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Validation of a GC-MS method for the determination of pesticides in drinking water.

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Abstract

According to the drinking water legislation in Austria there are 50 pesticides which have to be monitored because their abundance in water supplies has to be expected and therefore accurately measured. For quantifying these analytes by gas chromatography mass spectrometry (GC-MS) systems, different approaches for sample preparation have been proposed. Beside popular methods such as solid phase extraction (SPE) and solid phase micro extraction (SPME) the solid phase dynamic extraction (SPDE) has not been evaluated in depth. In this technique a syringe aspirates the sample solution through a needle, which is coated by a stationary phase. After ad- and absorption of the analytes they are desorbed in the GC inlet. A method development, followed by a validation, for analyzing 7 pesticides namely aldrin, dieldrin, heptachlor, heptachlor epoxide cis and trans, pethoxamid and tolylfluanid was conducted. Optimizing of extraction parameters was carried out by comparing respective peak areas of 5 varying measurements. Results revealed that a validation at low (0,01 / 0,05 µg/l), medium (0,03 / 0,1 µg/l) and high (0,1 / 1 µg/l) concentration levels yielded satisfactory results for the majority of analytes, except for heptachlor at a medium and pethoxamid at a high concentration level. Moreover tolylfluanid was only validated at a medium concentration level, due to poor reproducibility and recovery.

Zusammenfassung

Laut österreichischer Trinkwasserverordnung gilt es 50 Pestizide zu überwachen, deren Vorhandensein in Trinkwasserversorgungen anzunehmen ist. Um diese, meist mit Gaschromatographie-Massenspektrometrie- (GC-MS) Systemen analytisch quantifizieren zu können bedient man sich unterschiedlicher Methoden zur Probenvorbereitung. Neben populären, wie der Festphasenextraktion (SPE) und der Festphasen-Mikroextraktion (SPME) gibt es die nicht ausführlich untersuchte dynamische Festphasenextraktion. Diese Technik arbeitet mit einer Spritze, die über eine innen beschichtete Nadel Probenflüssigkeit aufzieht. Nach Ad- und Absorption der Analyten werden diese in einem heißen GC-Injektor desorbiert. Es wurde eine Methodenentwicklung mit anschließender Validierung für 7 Pestizide durchgeführt: Aldrin, Dieldrin, Heptachlor, Heptachlor cis- und trans-epoxid, Pethoxamid und Tolylfluanid. Die Extraktionsparameter wurden optimiert indem die jeweiligen Peakflächen von 5 Messungen verglichen wurden. Der Großteil der

Pestizide konnte bei niedrigen (0,01 / 0,05 µg/l) mittleren (0,03 / 0,1 µg/l) und hohen (0,1 / 1 µg/l) Konzentrationen validiert werden. Außnahmen waren Heptachlor bei mittlerem und Pethoxamid bei hohem Konzentrationslevel. Tolylfluanid, das nur auf 0,1 µg/l validiert werden konnte, zeigte generell fluktuierende Wiederfindungen und konnte teilweise gar nicht analysiert werden.

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Index of abbreviations

AC…acetone

ACN…acetonitrile

DCM…dichlormethane

EA…ethyl-acetate

GC…gas chromatograph

LOD…limit of detection

LOQ…limit of quantification

Milli-Q®…ultrapure water

MS…mass spectrometer

MS/MS…tandem mass spectrometer

RSD…relative standard deviation

Scan…monitoring of all m/z transitions within a predefined area

SD…standard deviation

SIM…single ion monitoring

SPDE…solid phase dynamic extraction

SPE…solid phase extraction

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1. Introduction

1.1. Pesticides, health and environment

The analytes of interest in this study are aldrin, dieldrin, heptachlor, heptachlor-epoxide -cis and -trans (organochlorine, cyclodiene insecticides), pethoxamid (herbicide) and tolylfluanid (fungicide). Except of the latter the remaining analytes of interest are banned for agricultural use (*IUPAC* 2018). A persisting problem is that developing countries might still apply some of these banned chemicals. These compounds remain in soil and water of developed countries, which refer to the prohibition (*WHO* 2018).

Not only environmental systems are affected by contaminants like pesticides, also living organisms – humans – are reacting to potentially toxic effects. A meta-analysis of a cohort of 84.739 people, applying pesticides, showed an association between overall pesticides and a neuronal disease, the amyotrophic lateral sclerosis (ALS). Pesticide groups, for example herbicides (pethoxamid in this study), the organochlorine insecticides (aldrin, dieldrin, heptachlor and heptachlor-epoxide analyzed in this study) showed a similar association. Aldrin and dieldrin showed specific correlations within these groups. Although the findings were not significant, they clearly show potential side effects. (*Kamel et al.* 2012) More effects by pesticides like heptachlor are shown by a meta-analysis from *Evangelou et al.* 2016. They found an association between pesticides and diabetes with especially heptachlor yielded an increased risk. (*Evangelou et al.* 2016) Breast cancer is another disease, which could be modulated by cyclodiene pesticides (subgroup of organochlorine insectides) like aldrin, dieldrin and heptachlor when only the latter showed significant associations (*Khanjani et al.* 2007). The effects of pesticides and moreover specifically the 7 substances analyzed in this study are more widespread and include sexual and reproductive dysfunction like contamination of breast milk (*Müller et al.* 2017) or effects on serum levels of sex hormones (*Freire et al.* 2014). Parkinson's Disease also seems to be stimulated by chemicals like dieldrin (*Richardson et al.* 2006, *Rhodes et al.* 2013, *Baltazar et al.* 2014). Further studies suggest that aldrin might alter endocrine functions, more specifically the thyroid (*Lerro et al.* 2018) or even act as endocrine disruptors in neurodevelopment in the case of heptachlor (*Roncati et al.* 2016).

1.2. Austrian legislation for drinking water

Pesticides are biochemical substances which are commonly used in agriculture. Because of negative side effects on the environment and even on living organisms like humans governments around the world specified regulatory limits for these contaminants.

There are statutory orders for food as well as drinking water, containing different regulations. Since this work's aim was to develop a method for analyzing 7 pesticides for the company „Lebensmittelversuchsanstalt GmbH", which might occur in water for human usage, the legislation for drinking water regulation in Austria is used (*TWV* 2001).

The chemical parameters lists pesticides with following parameter values:

- Pesticides: 0,10 µg/l
- Exceptions for Aldrin, Dieldrin, Heptachlor and Heptachlor epoxide: each 0,030 µg/l
- Pesticides overall: 0,50 µg/l

„Pesticides" in this legislation are defined as organic insecticides , organic herbicides, organic fungicides, organic nematicides, organic acaricides, organic algicides, organic rodenticides, organic slimicides and related products like growth regulators as well as all relevant metabolites, degradation and reaction products. (*TWV* 2001) Among 50 substances whose occurrence in drinking water must be assumed 7 have not yet been validated by the company. While the others already have been validated using a HPLC-MS/MS system the analytes of this work are analyzed more conveniently by GC-MS because of their physicochemical properties (EURL 2018). Therefore the validation of aldrin, dieldrin, heptachlor, heptachlor epoxide-cis, heptachlor epoxide-trans, pethoxamid and tolylfluanid completes the multi-method for testing drinking water for pesticides.

The following minimum performance characteristics values are always stated in percent of the parameter value of the analyte. For the measuring uncertainty it is 30% and for accuracy, precision and limit of detection (LOD) it is 25% each. Accuracy and the more often used term "recovery" are frequently applied simultaneously. Accuracy is high when the combination of precision (standard deviation - SD) and recovery (closeness of measured value to true value) are also high. Therefore the maximum relative SD (RSD) of many measurements from the true value should be plus, minus 25%. "Precision" is the random error that is typically expressed as the SD (within a series of measurements and between series of measurements) of the variation of results around the mean value. LOD is either the triple standard deviation (within a series of measurements) of a natural sample

with a low concentration of the parameter or five times the SD of a blank (within a series of measurements). (*TWV* 2001)

1.3. Theoretical Framework

Most of the studies conduct their sampling at lakes, rivers and wastewaters in varying areas (*Na et al.* 2006, *Pitarch et al.* 2007, *Chormey et al.* 2017).

DLLME (dispersive liquid liquid micro extraction) is a method, where the analytes are extracted by injecting two solvents (extraction solvent and disperser solvent) into an aqueous sample, forming a cloudy solution of the particles which are completely dispersed in the aqueous phase. After centrifuging the particles will build a sediment phase on the bottom, which is taken up by a micro syringe and is injected into the GC. (*Rezaee et al.* 2006) The LODs are with 2,25 µg/l for heptachlor, 2,98 µg/l for aldrin and 0,31 µg/l for dieldrin too high compared to the respective maximum residue values of the drinking water legislation. (*Chormey et al.* 2017) Other studies, using liquid-liquid extraction quantified pesticides at a much lower levels in the ng/L range (*Robles-Molina* 2013). DLLME's capability of yielding very low LODs with organochlorine pesticides (OCPs) in general is shown in another study. It was possible to reach even lower limits than with solid phase extraction (SPE) or solid phase micro extraction (SPME), but aldrin and heptachlor seem to act differently because of their physicochemical properties, as their relative recoveries were very low. (*Zhao et al.* 2011)

There are basically two ways of extraction within the SPE: cartridges and disks. While it is possible to allow a higher sampling flow on SPE disks there are influences on analytical quality by the drying step of the disks. Therefore it is important to dry disks by high vacuum pumps and a stream of nitrogen for reaching a lower residual water content in the disks and therefore higher recovery rates of the analytes. (*Günter et al.* 2016) SPE itself also shows very low LODs in the ng/l range for OCPs despite the recoveries are not always within the desired range. Especially heptachlor epoxide seems to be widespread throughout different water sources (in China) but the mean recoveries (in %) are just between 63.0 and 79.6. At the same time the LOD is very low with 0.04 ng/l. (*Na et al.* 2006) The sensitivity of SPE as an extraction technique is further dependent on the used mass-spectrometer. Earlier findings show lower LODs and better recoveries within GC-(EI)MS/MS (gas chromatography – electron impact tandem mass spectrometry) for

heptachlor and dieldrin while GC-(NCI [negative chemical ionization])MS is more sensitive for aldrin, heptachlor epoxide cis and trans. (*Pitarch et al.* 2007)

In a recent study regarding SPME, where besides a few OCPs, including aldrin and dieldrin, mainly polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and brominated diphenyl ethers (BDEs) were investigated, they also quantified analytes like aldrin and dieldrin at low quantification limits (LOQs) of 5ng/l and 1 ng/l respectively. The recoveries where in an excellent range between 96% and 116% for two different validation levels (5 ng/l and 50 ng/l) as well as intermediate random errors of maximum 16%. (*Dominguez et al.* 2017)

Many more techniques like MEPS / micro extraction in packed syringe (*Taghani et al.* 2016), HF-LPME / hollow fiber liquid phase micro extraction (*Garrido-Frenich et al.* 2011) MSPE / micro-solid-phase extraction (*Zare et al.* 2016), combinations of SPE and DLLME (*Shamsipur et al.* 2016) as well as DLLME and SPME (*Jafari et al.* 2016), USAEME-SFO / ultrasound-assisted emulsification microextraction - solidification of floating organic droplets (*Shu et al.* 2016) are the basis for further scientific investigation.

According to reviews the SPE and QuEChERS (quick, easy, cheap, effective, rugged and safe) methods for sample preparation are the most frequently used ones for pesticide analysis in various matrices, whereby for water as matrix SPE is commonly applied (*Elbashir et al.* 2018). Interestingly Headspace (HS-) SPME is not covering a wide range of analytes while liquid-liquid extraction (LLE) and SPE are more versatile. LLE yields recovery rates within the drinking water legislation's limit values but lacks in saving on solvent consumption. When eluting solvents are carefully selected for the target analytes SPE offers similar recovery rates like LLE. In wastewater samples the main drawback of SPE is the need of filtering and/or reducing particles, where some of the substances of interest could be bound. There are various HS-SPME coatings available, which need to be selected properly for the confident determination of the target analytes. Carryover effects are observed with some molecules having a strong affinity to the fiber. The main advantage with this method is less usage of solvents and sample volume while disadvantages are seen with a limited coverage of analytes at the same time. (*Robles-Molina et al.* 2013)

Basis of the subsequent work was an application note of Chromtech GmbH, related to the SPDE (solid phase dynamic extraction) technique combined with GC-MS. With this technique it is possible to quantify pesticides at low concentrations (e.g. ng/l) in drinking water. (*Chromtech Application Note SP303*, 2003) While there is a lot of literature of common used sample preparation techniques like SPE or SPME, there are not many investigations into the SPDE method.

In the early 2000's first experimental studies on enhancing the already used SPME technique were conducted. Extraction speed and mechanical stress could be improved when compared to the fragile fiber of SPME due to a coating positioned inside a steel needle. The thinner coating used in SPDE was compensated by an increase in surface area. This increase was achieved by applying the coating to the inner wall of the needle leading to shorter extraction times with similar sample capacity. (*Lipinski* 2001) The mechanism of today's SPDE technique can be described as follows (see detailed description in SPDE chapter): A SPDE needle, which is mounted to a 2.5ml syringe is moving to a heatable magnetic station, containing the 20ml sample vial (which includes a magnetic stir bar). While the sample is continuously mixed, the needle penetrates the septum of the vial cap and starts extraction (i.e.: pulling and pushing the plunger up and down). After extraction a defined volume of carrier gas (usually nitrogen) will be aspirated followed by inserting the needle in the GC inlet and starting desorption of the analytes. (*Jochmann et al.* 2007) In 2001 carryovers were observed when using one out of 2 desorption methods. There was no gas station back then, therefore *Lipinski* conducted desorption process by aspiring a fixed volume of 2.5ml of air pre desorption and pushing down the plunger with a speed of 30µl/s or desorbed by applying a nitrogen stream with a pressure of 0,5 bar by opening the flushing valve (positioned at the back of the syringe holder / heater). The column head / carrier gas pressure had to be reduced otherwise the plunger head was not able to push down the plunger against a higher pressure at least at a plunger speed of 30 µl/s. Because of this low pressure the analytes migrated backwards into the carrier gas line, showing carryovers for high sample concentrations. At higher plunger speeds, the carrier gas pressure also could be increased but desorption process could not be finished. When desorption was conducted by applying a 0,5bar nitrogen stream, the column head / carrier gas pressure also could be held high and therefore no carryover was observed. (*Lipinski* 2001) Following works used a gas station and taking up nitrogen for desorbing. While

desorption within these works were conducted, except of using nitrogen instead of air, the same way where *Lipinski* observed memory effects no carryovers where observed in later paper works. (*Jochmann et al.* 2006 and 2007, *Van Durme et al.* 2007, *Bagheri et al.* 2009, *Rossbach et al.* 2012, *Laaks et al.* 2012) Since SPDE is representing an improvent of SPME, which itself is the most important micro extraction technique within chemical and analytical investigations of liquid and gaseous samples (*Merkle et al.* 2015), SPDE can be and is applied for a wide range of analytes. Several analyzes were carried out (extracting from head space – HS, or liquid space – LS), for example on pesticides in water LS (*Lipinski* 2001), polar volatile compounds in water HS (*Jochmann et al.* 2006), volatile organic hydrocarbons in water HS (*Jochmann et al.* 2007), toluene from air HS (*Van Durme et al.* 2007), PAHs from water HS (*Bagheri et al.* 2009), n-heptane and its mono-oxygenated metabolites in blood HS (*Rossbach et al.* 2012) and volatile compounds of red wine HS (*Laaks et al.* 2012). The major findings of all this paper works have in common that SPDE is comparable to SPME according to analytical efficiency, with lower extraction times and higher stability. Furthermore while most of the actual works are consistent in higher yields when using a higher amount of extraction strokes, a higher extraction temperature, a low initial oven temperature of about 40°C for trapping the analytes and a 20% addition of NaCl for increasing ionic strength, other parameters like extraction stroke speed, desorption flow rate, desorption volume and desorption temperature depend on the respective analytes of interest. (*Lipinski* 2001, *Jochmann et al.* 2006 and 2007, *Bagheri et al.* 2009, *Rossbach et al.* 2012, *Laaks et al.* 2012) For polar volatile compounds it was observed that a higher extraction temperature can also influence extraction yield negatively, since the extracted analytes on the extraction phase are decreasing in relation to the concentration in the headspace. In contradiction to other paper-works, where the syringe temperature is usually set 10°C higher than extraction temperature in order to prevent condensation of water vapor on the syringe wall and therefore inducing carryover, in this work this issue was prevented by constantly holding the syringe temperature at a low level, when increasing extraction temperature. (*Jochmann et al.* 2006)

The patent for the SPDE technique was registered in April 2000 and can be found on the patent scope of the world intellectual property organization's website by searching for the patent number "PCT/DE00/01376" (*WIPO* 2018).

1.4. Research question

The aim of the following work was to develop a multi-method for validating 7 pesticides, namely aldrin, dieldrin, heptachlor, heptachlor cis- and trans epoxide, pethoxamid and tolylfluanid. While the other 43 analytes were validated before a validation of these compounds would complete the drinking water legislations' specification for monitoring pesticides in drinking water supplies. There are scientific research questions which mainly concern the effect of the physicochemical properties of each analyte on the extraction, desorption and thus also on the overall analytical quality.

The main research questions are:

1.) When comparing solid phase extraction (SPE) to solid phase dynamic extraction (SPDE), which of these techniques is more effective, regarding analytical quality, time and costs for analyzing the following pesticides: aldrin, dieldrin, heptachlor, heptachlor cis- and trans epoxide, pethoxamid, tolylfluanid?

2) Which impact on analytical yield have extraction and desorption parameters of the SPDE technique like extraction strokes, extraction stroke speed, desorption volume and desorption flow speed?

The goal was to fulfill all analytical requirements set by the Austrian drinking water Regulation.

1.5 Analytes of interest
1.5.1 Aldrin
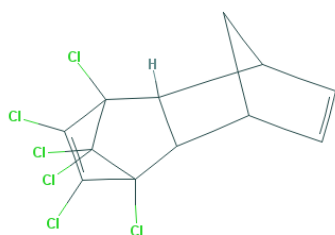
Figure 1 Chemical Structure of aldrin.
Source: *Pubchem* 2018.

Property	Value	Interpretation
Solubility – In water at 20°C	$0.027 \frac{mg}{l}$	Low
Melting point	104°C	
Boiling point	145°C	
Octanol-water partition coefficient at pH 7, 20°C	$P = 3.16 \times 10^6$ $\log P = 6.5$	High, very apolar
Vapour pressure at 25°C	8.6 mPa	Moderately volatile
Henry's law constant at 25°C	$5.35 \times 10^1 \frac{Pa\,m^3}{mol}$	Moderately volatile
Aqueous photolysis DT50 (days) at pH 7	-	-
Aqueous hydrolysis DT50 (days) at 20°C and pH 7	-	-

Table 1 properties of aldrin.   Based on: *IUPAC* 2018.

1.5.2 Dieldrin
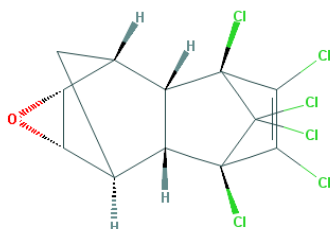
Figure 2 Chemical Structure of dieldrin.
Source: *Pubchem* 2018.

Property	Value	Interpretation
Solubility – In water at 20°C	$0.14 \frac{mg}{l}$	Low
Melting point	177°C	
Boiling point	385°C	
Octanol-water partition coefficient at pH 7, 20°C	$P = 5.01 \times 10^3$ $\log P = 3.7$	High, moderately apolar
Vapour pressure at 25°C	0.024 mPa	Low volatility
Henry's law constant at 25°C	$6.5 \times 10^{-2} \frac{Pa\,m^3}{mol}$	Non-volatile
Aqueous photolysis DT50 (days) at pH 7	-	-
Aqueous hydrolysis DT50 (days) at 20°C and pH 7	-	-

Table 2 properties of dieldrin. Based on: *IUPAC* 2018.

