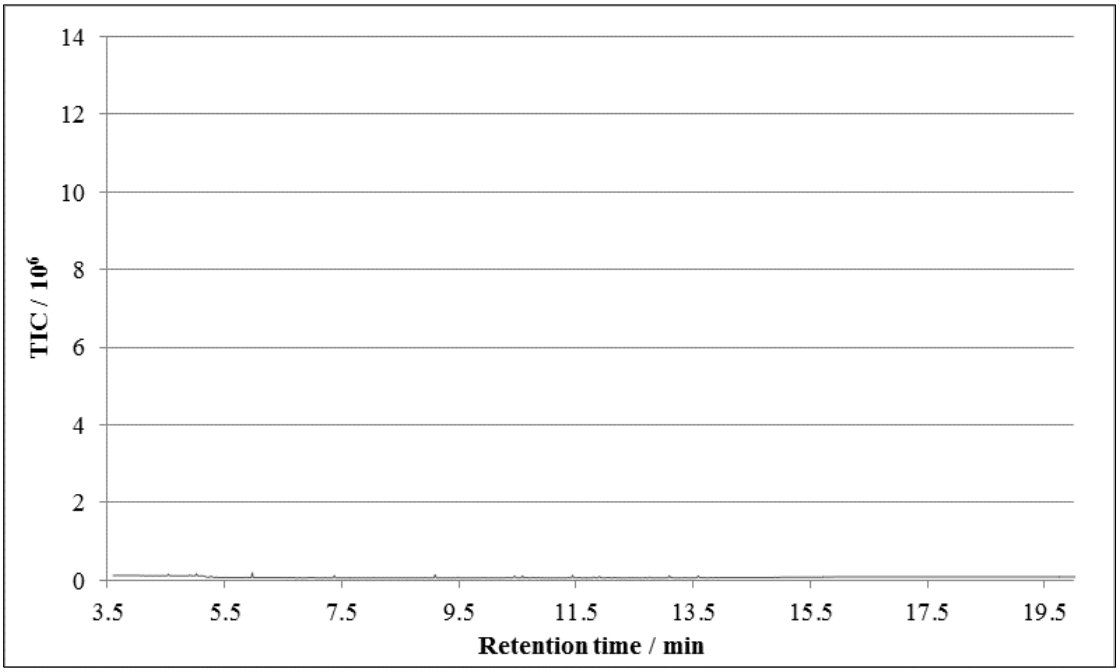

Fig. S 5-2 Total ion current chromatogram in scan mode for a blank sample (pure water)

6 General Conclusions and Outlook

Extractable and leachable studies are standard tests for the evaluation of plastic packaging materials, especially in the case of pharmaceutical solutions, where contaminations may pose a risk to the patients' health.

In this thesis 12 unknown substances, which were found in an extraction study on a new multilayer packaging material, were successfully identified as cyclic ester. In addition a diurethan was clearly identified. All of them were formed as byproducts during the production of the polyurethane adhesive that was used to laminate the multilayer film. The study illustrated that only the combined use of different analytical methods made it possible to unambiguously identify the extracted substances. This extractable study also showed that byproducts, which form in trace amounts during the production process of a single- or multilayer film, can become the major leachable. Even low molecular weight trace contaminations in raw materials, which are used in the very beginning of a multi-step production process, may finally be detected as a leachable. This was observed in the case of a cyclic oligomer that consists of tetraethylene glycol, which in turn was a trace contaminant of the diethylene glycol. Finally the results demonstrated that the identification of unknown extractable components can be simplified by a sound knowledge about solvents, raw materials and possible contaminants used in the production process of a film or multilayer film.

Aiming at leachable studies on dialysis solutions two trace analysis GC-MS methods were developed and validated. Both, SBSE and DHS, have the clear potential to be employed in routinely performed quality controls and should accompany further developments of packaging materials or their raw materials.

SBSE exhibited a superior LOD in a simultaneous determination of several ten components down to a few ppt (ng/kg). This is low enough to decide if a particular substance is below the safety concern threshold and thus its toxicological assessment is not necessary. Measurements performed with real samples demonstrated that the achieved LOD is necessary for a complete leachable study. Most of the substances quantified here would have not been detectable with a standard, less sensitive liquid-liquid-extraction. The results also reveal that a toxicological assessment is necessary for most of the components. Probably the analysis of a structure-activity-relationship would indicate that these are of no carcinogenic concern and thus a higher threshold is allowed without a toxicological assessment, but this was beyond of the scope of this work. In accordance with the extractable study, once more the important of a detailed knowledge about the sources of the leachables comes into the focus. As soon as the source is known, leachable concentrations can probably be reduced and thereby improving the quality of the dialysis solution.