1.5.3 Heptachlor
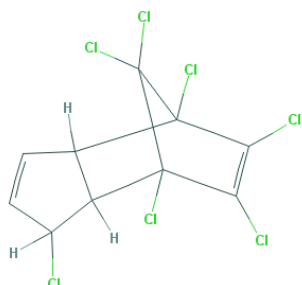
Figure 3 Chemical Structure of heptachlor.

Source: *Pubchem* 2018.

Property	Value	Interpretation
Solubility – In water at 20°C	$0.056 \frac{mg}{l}$	Low
Melting point	95°C	
Boiling point	135°C	
Octanol-water partition coefficient at pH 7, 20°C	$P = 2.75 \times 10^5$ $\log P = 5.44$	High, very apolar
Vapour pressure at 25°C	53 mPa	Highly volatile
Henry's law constant at 25°C	$3.53 \times 10^2 \frac{Pa\ m^3}{mol}$	Volatile
Aqueous photolysis DT50 (days) at pH 7	-	-
Aqueous hydrolysis DT50 (days) at 20°C and pH 7	1	Non-persistent

Table 3 properties of heptachlor. Based on: *IUPAC* 2018.

1.5.4 Heptachlor epoxide cis, trans
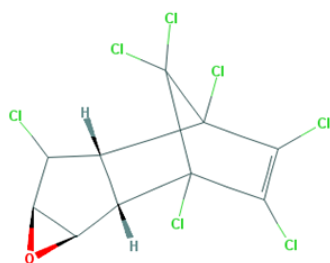
Figure 4 Chemical Structure of heptachlor epoxide cis.

Source: *Pubchem* 2018.
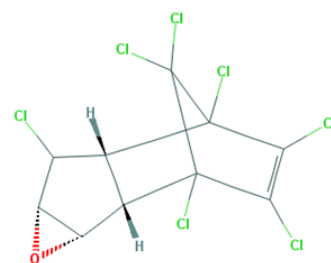
Figure 5 Chemical Structure of heptachlor epoxide trans.

Source: *Pubchem* 2018.

Property	Value	Interpretation
Solubility – In water at 20°C	$0.2 \frac{mg}{l}$	Low
Melting point	-	-
Boiling point	-	-
Octanol-water partition coefficient at pH 7, 20°C	$P = 9.55 \times 10^4$ $\log P = 4.98$	High, very apolar
Vapour pressure at 25°C	-	-
Henry's law constant at 25°C	-	-
Aqueous photolysis DT50 (days) at pH 7	-	-
Aqueous hydrolysis DT50 (days) at 20°C and pH 7	-	-

Table 4 properties of heptachlor epoxide. Based on: *IUPAC* 2018.

1.5.5 Pethoxamid
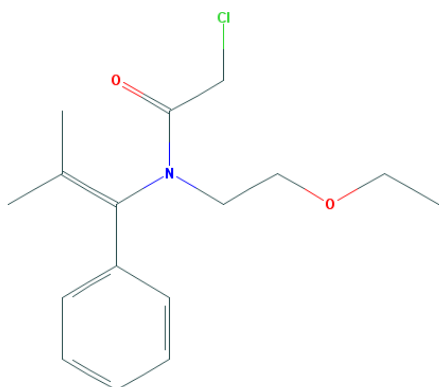
Figure 6 Chemical Structure of pethoxamid.

Source: *Pubchem* 2018.

Property	Value	Interpretation
Solubility – In water at 20°C	$400 \frac{mg}{l}$	Low
Melting point	37.5°C	
Boiling point (Degradation Point)	- (200°C)	
Octanol-water partition coefficient at pH 7, 20°C	$P = 9.12 \times 10^2$ $\log P = 2.96$	Moderate, apolar
Vapour pressure at 25°C	0.34 mPa	Low volatility
Henry's law constant at 25°C	$7.60 \times 10^{-6} \frac{Pa\ m^3}{mol}$	Non-volatile
Aqueous photolysis DT50 (days) at pH 7	14	Slow
Aqueous hydrolysis DT50 (days) at 20°C and pH 7	Stable	-

Table 5 properties of pethoxamid.   Based on: *IUPAC* 2018.

1.5.6 Tolylfluanid
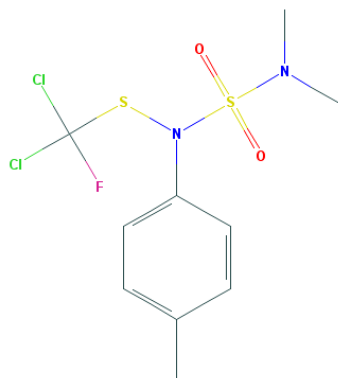
Figure 7 Chemical Structure of tolylfluanid.

Source: *Pubchem* 2018.

Property	Value	Interpretation
Solubility – In water at 20°C	$0,9 \frac{mg}{l}$	Low
Melting point	93°C	
Boiling point (Degradation Point)	- (200°C)	
Octanol-water partition coefficient at pH 7, 20°C	$P = 7.94 \times 10^3$ $\log P = 3.9$	High, apolar
Vapour pressure at 25°C	0.2 mPa	Low volatility
Henry's law constant at 25°C	$7.70 \times 10^{-2} \frac{Pa\,m^3}{mol}$	Non-volatile
Aqueous photolysis DT50 (days) at pH 7	Stable	Slow
Aqueous hydrolysis DT50 (days) at 20°C and pH 7	1.9 pH-sensitive: DT50 11.7 days at pH4 and 22 degC, 10 minutes at pH9 and 20 degC	**Non persistent**

Table 6 properties of tolylfluanid.   Based on: *IUPAC* 2018.

1.6 Comparison: SPE - SPME – SPDE

SPE	**SPME**	**SPDE**
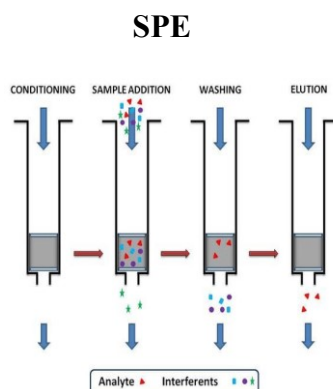
Figure 8 SPE procedure. Source: *Al-Karawi* 2016, Figure 4.
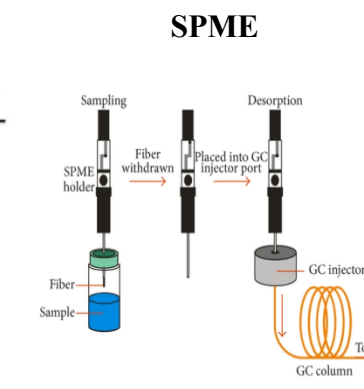
Figure 9 SPME procedure. Source: *Schmidt and Podmore* 2015, Figure 2.
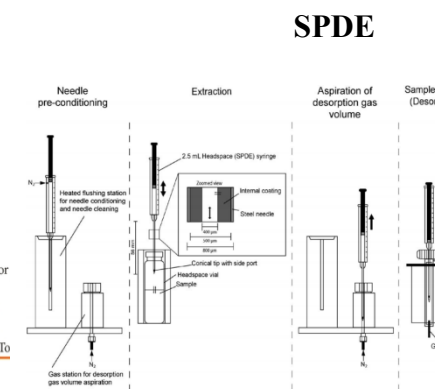
Figure 10 SPDE procedure. Source: *Jochmann et al.* 2006, Figure 1.

Solid phase extraction is basically carried out by activating / conditioning the stationary phase followed by addition of the sample, washing the interferents out of the adsorbent and in the end eluting the searched analytes out of the cartridge.

The stationary phase in solid phase micro extraction is protected by a cover which penetrates the septum of a sample container. After that a piston is pushing down the phase which is now exposed to the headspace or liquid phase of the sample container. After the analytes are adsorbing on the phase they will be desorbed in an injector / inlet of a GC.

Solid phase dynamic extraction works in a similar manner to SPME. The stationary phase is coated on the inner wall of a needle, which is itself connected to a syringe. Extraction follows by penetrating the needle through the septum of the sample container and moving the plunger up and down for a predefined stroke count. Meanwhile the sample will be mixed by a magnetic stir bar. After extraction the syringe takes up some nitrogen gas and desorbs by pushing down the plunger while the needle is in the hot inlet. The needle will be conditioned afterwards in a condition station to prevent carryover.

1.7 Direct Injection of Analytes

Before taking investigations into method development direct injections of the analytes at 7 levels, referring to the Austrian drinking water legislation, were investigated. These were 0,005 µg/l, 0,01 µg/l, 0,03 µg/l, 0,1 µg/l, 0,5 µg/l, 1 µg/l and 5 µg/l. The dilution series was carried out in acetone (AC), despite the stock solutions were in acetonitrile (ACN).

2. Experimental

2.1. Material and Methods SPE

2.1.1. Reagents

All of the organic pollutants used in this work were already ordered before (Sigma Aldrich) the investigation started. The target analytes were aldrin, dieldrin, heptachlor, heptachlor epoxide cis, heptachlor epoxide trans, pethoxamid and tolylfluanid. Stock solutions of 1000 mg/l in ACN also already have been prepared before and are stored in a freezer at -20°C. Working solutions were prepared by dissolving the stock solutions in ACN and ultra clean water (Milli-Q®) in Erlenmeyer flasks until the desired concentration was reached. Solvents like AC, ACN, dichlormethane (DCM), ethyl acetate (EA), Milli-Q® and other consumables were already available in the laboratory.

Analyte	Concentration [mg/l]	Manufactured on
Aldrin	1000	08.02.2017
Dieldrin	1000	08.02.2017
Heptachlor	1000	08.02.2017
Heptachlor (exo) epoxide cis	1000	23.03.2017
Heptachlor (endo) epoxide trans	1000	08.02.2017
Pethoxamid	1000	15.02.2017
Tolylfluanid	1000	17.02.2017

Table 7 concentration and manufacturing date of used analytes. Source: *LVA GmbH* **2018.**

The working standard solutions for calibration and sample fortification were prepared using the following scheme with applying the formula for volume calculation:

$$c_1 \times V_1 = c_2 \times V_2 \rightarrow V_1 = \frac{c_2 \times V_2}{c_1}$$

c_1 = Concentration 1

c_2 = Concentration 2

V_1 = Volume 1

V_2 = Volume 2

Type	Initial conc. c_1 [µg/l]	Desired conc. c_2 [µg/l]	Desired volume V_2 [ml]	Needed volume V_1 [µl]
Stock 1	1.000.000	10.000	10	**100**
Stock 2	10.000	1.000	10	**1.000**
Cal 1	1.000	50	10	**500**
Cal 2	1.000	25	10	**250**
Cal 3	1.000	10	10	**10**
Cal 4	1.000	5	10	**50**
Cal 5	10	1	10	**1.000**
Cal 6	10	0.5	10	**500**
Cal 7	10	0.1	10	**100**
Smpl 1	10	0.01	200	**200**
Smpl 2	10	0.01	200	**200**
Smpl 3	10	0.01	200	**200**

Table 8 Dilution series SPE. Stock = Stock solution; Cal = Calibration; Smpl = Sample. Source: *Matthias Reis* 2018.

Above a dilution scheme is illustrated. The stock and calibration standards were prepared in ACN while for the SPE samples 200µl of a 10 µg/l were transferred to a 200ml Erlenmeyer flask and filled up until the 200ml mark.

Following conditions of the multi reaction monitoring (MRM) transition of the compounds were investigated by former developed methods which are currently applied.

Compound name	Precursor ion	Product ion	Dwell (min)	CE (eV)
Aldrin – Q2	293	257	15	15
Aldrin	263	193	15	30
Aldrin – Q1	263	191	15	30
Dieldrin – Q1	276.8	205.8	15	20
Dieldrin – Q2	276.8	240.8	15	10
Dieldrin	263	193	15	40
Heptachlor – Q2	274	239	15	20
Heptachlor – Q1	273.7	236.9	15	15
Heptachlor	271.7	236.9	15	15
Heptachlor-cis – Q1	353	282	15	15
Heptachlor-cis	353	263	15	15
Heptachlor-cis – Q2	350.8	260.8	15	15
Heptachlor-trans – Q2	217	182	15	20
Heptachlor-trans – Q1	183	119	15	30
Heptachlor-trans	183	155	15	30
Pethoxamid	260	119	15	30
Pethoxamid – Q1	260	147	15	20
Pethoxamid – Q2	131	91	15	20
Tolylfluanid – Q2	238	137	15	8
Tolylfluanid – Q1	137	65	15	30
Tolylfluanid	137	91	15	18

Table 9 Experimental conditions of the MRM transitions.
CE = collision energy. Q1,2 = Qualifier 1,2. Source: *LVA GmbH* 2018.

2.1.2. Sample material

No sample material from Rivers, Lakes etc. was used. Milli-Q® water samples were spiked with a defined concentration of 0.01 µg/l in a triple approach. A calibration was established and recoveries were evaluated by comparing the samples with the calibration curve.

2.1.3. Methods

2.1.3.1. Used cartridges

The SPE preparation method was carried out with two different phases. A 500mg C18 stationary phase with a volume of 6ml (Agilent Technologies). The Strata^{TM} − X (Phenomenex) is a 500mg 33µm polymeric reversed phase cartridge, which also has a volume of 6ml. Considering the manufacturer's features the Bond Elut (Agilent Technologies) should be "extremely retentive for nonpolar compounds; effective for desalting aqueous mixtures and the most hydrophobic, bonded silica sorbent" (*Agilent* 2018). Also 500mg C18 cartridges are

Figure 11 SPE cartridges.
Source: *Matthias Reis* **2018.**

often used within scientific paper works related to pesticide analytics (*Pitarch et al.* 2007, p.252). The polymeric reversed phase of the **Strata^{TM} − X** is designed for giving a strong retention for neutral, acidic or basic compounds, that will undergo aggressive and high organic wash conditions. There are 3 mechanism of retention of this sorbent: hydrogen bonding (dipole-dipole interactions), pi-pi bonding and hydrophobic interaction. (*Phenomenex* 2018)

2.1.3.2. GC-instrumentation

An Agilent 7890B GC system combined with an Agilent 7693 Autosampler and an Agilent 7010 GC-MS triple quadropole mass detector was used for analysis. The instrumentation was operating in electron impact (EI) mode. 2 preinstalled 15m x 250µm x 0.25µm Ag-

Figure 12 GC-MS/MS system for SPE analysis.
Source: *Matthias Reis* 2018.

ilent HP-5MSUI column were used for separating the analytes, with one column acting as a helium (He) backflush column. Following oven setup was programmed: 70°C initial temperature (1min hold time); $50\,\frac{°C}{min}$ to 150°C (0min); $6\,\frac{°C}{min}$ to 200°C (0min) and $16\,\frac{°C}{min}$ to 280°C (8min). The total run time was 23.933 minutes. 3µl splitless injections were performed using an Agilent 5190-2297 200µl dimpled, splitless, ultra inert liner while the inlet was tempered to 320°C. A septum purge flow of $1\,\frac{ml}{min}$ was set up in addition to a purge flow to split vent of $60\,\frac{ml}{min}$ at 1.5min. After the analytes were "washed" out of the inlet gas saver mode was turned on after 4min with a flow rate of $20\,\frac{ml}{min}$. Helium was used as carrier gas with a flow rate of $1.76\,\frac{ml}{min}$.

The mass spectrometer was operating in MS/MS (MRM) positive chemical ionization EI mode, with a source temperature of 300°C. A dwell time of 333 ms per cycle were chosen, measuring 3 cycles per second. Quantitative data for the calibration and samples was obtained using Agilent's Mass Hunter Quantitative Analysis for QQQ. Signals were included with a signal to noise ratio not lower than 3.

2.1.4. Sample preparation

P…vacuum pump

T…silicon tube

M…manometer

V…valve

C…SPE cartridge

S… stopcock

G…glass container

E…elution container

W…waste container
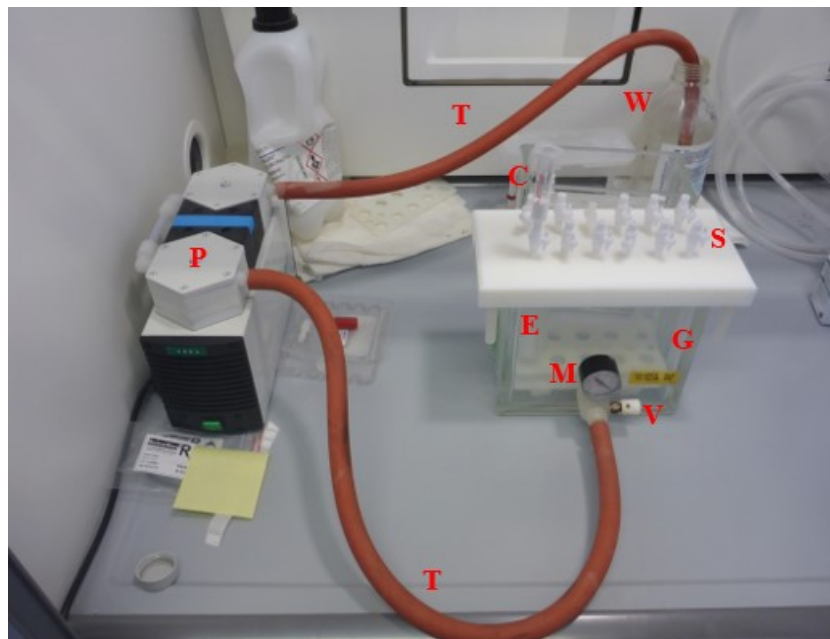
Figure 13   Experimental setup for solid phase extraction.
Source: *Matthias Reis* 2018.

The accomplished sample preparation was based on former studies (*Pitarch et al.* 2007) and was conducted as follows:

- Addition of 3 sets of 0.01µg/l spiking mixture of the analytes to 100ml Milli-Q® water and expose the containers to 5min of an ultrasonic bath (to ensure mixing of the in ACN dissolved analytes with Milli-Q® water)

- Conditioning of the SPE phases with 6ml DCM and 6ml Milli-Q® water avoiding dryness.

- Adding 6ml of sample (max. volume of the SPE cartridges) onto the cartridges until the whole 100ml were added.

- Washing of the cartridges with 3ml of Milli-Q® water.

- Drying by air for 10 minutes under vacuum (vacuum pump).

- Elution was performed by passing 6ml of ACN through the cartridge.

- The collected extract was evaporated under a nitrogen stream of 10psi at 40°C.

- 250µl of ACN was used to redissolve the analytes, achieving a preconcentration factor of 400. Therefore with a spiked sample concentration of 0.01µg/l it is just necessary to analyze 4µg/l concentration after redissolving.
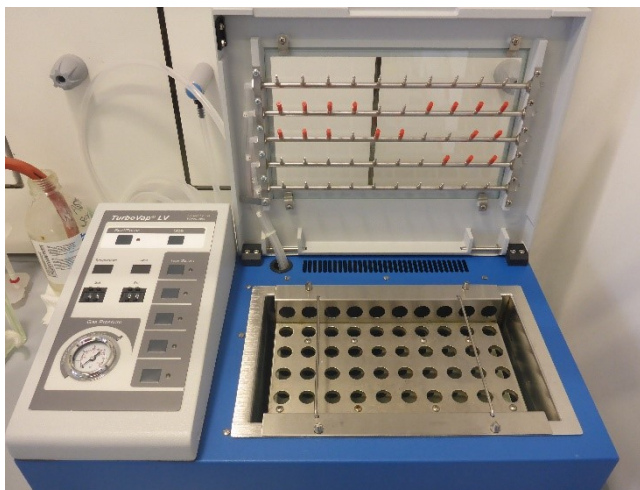
Figure 14 Opened Biotage TurboVap® LV evaporator. Source: *Matthias Reis* **2018.**

- The obtained extract was finally injected into the GC-MS/MS system under the beforementioned conditions. Quantification was carried out by comparing the sample results to these of the 7 point calibration curve, which also was prepared in ACN containing a mixture of the desired analytes.

2.1.5. Validation study

No validation study was conducted due to the tedious and time consuming sample preparation and the failing of desired low parameter values.

2.1.6. Results and discussion

2.1.6.1. Direct injection of analytes

The levels 0,005 µg/l, 0,01 µg/l, 0,03 µg/l were out of range as the signal to noise ratio was below 3, either for the precursor ion and/or the product ions. While the peaks are clearly visible at 5 µg/l for all analytes the noise at lower concentration levels exceeds the responses of most of the desired compounds. Without any preconcentration a further investigation in this simple method therefore seemed to be pointless and can be just used to examine mass transitions of each analyte, measured at a high concentration.
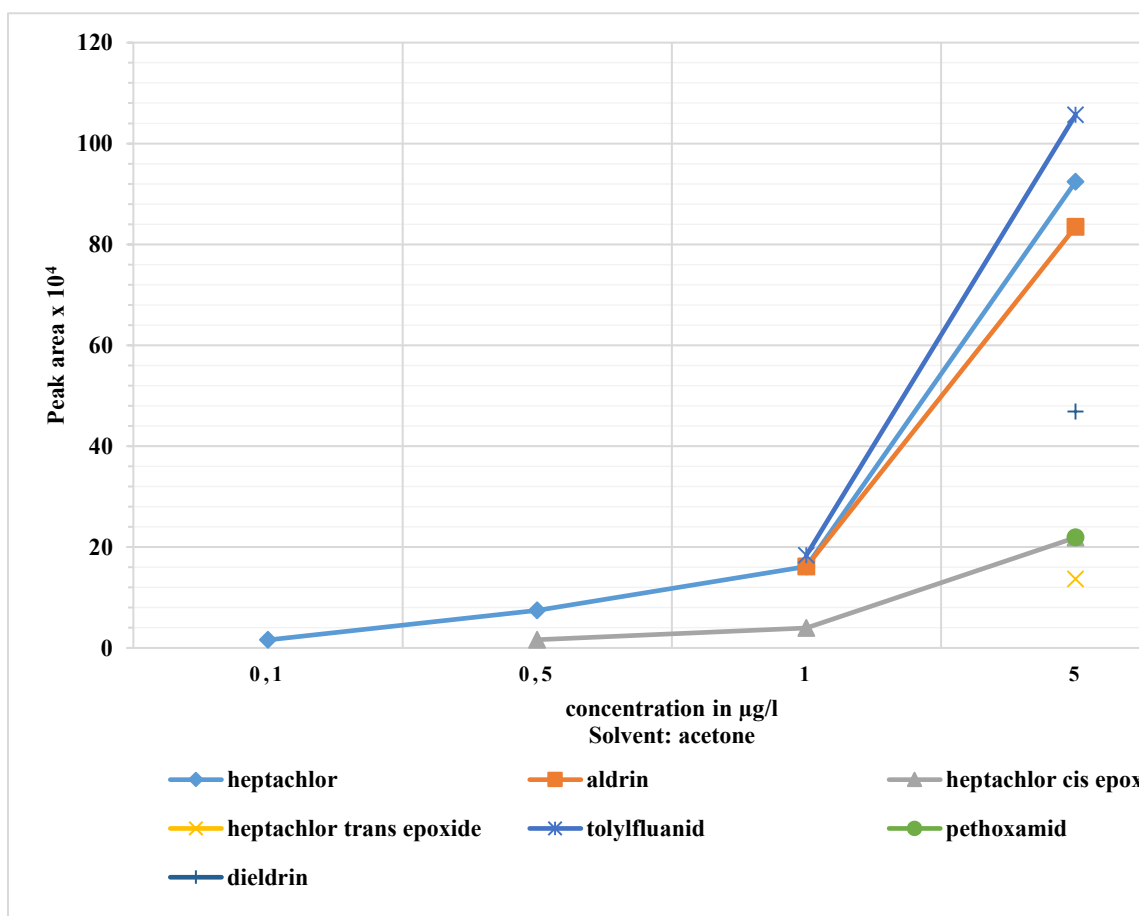
Figure 15 Direct injection of analytes.
Source: *LVA GmbH* 2018

2.1.6.2. Optimizing calibration solvents

Acetone
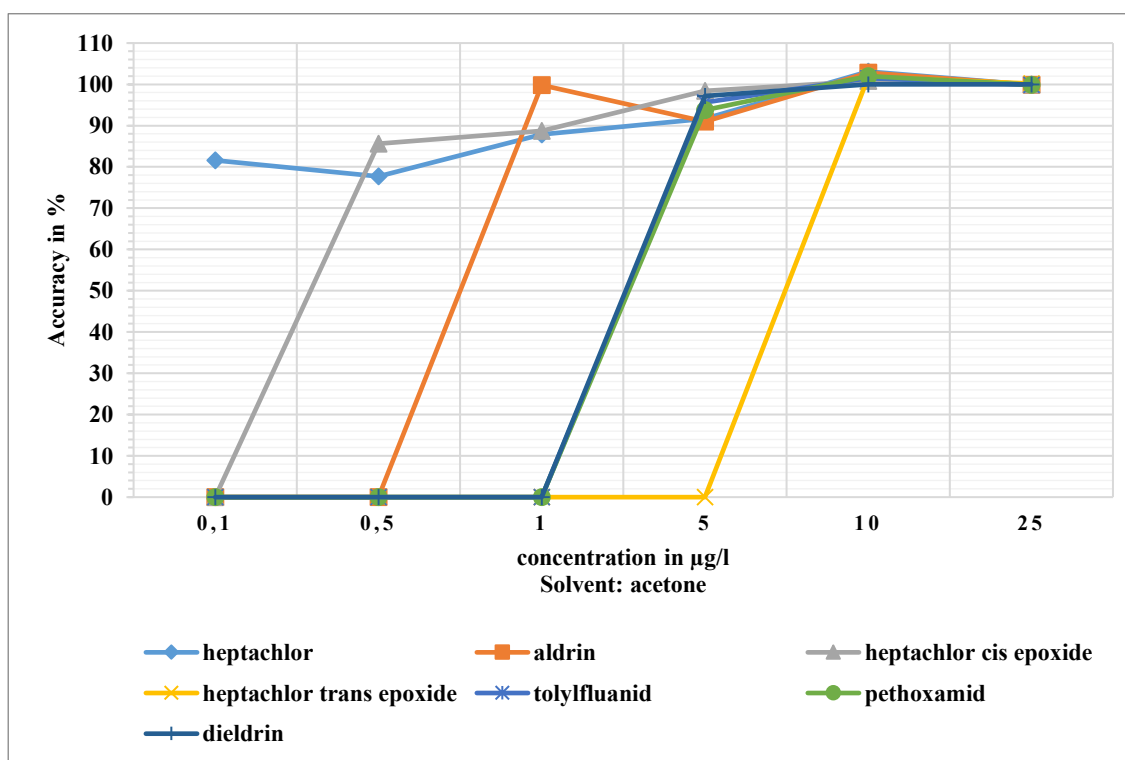
Figure 16 Accuracy of calibration in acetone for SPE.
Accuracy = calculated concentration / expected concentration x 100; the accuracy of not observed peaks or peaks with a S/N ratio below 3 were displayed as 0 for better interpretation of the figure.
Source: *Matthias Reis* 2018.

When a calibration series from 0,1 to 25 µg/l has been established for SPE, first measurements have been conducted. The results revealed that, when using AC as calibration solvent, most of the compounds are detected starting at a concentration of 5 µg/l. While heptachlor is analyzed at the lowest level of 0,1 µg/l, heptachlor cis epoxide and aldrin can be demonstrated at the second lowest levels of 0,5 and 1 µg/l. If the analytes are recovered, they show a good accuracy (trueness) between 80 and 100 %,

Acetonitrile
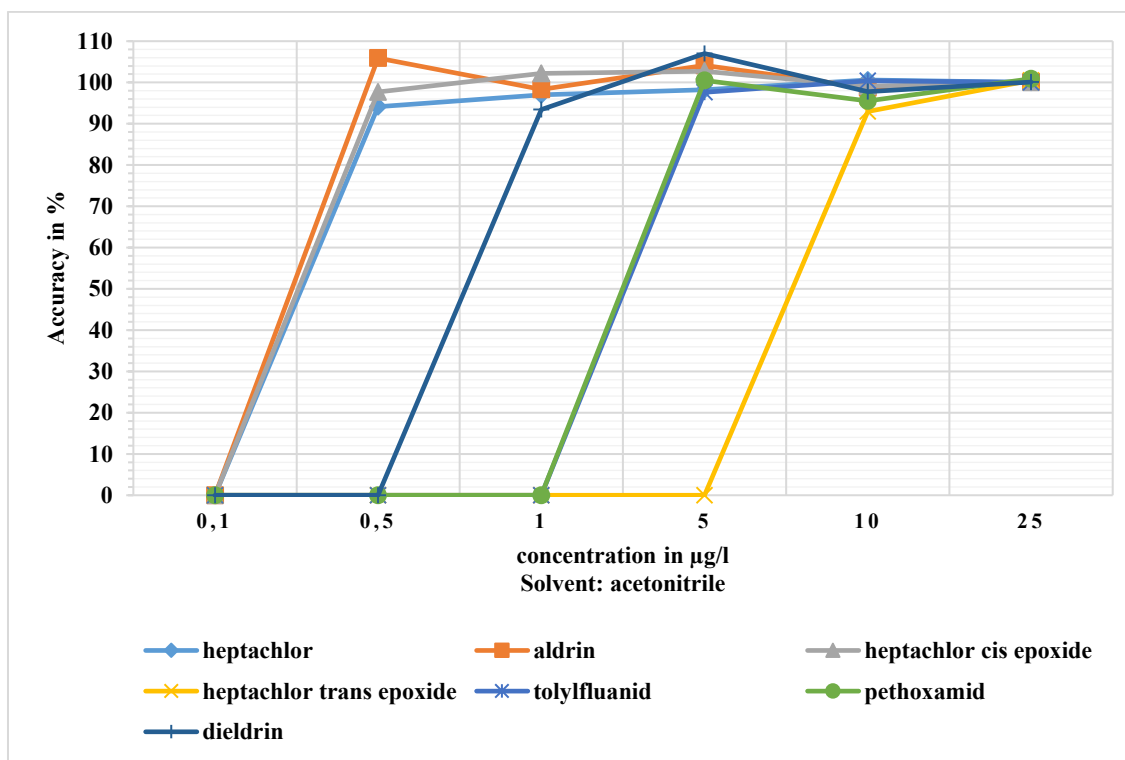
Figure 17 Accuracy of calibration in acetonitrile for SPE.
Accuracy = calculated concentration / expected concentration x 100; the accuracy of not observed peaks or peaks with a S/N ratio below 3 were displayed as 0 for better interpretation of the figure.
Source: *Matthias Reis* **2018.**

For the same calibration series of 0,1 to 25 µg/l ACN used as calibration solvent showed a slightly better recovery at lower concentration levels, starting at a concentration of 1 µg/l for over half of the compounds. While with ACN no analyte can be recovered at 0,1 µg/l. Aldrin, heptachlor and heptachlor cis epoxide are demonstrated at a concentration of 0,5 µg/l. The recovered compounds show satisfactory recovery rates between 80 and 100 %. Since ACN showed slightly better analytic behavior it was chosen for further experiments.

2.1.6.3. Optimizing SPE procedure – elution solvents

Acetone

In the next step a solid phase extraction was carried out by spiking 200ml of Milli-Q® with 0,01 µg/l of the compound mix. After solid phase extraction and evaporation the analytes were redissolved in ACN. When eluting the analytes with AC from the SPE column no analyte can be recovered in the allowed range of 75-125 % and or a relative standard deviation below 25 %. AC therefore turned out to be not suitable.
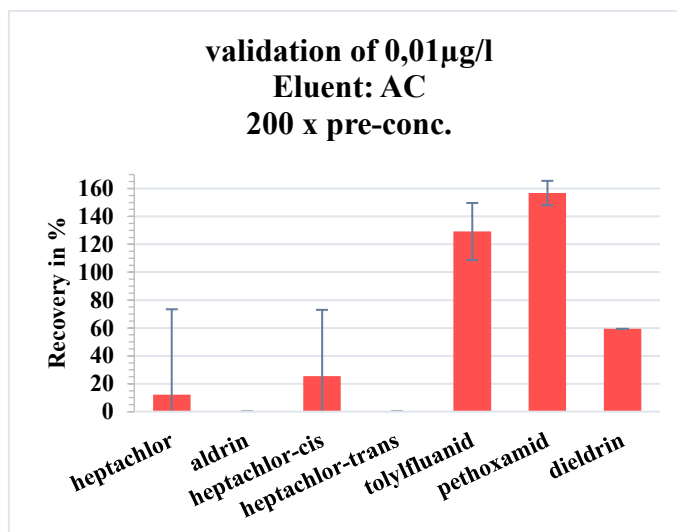
Figure 18 SPE triple approach of 0,01µg/l. AC as eluent.
Red bars highlight a recovery and / or relative standard deviation out of range; if the error bar is 0 just one out of three measurements gave a result; "negative" error bars are not shown for better illustration. Source: *Matthias Reis* 2018.

Ethylacetate-Dichlormethane

According to other observations a mixture of EA-DCM showed best results for the recovery of pesticides in water (*Pitarch et al.* 2007). In the case of the analytes of investigation only pethoxamid showed acceptable results. While two compounds couldn't be recovered at all heptachlor was only observed in one out of three measurements. Therefore, no (relative) standard deviation could be calculated.
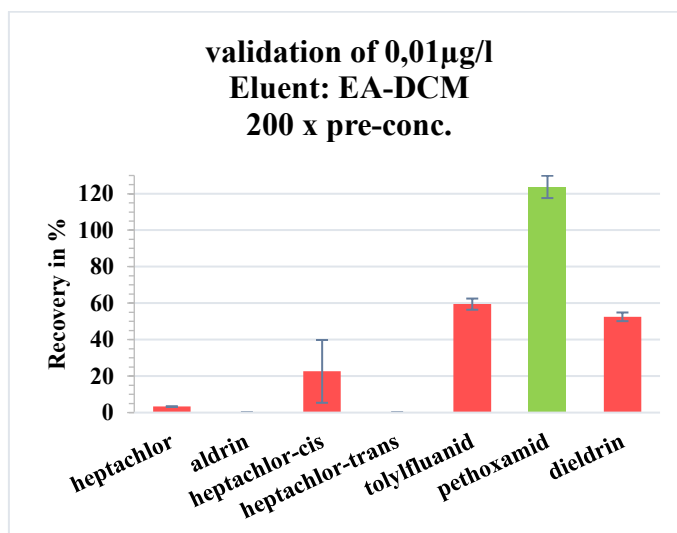
Figure 19 SPE triple approach of 0,01µg/l. EA-DCM as eluent.
Red bars highlight a recovery and / or relative standard deviation out of range; for green bars the recovery and relative standard deviation is within the range. if the error bar is 0 just one out of three measurements gave a result; "negative" error bars are not shown for better illustration. Source: *Matthias Reis* 2018.

Regarding the other compounds, which are visible as bars, the relative standard deviation is always below 25 %, which is an improvement to AC as the elution solvent.

Dichlormethane

Finally an investigation in DCM as the elution solvent revealed the best recovery for all analytes. At least all of them could be recovered, except for heptachlor trans epoxide. Moreover heptachlor and aldrin were within the acceptable range of recovery and relative standard deviation. Furthermore the other compounds showed recoveries in the range of 140% with low standard deviations.

**validation of 0,01µg/l
Eluent: DCM
200 x pre-conc.**
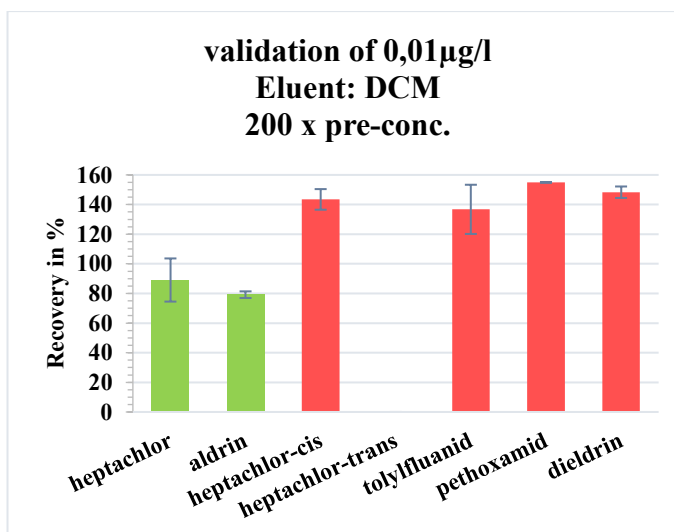
Figure 20 SPE triple approach of 0,01µg/l. DCM as eluent.
Red bars highlight a recovery and / or relative standard deviation out of range; for green bars the recovery and relative standard deviation is within the range; if the error bar is 0 just one out of three measurements gave a result; "negative" error bars are not shown for better illustration. Source: *Matthias Reis* 2018.

Residue after evaporation

After evaporation residues were observed. These could be solvent residues or crystals of the dried analytes, because when substances are ordered for preparing stock solutions they are usually also delivered in crystalline form. While the residues were noticed in one out of 3 AC- and 2 out of 3 DCM-eluent samples, they
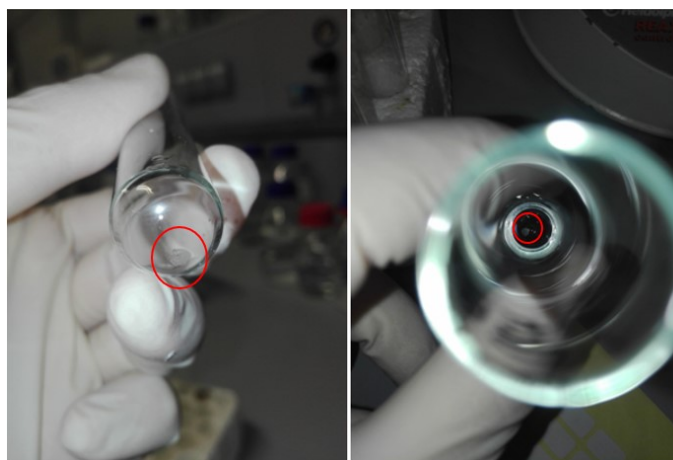
Figure 21 Residue after evaporation.
Source: *Matthias Reis* 2018.

couldn't be found in the EA-DCM-eluent trial. Moreover the analytical results revealed that the attributed sample of AC showed no recovery for all analytes but the two attributed DCM samples were responsible for the observed recoveries in the DCM trial, while the third DCM sample (with no noticed residue) showed no peaks for any analyte. These contradictory results lead to the question where these residues really come from and if they play a role in the poor recovery rates.

2.1.6.4. Optimizing SPE procedure – losing analytes in evaporation

AC, ACN and H2O as solvents

3 test tubes were filled with either 1ml AC, ACN or H2O. Subsequently they were spiked with 10µg/l of analyte mix. When the liquid phase of each of the test tubes were evaporated under a gentle stream of nitrogen, they were redissolved in 1ml ACN. Therefore a precon-
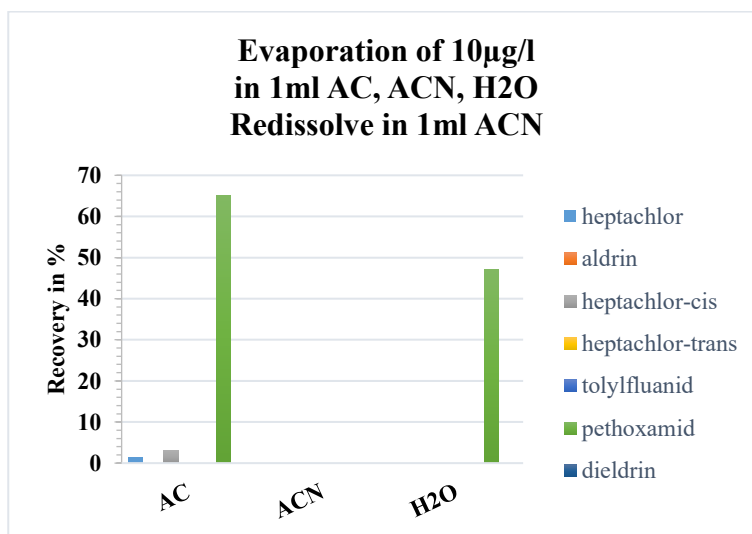
Figure 22 recovery of 10µg/l after evaporation. Source: *Matthias Reis* 2018.

centration factor of 1 leads to a detectable concentration of 10µg/l again. Evaluation of the data revealed that only pethoxamid could be recovered (insufficiently), when using H2O or AC as carrier solvent. Regardless the limitation that this experimental design was conducted only in a simple approach, further investigations were undertaken.