This is particularly important as these pharmaceutical solutions are infused into the patient most likely a lifetime long.
Advantaged of SBSE are the simple and at least partly automated handling and the requirement of only small sample volumes. Furthermore the method was developed as a screening method, which means, leachables that were not part of the standard solution used for the development and validation can be detected. Disadvantages are on one hand the preference of the PDMS to sorb non-polar components more efficiently than polar ones and on the other hand the complex cleaning process of used stir bars.
These disadvantaged were of no concern for the DHS method. Here Tenax was used as a sorbent and the LOD for most components was at 100 ppt (ng/kg) and thus clearly higher than LOD values for SBSE. But the DHS has the key advantage of a complete automation and a very simple handling, as just 10 mL of the sample have to be filled in a vial, which is afterwards placed on the auto sampler tray. In addition, DHS is a complete solvent less method, where SBSE employs small amounts of organic solvents for the stir bar cleaning.

In a future work SBSE can be further developed to be used as a fingerprinting method to identify raw materials of the packaging material within in the final product by representative leachables. This requires in the first step a database with typical amounts and chemical structures of substances leaching out of raw materials of several suppliers. In a second step plastic packaging materials made of these raw materials have to be studied regarding their leachables. If these leachables represent the sum of the leachables found in individual raw materials a fingerprinting method seems promising.
The DHS method can probably be used for the fingerprinting as well or can be used at last to deliver additional information about raw material for a fingerprinting database, because this method has the potential of a simple analysis directly of the solid raw materials without the need of an extraction process. Beside a scientific question DHS has the clear potential for a standard analysis method in a quality assurance laboratory with a high daily sample throughput.
For both methods the use of internal standards will simplify the quantifications. These internal standards have to be chosen to represent the wide variety of physical and chemical properties of the analyte mixture used in this work. Probably this mixture can be reduced to one or two substances per class so that in a future method a mixture of for instance 10 internal standards is added to every sample.
When focusing on the determination of just one substance class or even a single substance both methods can be further optimized and single ion monitoring might be employed. This should considerably lower the LOD even further.

7 Appendix

7.1 List of Figures

7.2 List of Supplement Figures

7.3 List of Tables

7.4 List of Supplement Tables

7.5 List of Abbreviations and Symbols

AA	adipic acid
BHT	butylhydroxytoluene
BPA	bisphenol A
CI	chemical ionization
CIS	cold injection system
CMC	critical micellization concentrations
CO	cyclic oligomer
DEG	diethylene glycol
DEHP	di-2-ethylhexyl phthalate
DHS	dynamic headspace
EI	electron impact
ESI	electrospray ionization
GC	gas chromatographic
HD	hemodialysis
HF	hemofiltration dialysis
IA	isophthalic acid
IPDI	isophorone diisocyanate
LC	liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
MEG	monoethylene glycol
MPS	multipurpose auto sampler
MS	mass spectrometer
m/z	mass-to-charge ratios
OINDP	orally inhaled and nasal drug products
PD	peritoneal dialysis
PDMS	polydimethylsiloxane
PEG	polyethylene glycol
ppb	part per billion
ppm	part per million
ppt	part per trillion
PQRI	product quality research institute

PUR	polyurethane
rpm	rate per minute
SBSE	stir bar sorptive extraction
SCT	safety concern threshold
SHS	static headspace
SPME	solid-phase microextraction
TDU	thermal desorption unit

$K_{PDMS/W}$	partition coefficient PDMS/Water
$K_{O/W}$	octanol-water partition coefficient
C_{PDMS}	analyte concentration in PDMS
C_{Water}	analyte concentration in water
m_{PDMSD}	analyte among in PDMS
m_{Water}	analyte among in water
V_{PDMS}	PDMS volume
V_{Water}	sample volume
β	phase ratio of sample volume to PDMS volume
pK_a	logarithmic acid dissociation constant
R	recovery
F	purge flow
t	purge time
K_{aw}	air-water partition coefficient, Henry's constant
V_m	sample volume
V_g	headspace volume
$K_{aw,T}$	air-water partition coefficient at the temperature T_0
$\Delta_{aw}H$	enthalpy the phase transfer
R_g	gas constant
A_t	total target ion peak area
A_i	target ion peak area for the i^{th} extraction
b	slope of ln (A_i) vs. $(i\text{-}1)$

7.6 Oral and poster presentations

Publications

Migrating components in a polyurethane laminating adhesive identified using gas chromatography-mass spectrometry.
Athenstädt B, Fünfrocken M, Schmidt TC., Rapid Commun Mass Spectrom. 2012 Aug 30;26(16):1810-6

Datenmanagement von Extractables und Leachables
Michael Fünfrocken, Behnusch Athenstädt und Richard Staab, Laborpraxis-Journal vom 11.05.2010

Poster

Anakon 2013 – Poster
Quantification of leachables from packaging in pharmaceutical solutions in the low pg/ml range by 'stir bar sorptive extraction'-GC-MS
Athenstädt, B., St. Wendel/D, Schmidt, T. C., Essen/D

Oral Presentations

„Identification of extractable components in a multi-layer packaging material"
Seminar "Lebensmittelrecht und Verpackungen in Europa (D, A, CH) – Fokus", Innoform Coaching GbR, Osnabrück, Germany,

7.7 Erklärung

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

„Development of GC-MS methods for the identification and quantification of leachables from plastic packaging in dialysis solutions"

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, Juni 2013

Behnusch Athenstädt