DCM mixed with ACN as solvents

The basis for these further investigations were thoughts about whether the analytes get lost be-cause of their volatility or their affinity to the solvent that they are still bound even when the solvent evaporates and switches to the vapor phase. Since first results suggested a

Figure 23 recovery of 10µg/l after evaporation – ACN/DCM mix. Source: *Matthias Reis* 2018.

loss of analytes within the drying process it was necessary to reevaluate the applied method.

Therefore we first tested the influence of the nitrogen stream and the water bath in which

the test tubes are positioned during drying. 1ml of a 10µg/l mixture in each: Milli-Q®
water, AC and ACN were evaporated. Afterwards they were redissolved in 1ml ACN and
analyzed by the GC-MS/MS system and compared to an untreated analyte mixture of
10µg/l in ACN. Interim results revealed that a loss of analytes happened during either the
drying process or SPE extraction. (*see Figure 22*) Considering this potential effect, a mix-
ture of 1ml ACN and the eluent, 6ml DCM, was prepared. In the course of this the 1ml
level was marked on the test tube and evaporation process was controlled until the solvent
level fell slightly below the 1ml mark. Observations revealed that DCM evaporates faster
than ACN, therefore, while the DCM was evaporated, the thought was that the com-
pounds might bind to the ACN solvent phase, which was conserved. After comparing the
initial weight from the test tube to the weight after evaporation, ACN was added until the
desired preconcentration volume of 1ml was reached again.

With a $0.786 \frac{g}{cm^3}$ density of ACN the residual volume was calculated by using the follow-
ing formula:

$m = m_2 - m_1$ with $\qquad$ m_1 = tare weight of test tube;

$\qquad\qquad\qquad\qquad\qquad$ m_2 = test tube with analytes in ACN after drying step;

$\qquad\qquad\qquad\qquad\qquad$ m = weight of analytes in ACN after drying step

$D = \frac{m}{V} \rightarrow V = \frac{m}{D}$ with $\qquad$ m = weight of analytes in ACN after drying step

$\qquad\qquad\qquad\qquad\qquad$ V = volume of ACN left in test tube

$\qquad\qquad\qquad\qquad\qquad$ D = density ($0.786 \frac{g}{cm^3}$ for ACN)

$V_a = V_e - V$ with $\qquad$ V_a = volume to add (needed to reach desired redissolving
volume)

$\qquad\qquad\qquad\qquad\qquad$ V_e = desired end volume

$\qquad\qquad\qquad\qquad\qquad$ V = volume of ACN left in test tube

In this experimental trial the eluted analytes were not evaporated until complete dryness.
A mark on the test tube, which was frequently controlled, ensured that there was some
ACN left. The results, which can be observed in *Figure 23*, revealed that prevention of
evaporation to complete dryness preserved adequate recoveries for all analytes except
tolylfluanid.

2.1.6.5. SPE considering evaporation investigation

In the end a similar experimental design to the comparison of elution solvents was set up, but this time conducting an actual SPE. The amount of the 0,01µg/l spiked MilliQ® water was 100ml and the evaporation residue was redissolved in 250µl, reaching a preconcentration factor not just of 200 like before, but of 400. After observing the results, which were, with low recovery rates of the analytes, comparable to previous SPE results this sample preparation method was evaluated as not satisfying for the desired compounds.
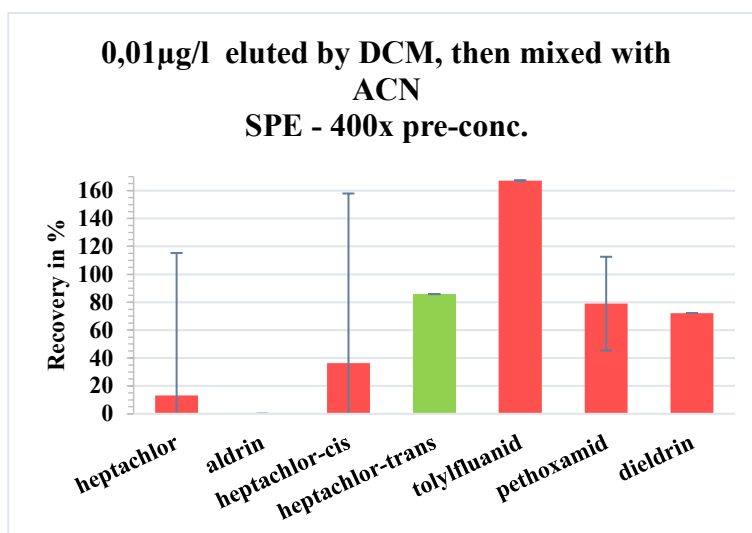
Figure 24 recovery of 0,01 µg/l after evaporation – ACN/DCM mix, SPE. Source: *Matthias Reis* 2018.

Below a sample table of the calculated volumes to add is illustrated. The used formulas can be found in the previous subchapter

Sample	Weight ACN (m) [g]	Volume ACN [µl]	Volume to add [µl]
1.1	0.3871	492	8
1.2	0.0389	49.491	450
1.3	0.3829	487	13

Table 10 Volumes to add for stopped drying process.
Desired volume in this experiment: 250µl. The bold line separates two different experiments / samples.   The SPE was carried out in a triple approach. Source: LVA GmbH 2018.

2.1.6.6. GC optimization

Because of the poor results no time was investigated into GC optimization. A standardized multi-method for pesticides in various food matrices, which is used in the company as an accredited analysis, was also used as a base for the temperature conditions as well as the mass transitions of the analytes.

2.2. SPDE
2.2.1. Overview – extraction mechanism

The needles and syringe were obtained from Chromtech GmbH (Idstein, Germany). Typical coatings are polydimethylsiloxane with 10% activated carbon (PDMS / AC), 25% Cyanopropyl / 25% Phenylpolysiloxane / 50% Methylpolysiloxane(CT-225), polyethylenglycol (carbowax) and many more – up to custom made coatings. They are positioned on the inner wall of the needle and act as a polar or nonpolar adsorbens for various analytes. On the conical top of the needle are two side ports for aspirating and ejecting from a headspace or liquid ana-
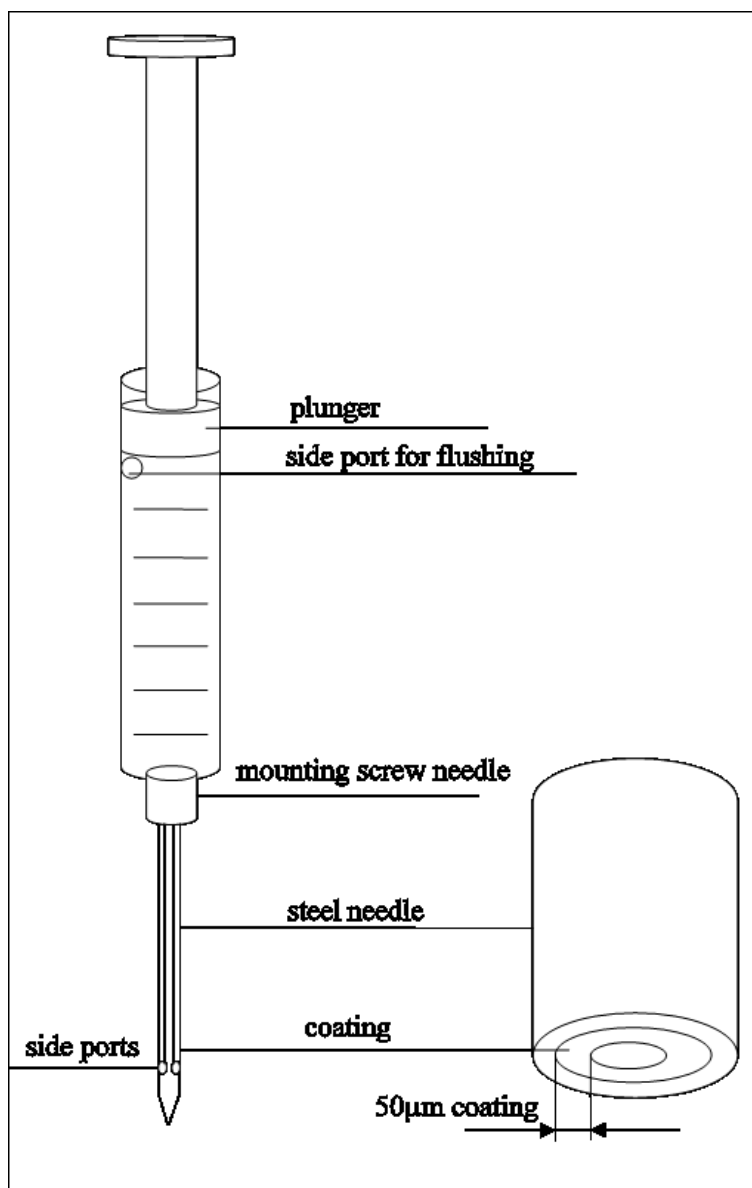
Figure 25 SPDE as a draft.
Based on: Chromtech GmbH 2018.

lyte space. Changing of needles (and therefore coatings) is made quick and easy by a mounting screw, which presses the needle on the syringe. On the top of the syringe is a side port, where carrier gas (in most cases nitrogen) can flow through the syringe and needle for transporting analytes from the coating. This mechanism of flushing the needle (at high temperatures) should reduce carryover effects.

Below the demounted needle and screw on figure 26 is shown. Figure 27 shows one of the two side ports just above the conical tip of the needle. While the plunger is on the highest possible position in figure 28, the syringe port which is now open for gas flow is visible. The syringe is positioned in the syringe heater, which itself is positioned on the pal head. There a small valve, which is disposed on the mounting area of the syringe heater provides nitrogen supply for flushing (figure 29).

Figure 26 SPDE syringe and needle.
Source: *Matthias Reis* 2018.

Figure 27 SPDE needle top.
two side ports for aspirating / ejecting.
Source: *Matthias Reis* 2018.
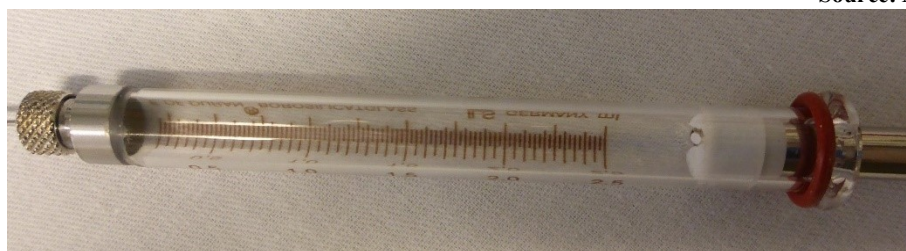
Figure 28 SPDE syringe.
plunger in top position, side port "opened". Source: *Matthias Reis* 2018.

Figure 29 gas valve at SPDE syringe heater mounting area.
Source: *Matthias Reis* 2018.
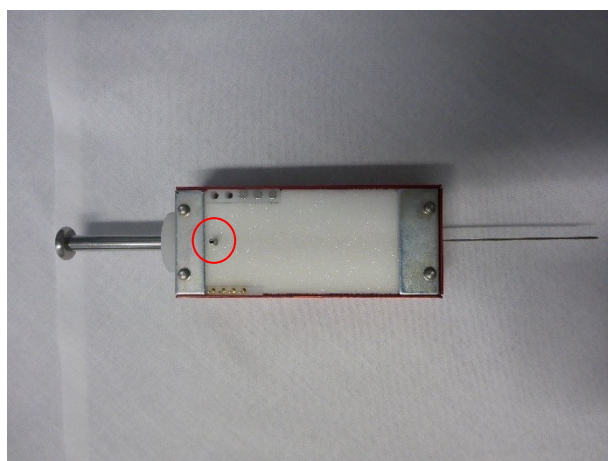
Figure 30 gas valve at SPDE syringe heater.
Source: *Matthias Reis* 2018.

2.2.2. Sample material

No sample material was used. Spiked Milli-Q® water acted as sample.

2.2.3. Stock solutions

Stock solutions of 1 g/l in ACN already have been prepared before and are stored in a freezer at -20°C (see table 7, SPE section). Intermediate stock solutions of 10000 µg/l, 1000 µg/l, 100 µg/l, 10 µg/l and 1µg/l were also prepared in ACN and stored at -20°C. A mix of all analytes was used for calibration and tolylfluanid, pethoxamid were diluted separately at higher concentrations than the other compounds because of the higher validation level according to the drinking water legislation. Working solutions were then prepared by dissolving the intermediate stock solutions in Milli-Q® in Erlenmeyer flasks until the desired concentration was reached. They were then transferred into 20ml vials with magnetic vial caps. Solvents and other consumables were already available.

Type	c1 [µg/l]	c2 [µg/l]	V2 [ml]	V1 [ml]
Cal1	1	0,005	20	0,1
Cal2	10	0,03	20	0,06
Cal3	10	0,05	20	0,1
Cal4	10	0,1	20	0,2
Cal5	100	1	20	0,2
Cal6	1000	2	20	0,04
Tolylfluanid, pethoxamid L	10	0,05	20	0,1
Tolylfluanid, pethoxamid M	10	0,1	20	0,2
Tolylfluanid, pethoxamid H	100	1	20	0,2
Ald., diel., hep., hep.ep. (cis & trans) L	1	0,01	20	0,2
Ald., diel., hep., hep.ep. (cis & trans) M	10	0,03	20	0,06
Ald., diel., hep., hep.ep. (cis & trans) H	100	0,1	20	0,02

Table 11 Dilution series for validation of SPDE method.
  L, M, H…Low, Medium and High validation level; Ald…aldrin; diel…dieldrin; hep…heptachlor; hep.ep…heptachlor epoxide (cis & trans); Cal1-5…calibration level 1-5; c1…initial concentration; c2…desired concentration; V1…volume needed; V2…desired volume. Source: Matthias Reis 2018.

2.2.4. Methods

2.2.4.1. SPDE needles

Following needles / phases were compared. The table is based on Chromtech's recommendations for the SPDE-phases (see Appendix).

Phase	Max. Temperature	Recommended Operating Temperature	Conditioning
SPDE PDMS / AC	280°C	200-280°C	30min @ 250°C
SPDE CT-225	230°C	160-210°C	30min @ 200°C
SPDE DVB-AC	-	-	-
SPDE CX-DVB	-	-	-

Table 12 Recommended operating and conditioning temperatures of SPDE needles. Source: *Chromtech GmbH* **2018.**

In consultation with Chromtech GmbH the needles were compared at inlet temperatures up to 280°C. As there was no information available for the maximum operating temperature for the DVB-AC and CX-DVB coating, they were conditioned at 250°C for 30min.

2.2.4.2. Needle-/ Phase-Conditioning

Before each run the PDMS/AC needle was conditioned in a conditioning / flushing station for at least 15min at 280°C. In the needle comparison run each of the needles were conditioned for just 5 minutes at 280°C to prevent degradation of the coating, as the recommended operating temperature was well below 280°C for some of them. De-

Figure 31 pressure valve for gas and flushing station.
Source: *Matthias Reis* **2018.**

spite that the nitrogen stream which "flushes" through the needle during conditioning should prevent from degradation itself since it cools down the needle / coating.

The pressure of the nitrogen stream for needle conditioning / flushing and for the gas station (where the desorption gas volume is taken up) was set to 0,5 bar.

2.2.4.3. GC instrumentation

An Agilent 6890N (G1530N) GC system combined with a CTC CombiPAL sampler and an Agilent 5973 inert mass selective detector was used for analysis. The instrumentation was operating in electron impact (EI) mode. 1 preinstalled 30m x 250µm x 0.25µm Agilent HP-5MS column was used for separating the ana-

Figure 32 GC-MS System with CTC-autopal sampler.
Source: *Matthias Reis* 2018.

lytes. The following oven setup was programmed: 40°C initial temperature for trapping the analytes in order to minimize peak broadening (2,60min hold time); $25 \frac{°C}{min}$ to 170°C (0min); $2 \frac{°C}{min}$ to 200°C (1min) and $50 \frac{°C}{min}$ to 320°C (5min). The total run time was 31,20 minutes. 1500µl splitless injections were performed using an Agilent 18740-80200 140µl direct, straight liner while the inlet was tempered to 280°C. While desorption efficiency is increasing with higher temperatures, 280°C was used as maximum temperature to prevent degradation of the coating. A purge flow to split vent of $30 \frac{ml}{min}$ at 2,8min was set. After the analytes were "washed" out of the inlet gas saver mode was turned on after 4,80min with a flow rate of $20 \frac{ml}{min}$. Helium was used as carrier gas with a flow rate of $1.2 \frac{ml}{min}$. The mass spectrometer was operating in single ion monitoring (SIM) negative chemical ionization EI mode, with a source temperature of 230°C. A dwell time of 100 ms per cycle (respectively 50ms for heptachlor-epoxide cis and trans as well as tolylfluanid) were chosen, measuring 3,09 and 2,65 cycles per second respectively. Qualitative and quantitative data for the calibration and samples was obtained using Agilent's Mass Hunter Qualitative Analysis and Agilent's Mass Hunter Quantitative Analysis for GCMS respectively. Signals were included with a signal to noise ratio not lower than 3.

2.2.4.4. SPDE extraction and desorption parameters

Injection volume: 1500µl

Drying SYR and
Needle Temperature: 30°C

Pre Incubation Time: 300s

Drying SYR and Needle Time: 60s

Syringe Temperature: 60°C

Desorption Gas Volume: 1500µl

Vial Needle Penetration: 25mm

Pre Desorption Time: 30s

Incubation Temperature: 50°C

Desorption Flow Speed: $10 \frac{\mu l}{s}$

Extraction Stroke Speed: $100 \frac{\mu l}{s}$

Post Inject Clean Solvent 1: 5 Strokes

Extraction strokes: 100

Post Inject Clean Solvent 2: 5 Strokes

Pre Inject Clean Solvent 1: 0 Strokes

Post Clean Volume Syringe: 40%

Pre Inject Clean Solvent 2: 0 Strokes

Conditioning Time: 900s

Pre Clean Volume Syringe: 40%

Wait for next sample: 600s

2.2.5. Sample preparation of the SPDE method
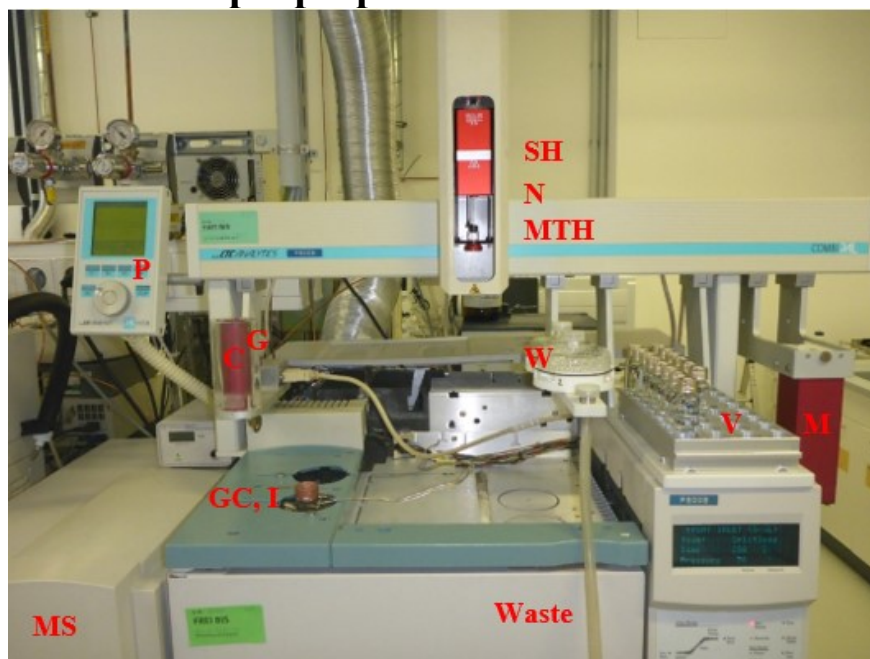
Figure 33 Overview of SPDE sample preparation system.
Source: *Matthias Reis* 2018.

MS…mass spectrometer

GC, I…gas chromatograph, inlet

M…single magnet mixer

V…vial tray

W…wash stations (2)

Waste…tube to waste container

MTH…magnetic transport head

N…needle

SH…syringe body heater

G…gas station

C…conditioning station

P…programming panel ctc pal
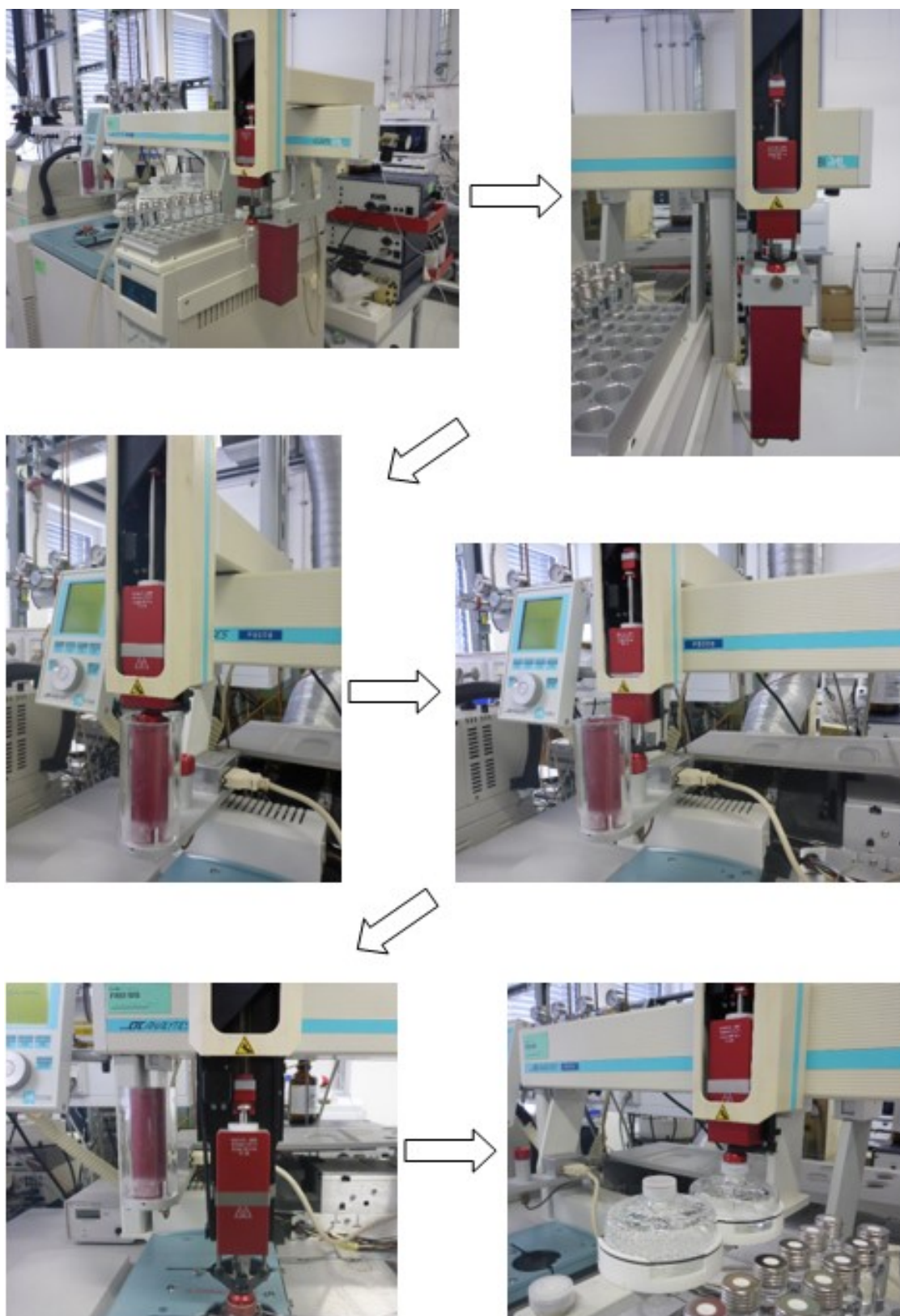
Figure 34 SPDE еxtraction and desorption process.
Source: *Matthias Reis* **2018.**

The automated sample preparation in solid phase dynamic extraction:

The pal autosampler is equipped with a single magnet mixer, a gas station, a conditioning station and a vial tray. Preprogrammed macros were used for the PAL's control, with some afterwards added cycles. While the gas station and PAL head are connected with nitrogen, the syringe body was held at 35°C. A 56mm long, PDMS-AC (polydimethylsiloxane- 10% activated carbon) coated (50μm thickness) SPDE needle was used. All needles were obtained from Chromtech GmbH and were preconditioned according to the recommended conditioning temperature datasheet of Chromtech (see Appendix or Table 12).

After the method parameters for the mass spectrometer, the GC (inlet, oven, column) and the CTC PAL were set and the sequence was written, the CTC PAL waits for a sync signal if the GC is ready (in explanation: temperature, pressure and so on reaches the method's start settings). Subsequently the PAL head moves to the vial tray, picks up (with the magnetic head) a vial (with a magnetic vial cap) and transports the vial to the single magnet mixer (SMM) (*upper left figure*). It moves back to the home position and waits for a defined period of time (pre incubation time) where the vial / sample can heat up and stirred by a magnetic stir bar until an equilibrium of analytes is reached. When the time is up, the pal head moves to the SMM again and starts extraction, with a predefined number of strokes (*upper right figure*) at a fixed volume of 1ml. After extracting the pal moves to the conditioning / flushing station for drying / flushing the needle by a stream of nitrogen gas (*middle left figure*). Subsequently the pal picks up the desorption gas (nitrogen) with a predefined volume (desorption gas volume) (*middle right figure*) followed by inserting the needle into the hot inlet of the GC, wait for a defined amount of time (pre desorption time) to let the needle equilibrate to the inlet temperature and start the desorption process by pulling down the plunger of the syringe with a predefined speed (desorption flow speed) (*lower left figure*). When the desorption process is finished the autosampler first takes back the vial from the SMM to the vial tray followed by washing the needle (*lower right figure*). There are two 100ml containers, which can be filled with wash solvent. Milli-Q® water and ACN were used for wash experiments. After each wash cycle the autosampler moves to the waste station, ejecting the waste into a tube, which is connected to a 2l container. Washing is followed by preconditioning the needle / coating for the next sample run. The sampler pulls up the plunger to the maximum position, to

uncover the syringe's side port. Through a side port adapter at the back of the syringe heater, nitrogen flows through the syringe and needle. This 15min conditioning step at 280°C removes all analytes from the phase and should prevent carryover.

2.2.6. Validation study

Linearity

Reference standard solutions were used for obtaining a calibration curve for each compound. The calibration levels for the low, medium and high level validation were 0,005µg/l, 0,03µg/l, 0,05µg/l, 0,1µg/l and 1µg/l whereby 2µg/l was added for high level validation. Medium level calibration was carried out a second time at the same levels but instead of 2µg/l as highest level, 1µg/l was chosen with another measurement at 0,5µg/l. Outliers were removed but at least 5 calibration points were included (one exception: pethoxamid at low level validation). A regression coefficient of greater than 0,999 was reached by a quadratic calibration curve at all levels and compounds.

Accuracy

7 measurements, excluding 2 outliers at a max, estimated accuracy of the method by taking the mean value and comparing it to the limiting conditions for recovery (75-125%) and precision (RSD < 25%) of the desired analytes, based on the drinking water legislation (*TWV* 2001).

Precision

Recovery experiments of 7 measures were carried out at each fortification level while outliers were removed, including at least 5 measurements. The RSD was calculated and therefore the repeatability of the method was expressed.

Selectivity

For each compound the ms/ms transitions (precursor and product ions) were identified by running a scan and comparing the peak's transitions with the EURL database (*EURL* 2018). Afterwards the quantifying and qualifying mass transitions were implemented in separated time windows, referring to the analyte's retention times. This resulted in a separation of the respective peak responses.

Limit of quantification (LOQ)

A full validation of the lowest concentration measured stated the LOQ for this method. The limiting conditions for recovery (75-125%) and precision (RSD < 25%), based on the drinking water legislation (*TWV* 2001) were considered.

Limit of detection (LOD)

A signal (peak of compound) to noise (background noise in chromatogram) ratio of at least 3 was chosen for precursor and product ions. The LOD therefore is an estimation for identifying the desired substances at the lowest fortification level.

Confirmation criteria

Within a scan method one precursor ion for quantification and two qualifying transition / product ions were chosen based on their intensities, compared to the specific transitions cited in EURL database (*EURL* 2018). The ratio of the intensity of the product ions to the intensity of the precursor ion of each analyte was determined. A quantifier / qualifier ratio (Q/q ratio) of ±20% was accepted in the method. These ratios were always in the desired range, with maximum deviations of ±20%.

2.2.7. Results and discussion

2.2.7.1. Scan and SIM of the final method

Figure 35 shows an analyte scan, with the respective mass transitions of the investigated-substances entered into the software, so that a large part of the scanned transitions (m/z 50-500) have already been removed. Furthermore the peak of tolylfluanid was selected for showing mass transitions at this retention time.
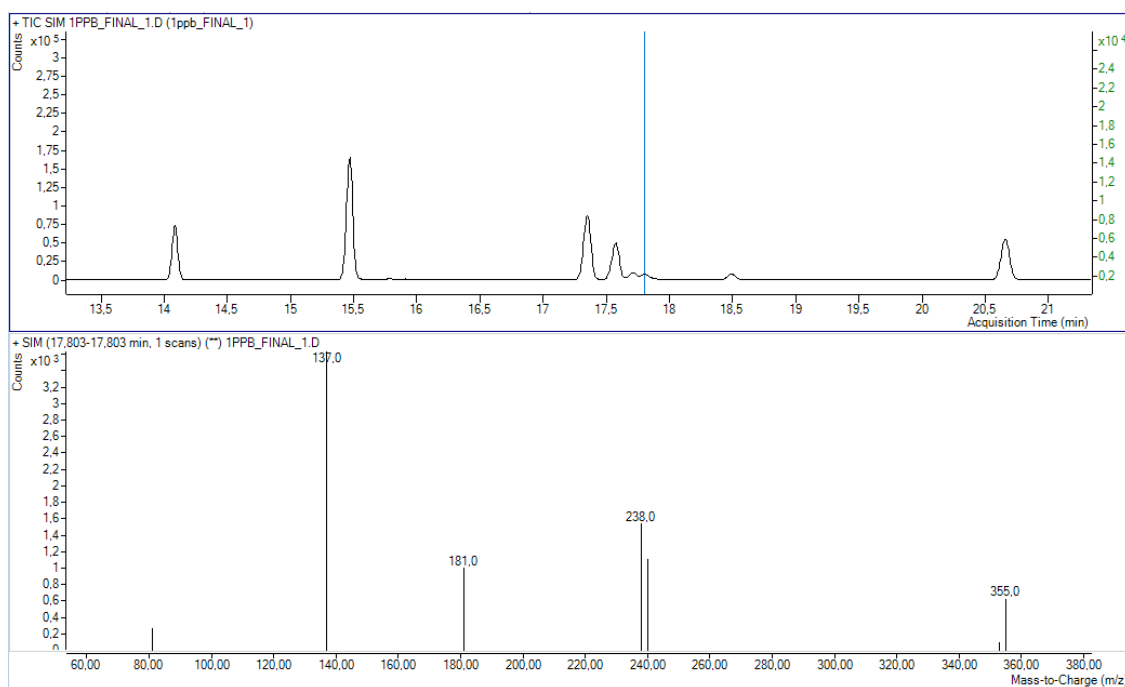
Figure 35 Scan of final method.
Shown is the peak area at 1µg/l analyte concentration as well as the mass transitions of tolylfluanid. Source: *Matthias Reis* **2018.**

In figure 36 a sim of the same method, but with 75°C extraction temperature is shown. Separate time windows for heptachlor, aldrin, heptachlor epoxide cis- / trans / tolylfluanid and pethoxamid were set. For all compounds the sensitivity / peak area increased. While tolylfluanid was selected again, the respective quantitative product ion is also highlighted in green colour, showing that especially heptachlor epoxide trans has similar mass transitions as tolylfluanid among the respective selected ones.
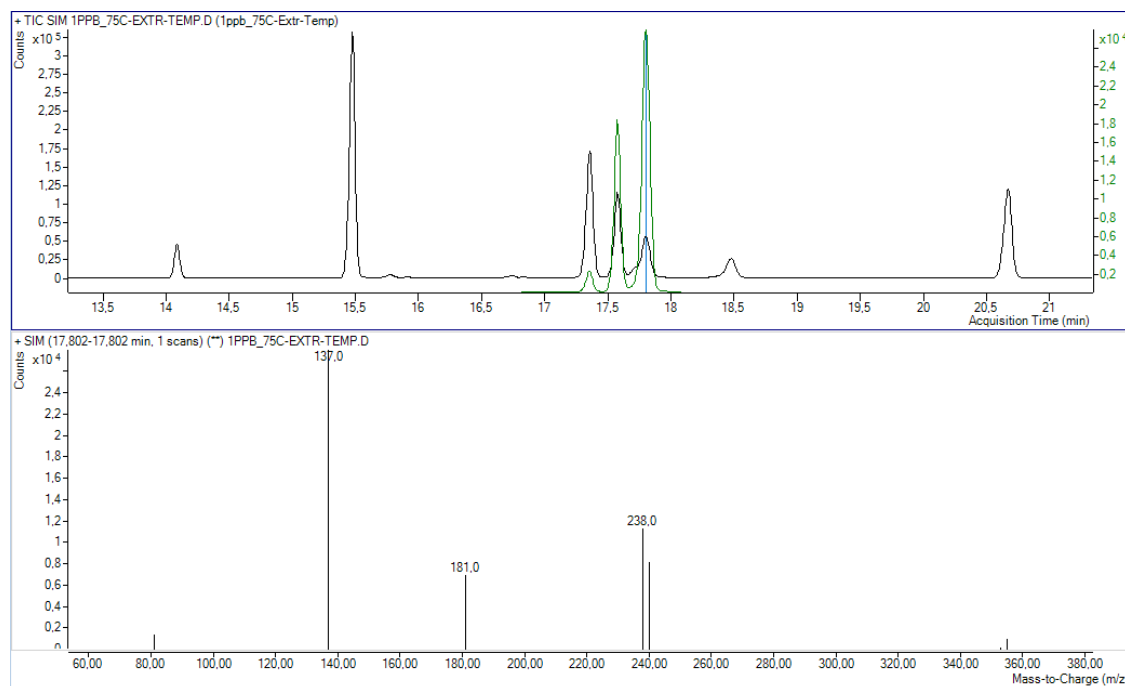
Figure 36 Sim of final method.
Shown is the peak area at 1µg/l analyte concentration as well as the mass transitions of tolylfluanid. Source: *Matthias Reis* **2018.**

2.2.7.2.   Comparison of SPDE coatings

Different SPDE selectivity coatings were investigated. Based on the IUPAC compound details all analytes of interest have a medium to high nonpolarity. Therefore nonpolar coatings should retain the analytes better than polar coatings. While there are two extraction mechanisms which appear on the polymer – active carbon mixes (absorption and adsorption respectively), the CT-225 and CX-DVB coating are just working by one of them – absorption. Both of the very nonpolar DVB coatings show a poor adsorption of the compounds while the best results were obtained by the medium polar PDMS/AC and CT-225 needle.
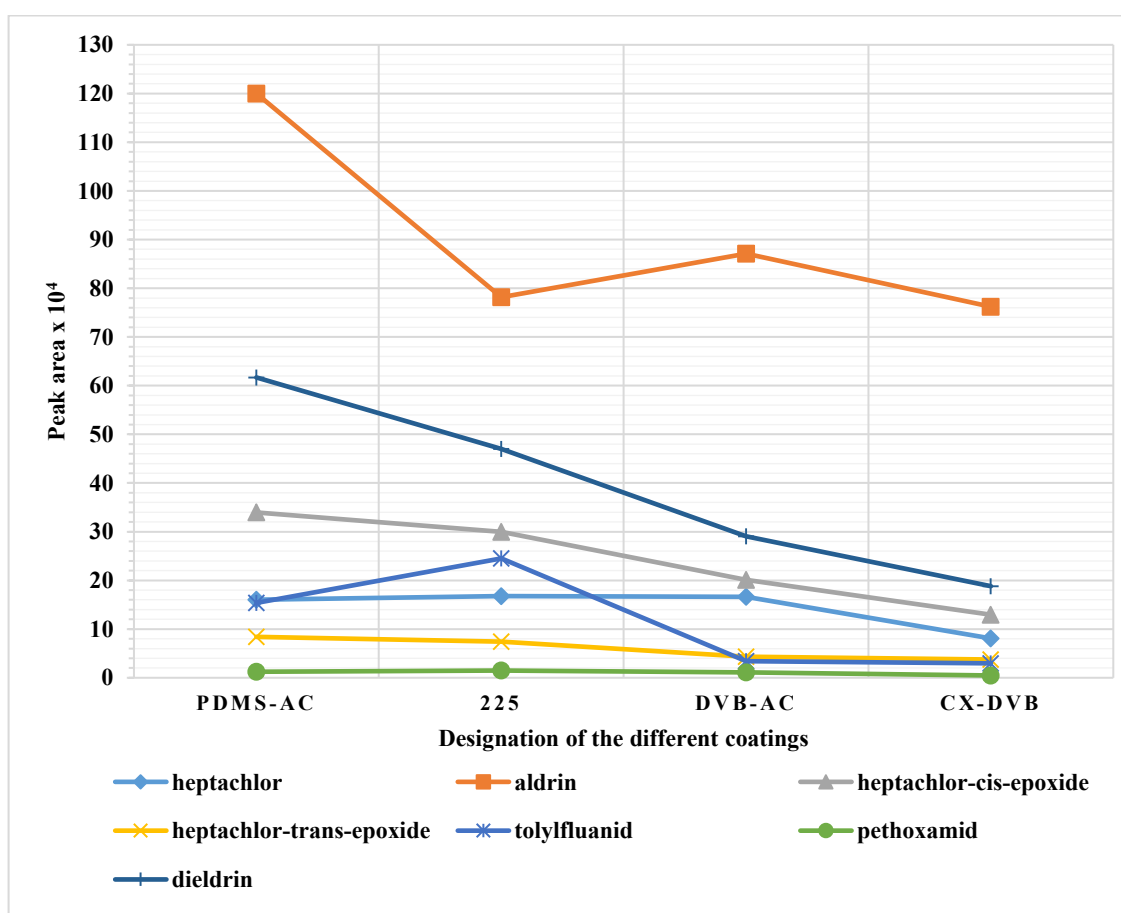
Figure 37 Comparison of 4 SPDE coatings.
Effect on peak area at 1μg/l concentration.Source: *Matthias Reis* **2018.**

2.2.7.3. Optimizing SPDE extraction parameters

Extraction out of head- or water space

Extraction of the analytes can be conducted by extracting out of the headspace of the vial or directly from the liquid phase where the analytes are dissolved. For testing the efficiency of both methods a 20ml vial was filled with a 10µg/l spiked solution of either 20ml or 10ml with a constant needle penetration of 25mm. Refering to the compound details the results verify the volatility of heptachlor and aldrin since these are the only compounds with a higher response when extracted out of headspace.
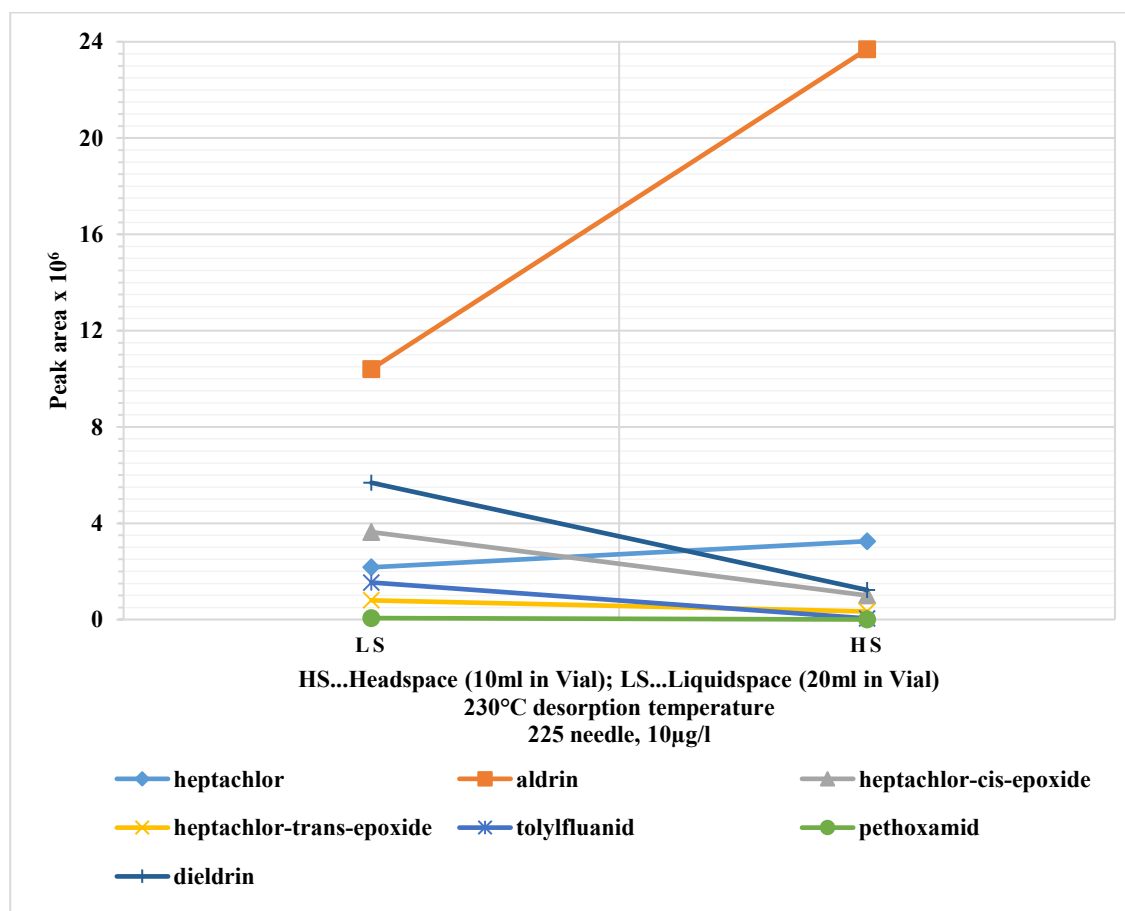
Figure 38 Effect on extraction: headspace – liquid space.
Effect on peak area at 10µg/l concentration. Source: *Matthias Reis* 2018.

Vial needle penetration

Based on the findings of *Jochmann et al.* 2007, who noticed an effect of temperature difference of the single magnet mixer and syringe / needle a similar investigation was carried out. While only a positive trendline could be obtained for tolylfluanid, because it showed a signal only at two measurements all other compound's peak area tend to negatively correlate with increasing needle penetration. Beside the 56mm long needles there are also 74mm long needles available, allowing a greater area exposed to the air and therefore possibly greater effects on extraction efficiency. Further experiments have shown that the temperature of the syringe seems to play a minor role, therefore the surrounding room air temperature could be responsible for this phenomenon. With purchasable extraction coolers the effects might be additionally reinforced.
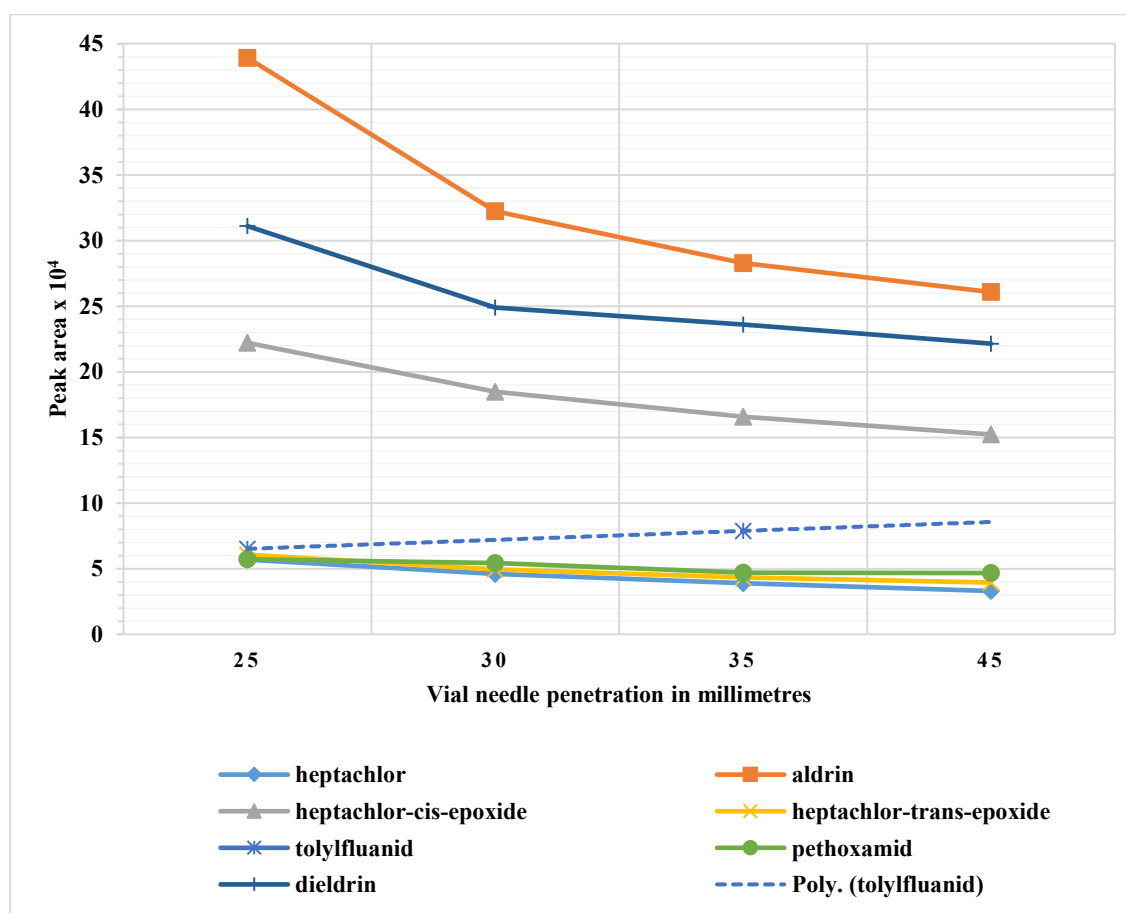
Figure 39 Effect on extraction: vial needle penetration.
Effect on peak area at 1µg/l concentration. Peak for tolylfluanid wasn't observed at 30 and 45mm.
Therefore an order 2 polynomial trendline was added to estimate the concentration at these levels.
Source: *Matthias Reis* 2018.

Extraction strokes

With a higher amount of cycles where the plunger of the syringe pulls up and pushes down the sample solution, the more analytes will be retained. The only limitation of using a high amount of strokes and therefore retaining a high amount of compounds is time and memory effects. With a stroke speed of 100μl/s it takes the syringe 33 minutes when aspirating 1ml of sample volume a hundred times.
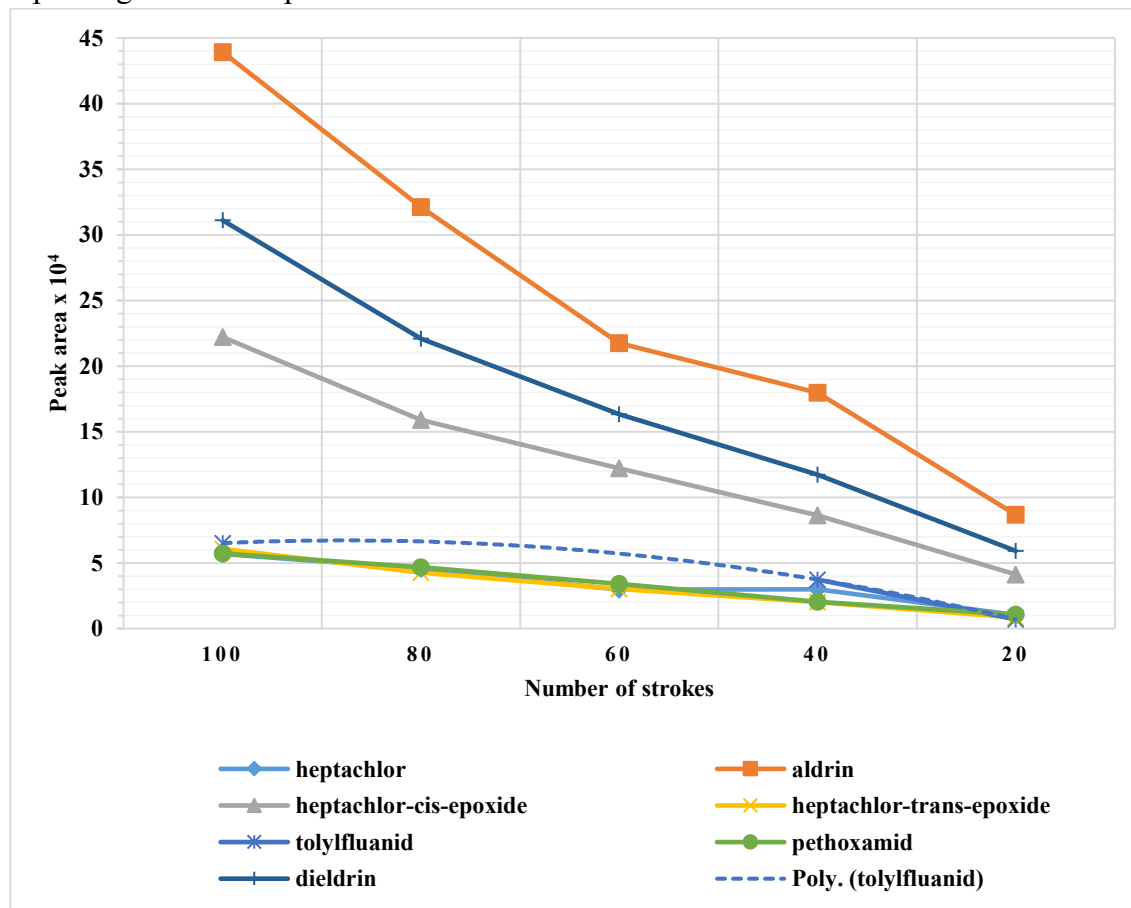
Figure 40 Effect on extraction: extraction strokes.
Effect on peak area at 1μg/l concentration. Peak for tolylfluanid wasn't observed at 80 and 60 strokes.
Therefore an order 2 polynomial trendline was added to estimate the concentration at these levels.
Source: *Matthias Reis* **2018.**

Extraction stroke speed

The speed of the plunger movement can be set in the CTC PAL's method parameters. It determines therefore the amount of time in which the analytes get in touch with the coating. Usually one would assume that the more time there is, the higher the retention rate. This assumption is supported by several SPDE studies (*Jochmann et al.* 2006 and 2007). In this work these empirical observings are only applicable for pethoxamid. For most of the analytes the highest response is obtained at 100μl/s whereby tolylfluanid obviously retains best at 150μl/s.
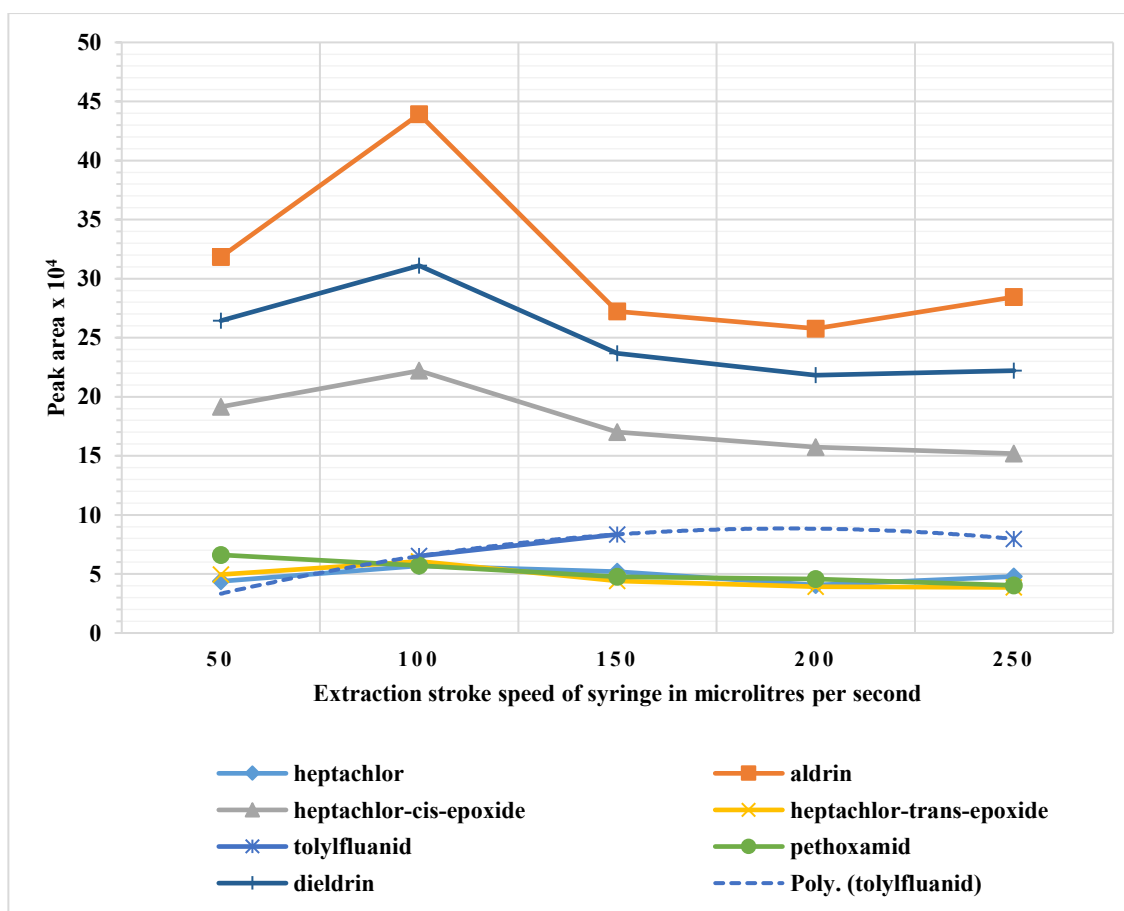
Figure 41 Effect on extraction: extraction stroke speed.
Effect on peak area at 1μg/l concentration. Peak for tolylfluanid wasn't observed at 50μl/s and 200μl/s.
Therefore an order 2 polynomial trendline was added to estimate the concentration at these levels.
Source: *Matthias Reis* 2018.

Extraction temperature

At a constant syringe temperature of 35°C the extraction efficiency is increasing with a higher sample temperature. While at 65°C a maximum is reached for most of the analytes, aldrin seems to further increase at 75°C whereby heptachlor's extraction efficiency starts to decrease again at this level.
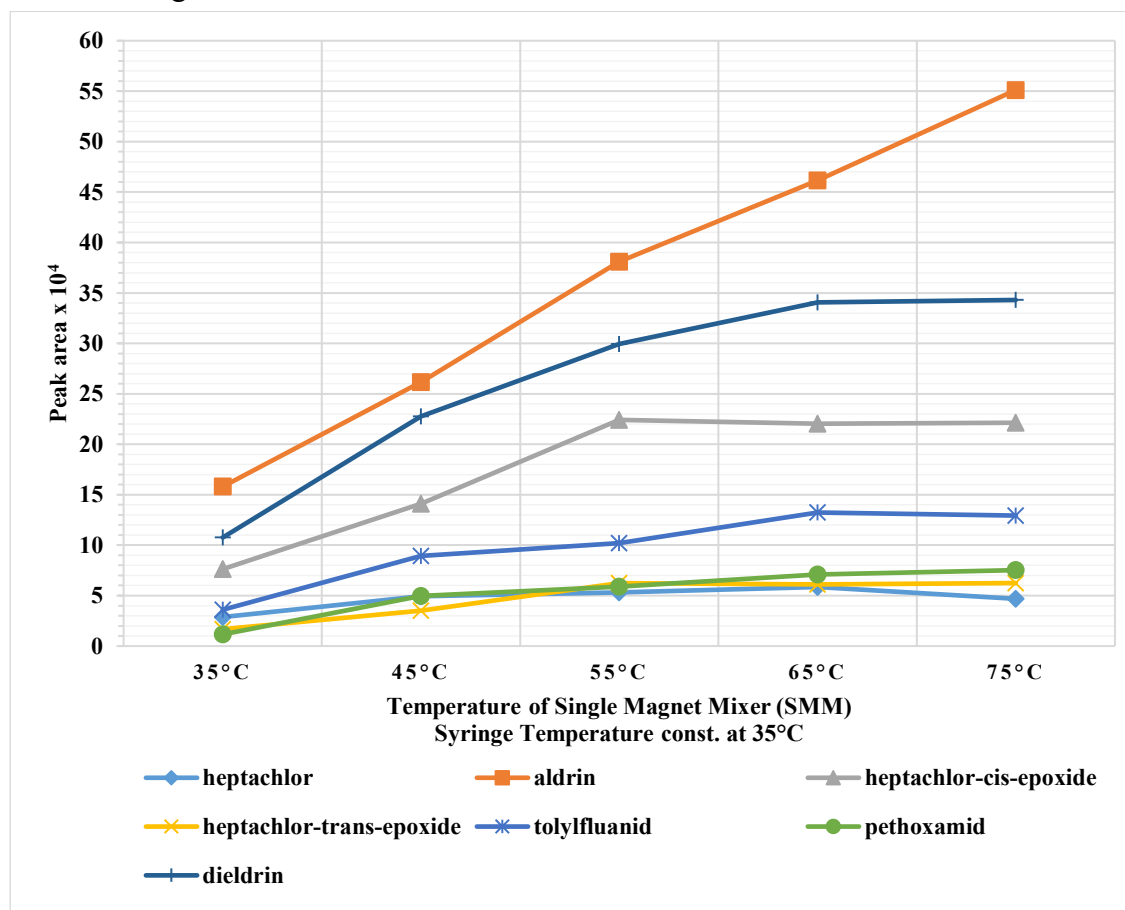
Figure 42 Effect on extraction: extraction temperature .
Effect on peak area at 1µg/l concentration. Source: *Matthias Reis* 2018.

Syringe Temperature

Contrary to the extraction temperature the syringe temperature does not have any effect on extraction efficiency. Aldrin shows a decrease in peak area at the lowest tested level of 35°C. This could be explained by an outlier because the limitation of all parameter tests was that each was carried out in a simple approach. Moreover a syringe temperature, which was set 10°C hotter than the SMM temperature led to a lesser extent of analyte carryover. This might be explained by repressed condensation of water on the syringe's surfaces.
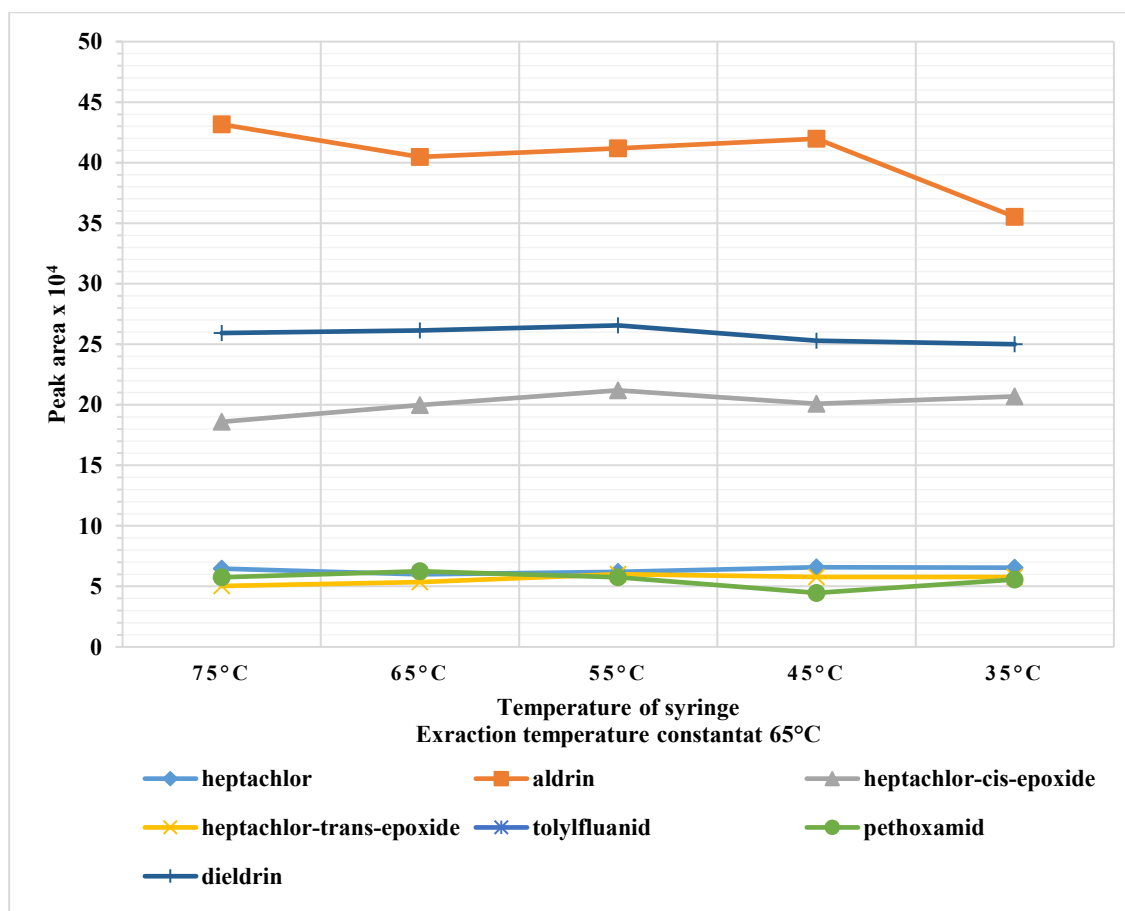
Figure 43 Effect on extraction: syringe temperature.
Effect on peak area at 1µg/l concentration. Peak for tolylfluanid wasn't observed. Source: *Matthias Reis* 2018.

2.2.7.4. Optimizing SPDE desorption parameters

Desorption temperature (inlet)

Increasing the temperature of the inlet leads to an increase in peak area of most analytes. Heptachlor was an exception (aside pethoxamid at 260°C), with the highest response noticed at 250°C inlet temperature. Besides, the metabolites heptachlor cis and trans epoxide are increasing with higher desorption temperatures. This leads to an assumption where metabolism rate of heptachlor is increasing by temperature rise.
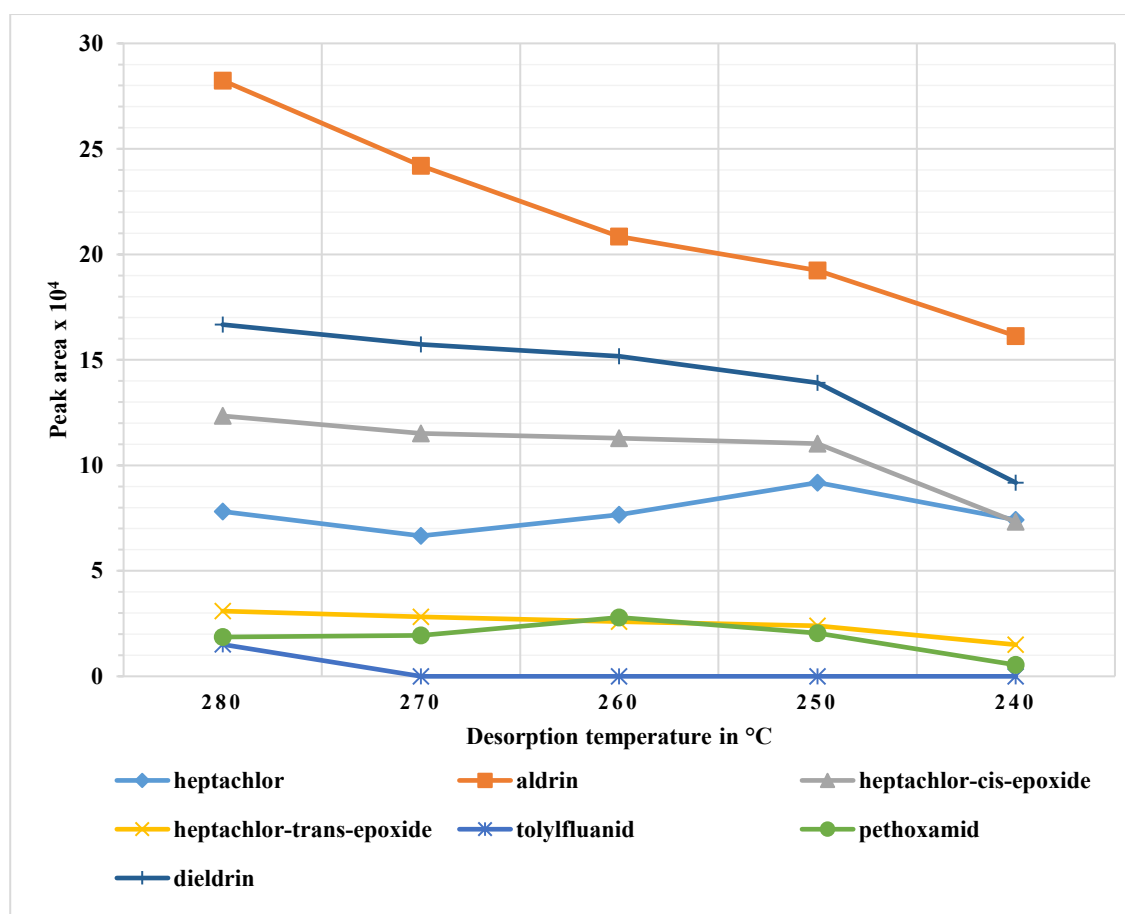
Figure 44 Effect on desorption: desorption temperature.
Effect on peak area at 1μg/l concentration. Peak for tolylfluanid was just observed at 280°C desorption temperature and therefore is highlighted as 0 at the other temperatures for better representation. Source: *Matthias Reis* 2018.

Pre desorption time

The pre-desorption time marks the interval between insertion of the SPDE needle into the hot inlet and starting the desorption process / ejecting the desorption gas. While other studies observed a peak splitting with higher desorption times, this phenomenon was not noticed in this work. Rather the highest desorption efficiency was reached by letting the needle equilibrate from room temperature to inlet temperature for 30 seconds. Heptachlor and pethoxamid were slightly better and tolylfluanid much better desorbed at 0s. Since no active nitrogen stream is applied during pre-desorption, the needle's coating is protected by nitrogen molecules hold by the vacuum of the previous desorption gas uptake. Therefore coatings with lower operating temperatures could be easily damaged with higher pre-desorption times.
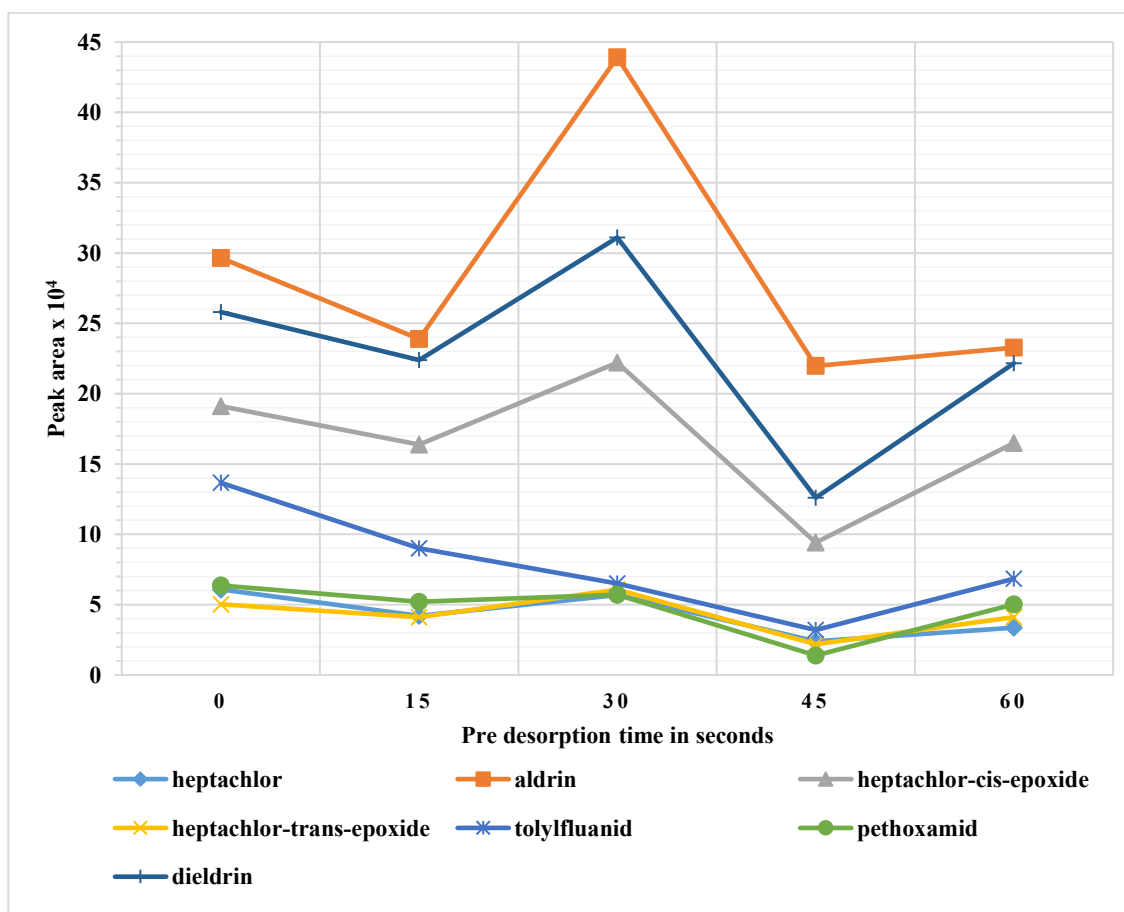
Figure 45 Effect on desorption: pre-desorption time.
Effect on peak area at 1µg/l concentration. Source: *Matthias Reis* 2018.

Desorption gas volume

When changing the desorption gas volume also the total time of desorption changes because it is the quotient of desorption gas volume and desorption flow speed. Because the oven is programmed for "start heating" after pre-desorption time one has to consider these constants when programming a temperature ramp. Two methods were investigated whereby in the first one the time intervals between end of desorption, start heating up the oven and purging the inlet kept the same. As an example when desorption time for 1500µl desorption volume was 2,50min with a 40°C oven hold time of 2,60min and a purge time of 2,80min then at 2500µl desorption volume the desorption time would be 4,17min with a 40°C oven hold time of 4,27min and a purge time of 4,47min.

Figure 46 Effect on desorption: desorption gas volume 1.
Effect on peak area at 1µg/l concentration, adjusted initial hold and purge time. Peak for tolylfluanid was just observed at 1500µl desorption volume and there-fore is highlighted as 0 at the other volumes for better representation. Source: *Matthias Reis* 2018.

In the second method the highest desorption parameters (these of 2500µl desorption volume) were used for each volume level. While the results of the first method show a clear advantage of 1500µl desorption volume when it comes to desorption efficiency, the results from method 2 remain unclear, since the desorption efficiency seems to increase with desorption volume but with a decrease at 2000µl. One explanation of this phenomenon could be a VARIABLE x TIME specific effect, meaning that desorption time, hold time of the initial oven temperature and purging of the inlet interact with each other.

Figure 47 Effect on desorption: desorption gas volume 2.
Effect on peak area at 1µg/l concentration, constant initial hold and purge time. Peak for tolylfluanid was not observed but is highlighted as 0 for better representation. Source: *Matthias Reis* 2018.

When comparing both methods it can be shown that the highest responses of each method (1500µl and 2500µl respectively) have about the same peak area values and at the same time the desorption time, hold time and purge time values were in the same specific temporal distance (as in the second method the times from the highest level were used constantly)

Desorption Flow Speed

For the investigation into the effects on desorption efficiency by changing the desorption flow speed the same two methods as in the desorption volume trial have been applied. The faster the plunger pushes down the nitrogen gas, the faster the desorption process is finished.

Therefore the parameters of purging and initial hold time have been adapted again in method 1 and held constant at the parameters obtained from the lowest desorption flow speed of 10μg/l (longest desorption time) in method 2. It is clearly recognizable that with

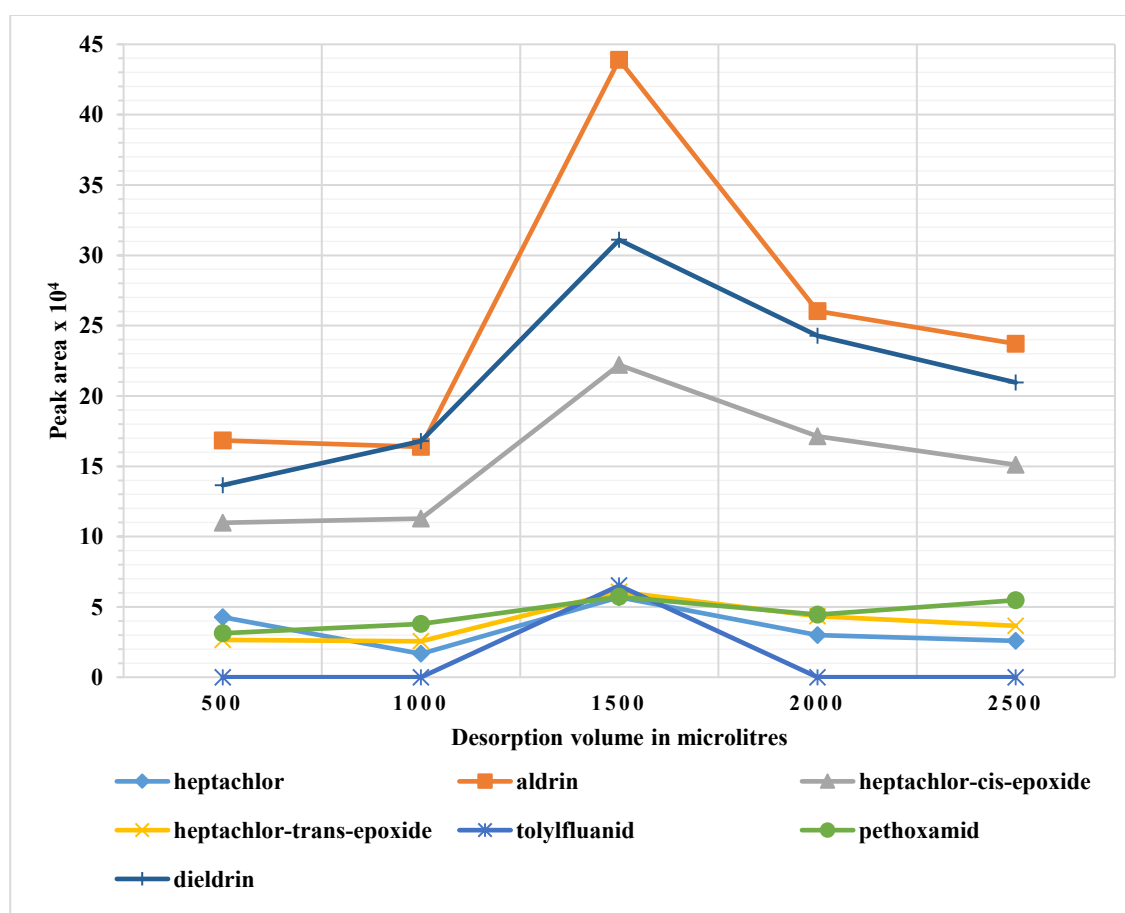**Figure 48 Effect on desorption: desorption flow speed 1.**
Effect on peak area at 1μg/l concentration, adjusted initial hold and purge time. Peak for tolylfluanid was just observed at 10μl/s desorption flow speed and there-fore is highlighted as 0 at the other desorption flow speeds for better representation. Source: *Matthias Reis* 2018.

higher desorption flow speeds the efficiency of desorption decreases. An explanation might be the lack of time where the analytes are exposed to the hot inlet and therefore not all of them come off the needle's coating. Moreover both of the diagrams show similar trendlines, in contradiction to the desorption volume. This leads to the suggestion that the desorption time of the 1500μl volume with 10μl/s desorption flow speed and 0,2min

longer hold time plus further 0,2min longer purge time builds a "threshold" for a maximum value. Longer times would change peak areas and shorter times would not change peak areas if 1500µl desorption volume is used. When looking on the adjusted 500µl desorption volume and adjusted 30µl/s desorption flow speed, which share the same desorption, purge and initial oven temperature times, both of them have almost exact the same peak areas for all analytes. Therefore an influence of the volume could be excluded and the interacting effect of desorption, hold and purge time is emphasized.
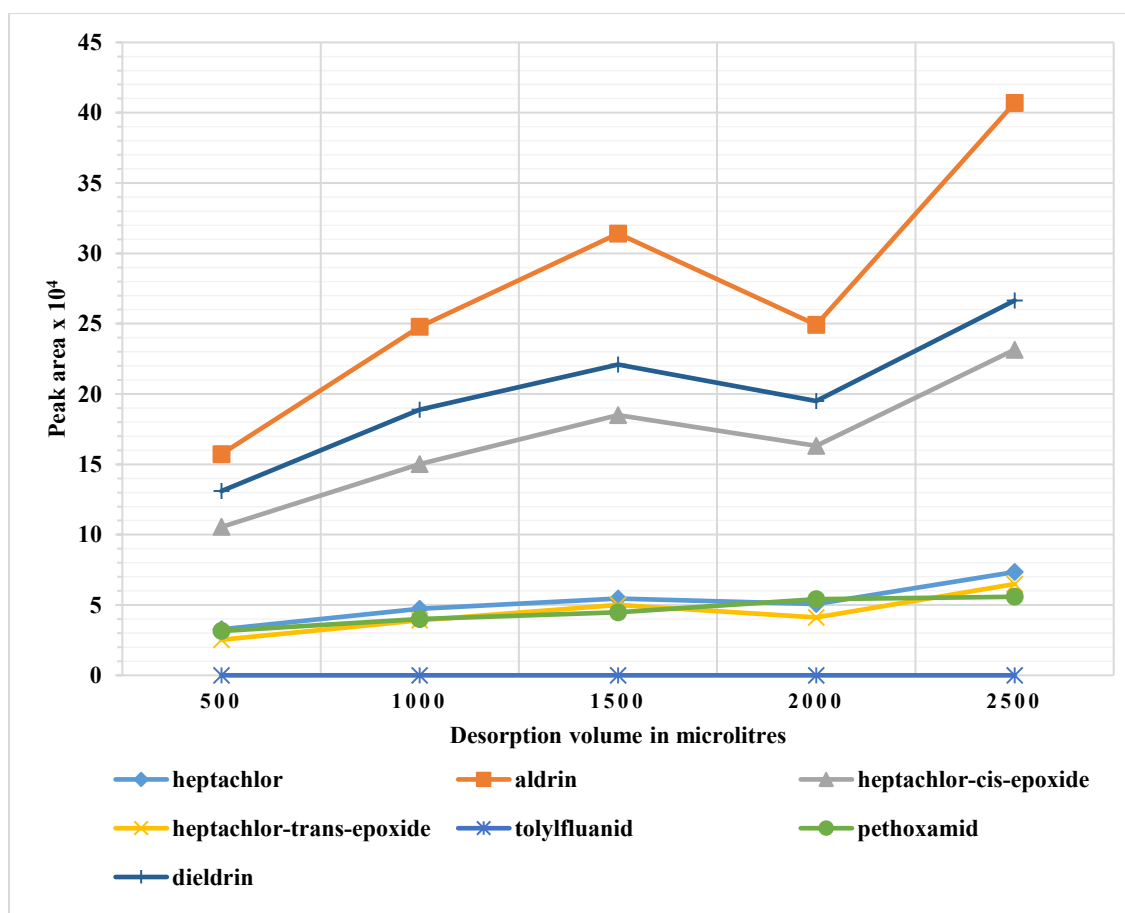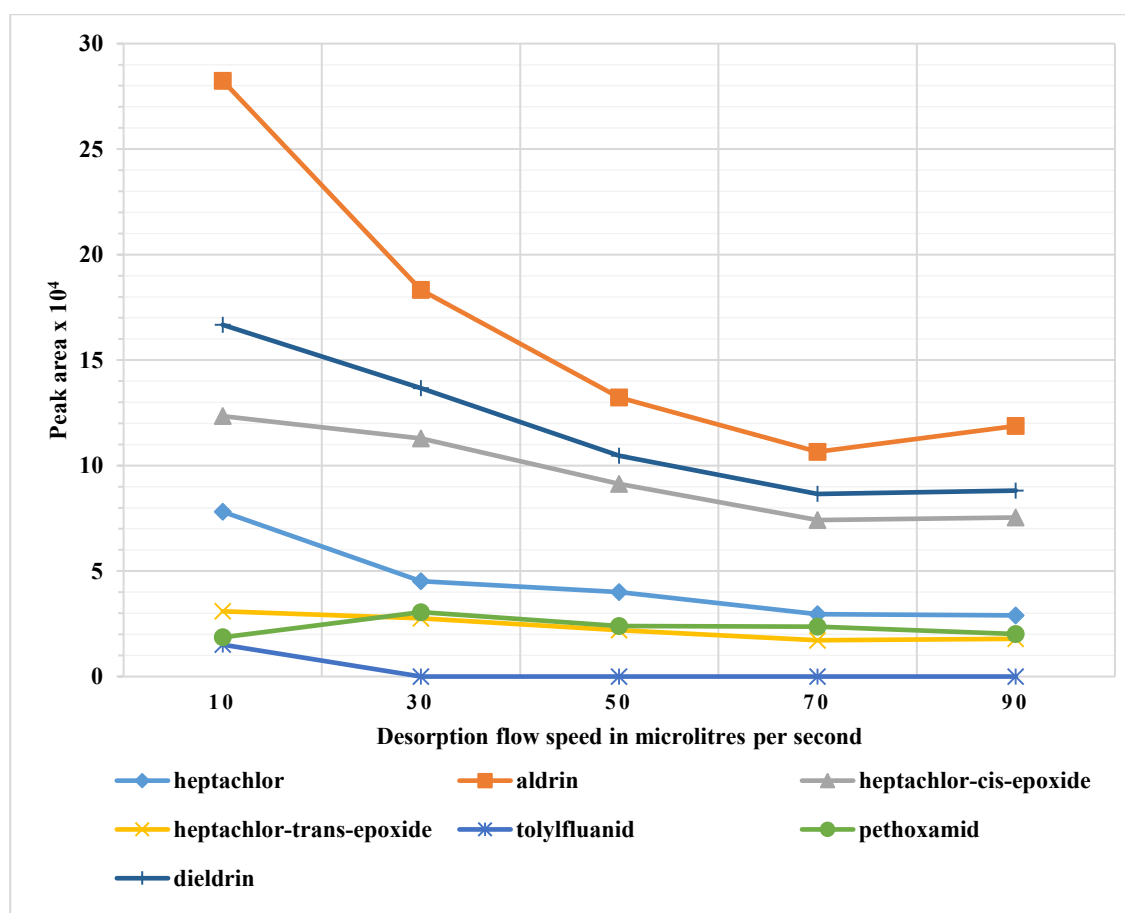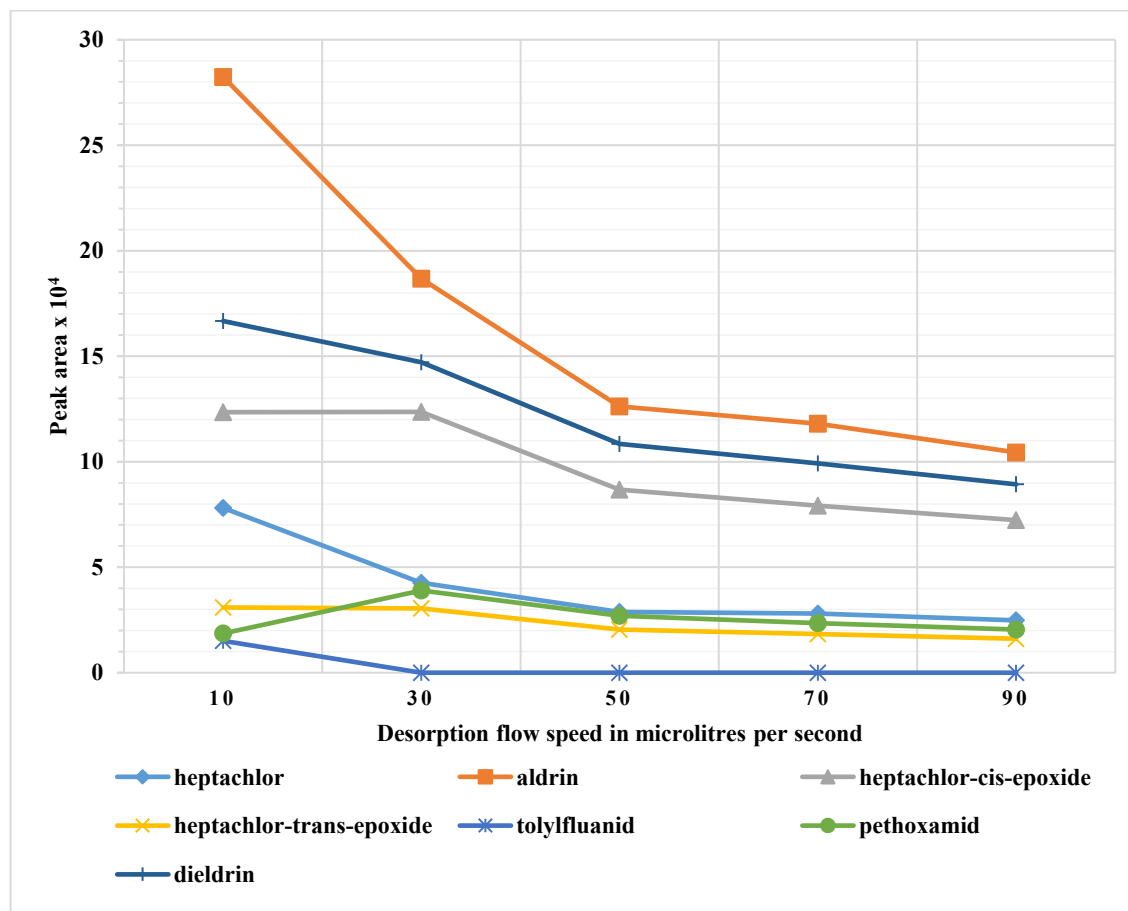

Figure 49 Effect on desorption: desorption flow speed 2.
Effect on peak area at 1µg/l concentration, constant initial hold and purge time. Peak for tolylfluanid was just observed at 10µl/s desorption flow speed and there-fore is highlighted as 0 at the other desorption flow speeds for better representation. Source: *Matthias Reis* 2018.

2.2.7.5. Optimizing oven program and mass spectrometer for SPDE

Initial hold time – "focus effect"

Holding a oven temperature, which is set about 10°C below the boiling point of the used solvent, for a certain amount of time leads to trapping of analytes. This is because the in the hot inlet immediately vaporized solvent, which is still bound to the analytes, builds liquid droplets again when it gets onto the colder column in the GC oven. When increasing the temperature in the oven, these droplets, filled with the abundance of analyte molecules, vaporize again and are therefore focused, which leads to sharp bands and widths. In the SPDE method there should be theoretically no solvent, since the analytes are desorbed from a stationary coating. Despite that an initial hold temperature of 90°C (10°C below the boiling point of water) was investigated with very poor results. Conducting experiments with 40°C led to a great increase of peak response. Afterwards trials on different hold times at 40°C were carried out, revealing that a hold time, ending just shortly after finishing the desorption process, yielded the best peak responses (see figure 50).
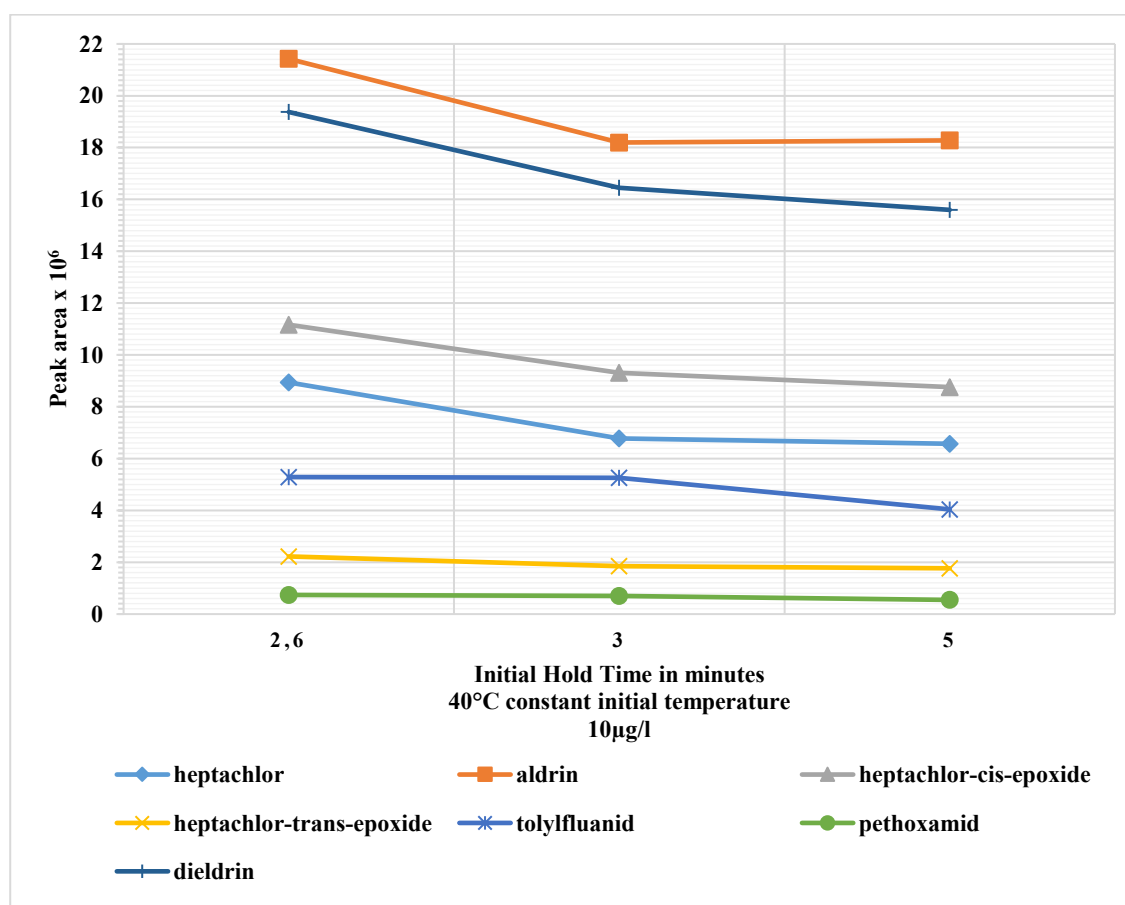
Figure 50 Effect of oven program: initial oven temperature, hold time.
Effect on peak area at 10µg/l concentration, constant initial hold and purge time. Source: *Matthias Reis* 2018.

Purge time

The purge time is determined by how long the split valve of the inlet stays closed. In this method the splitless mode was chosen because it allows measurements of very low analyte concentrations. When high concentrations are expected the split mode is chosen where a ratio of how many analytes reach the column and how many analytes are discarded into the split line can be set up. Operating in splitless mode means that the valve stays closed until all analyte molecules reached the column. Afterwards the valve opens for cleaning the inlet of residues with a high flow rate of helium gas. In some cases the desired compounds are moving slowly onto the column and "floating" around in the inlet for some amount of time, when for example depending on flow rate. Therefore it should be experimentally determined when the split valve opens as all analytes should be transferred onto the column at this time. Two purge times were investigated at a total desorption time of 2,6 minutes. When purging just 0,2 minutes after the desorption process was finished, better results are obtained than with 3,8min purge time.
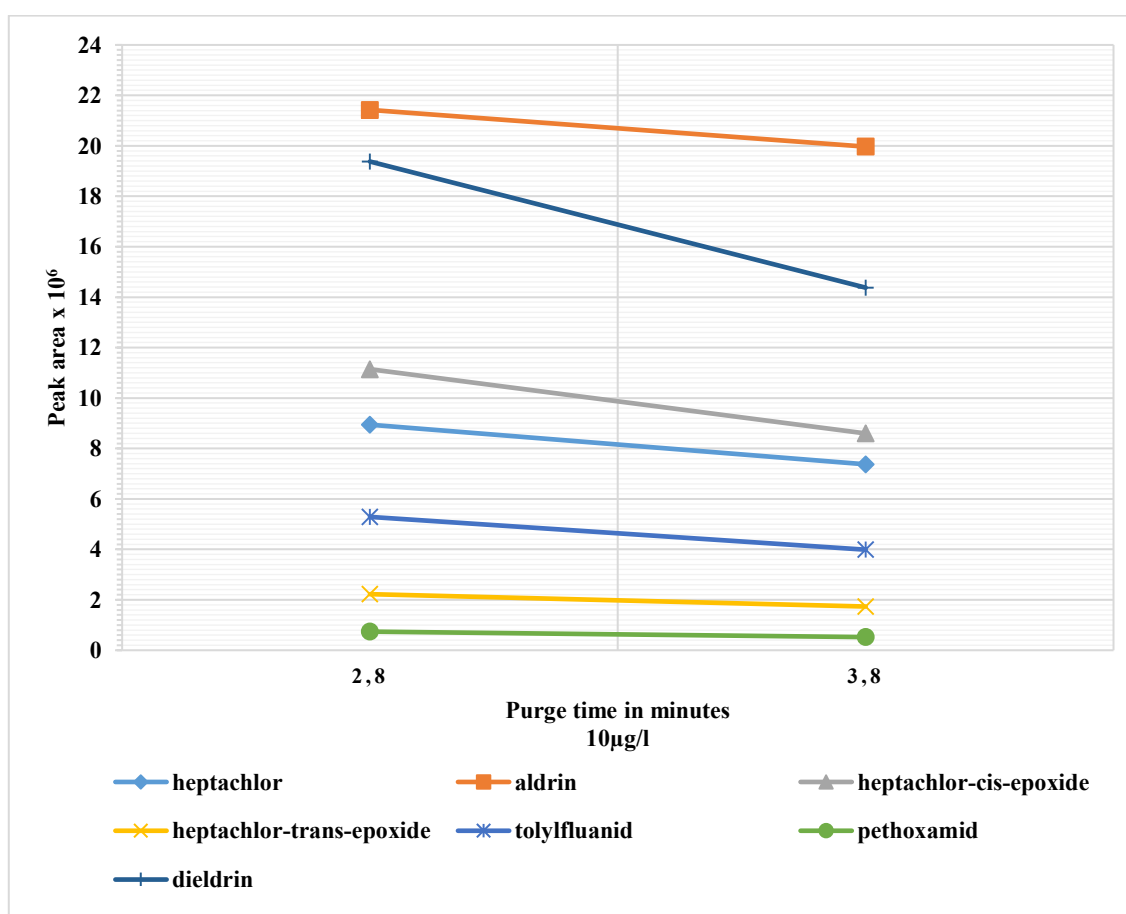
Figure 51 Effect of oven program: purge time.
Effect on peak area at 10µg/l concentration. Source: *Matthias Reis* **2018.**

2.2.7.6.   Optimizing SPDE cycles – reducing carryover

Washing steps

While the SPDE coating is mainly cleaned of analyte residues by conditioning under a gentle nitrogen stream at high temperatures up to 280°C furthermore the syringe itself also has to be cleaned. At the conditioning process the syringe is passed by the nitrogen stream for flushing the needle Therefore, cleaning by solvents should be conducted to reduce carryover. It is important to investigate solvents which are compatible with the individual SPDE coating. *Seethapathy and Gorecki* (2012) showed that PDMS is compatible with H2O as well as with ACN. Therefore, both of these solvents were used for washing the syringe. The cleaning step

Figure 52 washing containers.
Two washing containers on the upper middle of the picture. One waste station, with a connecting tube to a waste container. Source: *Matthias Reis* **2018.**

was programmed just before conditioning the needle and a variable number of washing cycles (up to 10 per wash container) can be set. When extracting dirty liquid samples it could be considered also washing the needle and syringe with ultraclean MilliQ® water before desorption to prevent pollution of the inlet, column and mass spectrometer. Therefore a similar washing step was programmed.

While the needle penetration into the wash containers should be limited due to prevention of damage, the number of washes must be calculated according to the volume of the containers, otherwise the needle will not aspirate any wash solvent anymore after the level falls below the needles side port.

Figure 53 washing containers – needle penetration.
Shown is the SPDE needle, penetrating wash container 2. Source: *Matthias Reis* **2018.**

Drying step

One of SPDE's main issues is that when extraction process is ended there are still very small amounts of water droplets inside the needle due to the plunger's inability to push out all of the water residue. Only one small droplet (of about 1µl) can expand extensively when heated up within seconds in the inlet. This could lead to a spillover of the GC liner, because in SPDE often straight liners with very small volumes are used for transporting the analytes onto the column. Therefore a "pre-conditioning" process was programmed in which the needle is flushed in the conditioning station by a nitrogen stream. The maximum temperature in this process was set to 50°C as loss of analytes previous to desorption should be prevented. When programming a test-cycle to investigate the appropriate function of this method it was observed that water residues immediately splashed out of the syringe's side port after the flushing process was started.

Inlet and gas station septum

A further source of carryover might be the septum of the inlet but moreover the septum of the gas station since the (with analytes loaded) syringe takes up gas at this station directly after the extraction process. When carryover is observed it's worth cleaning the inside area of the gas station with solvents of different polarities as well as replacing the septum. After cleaning the gas valve should be turned on (without the septum and mounting screw mounted) to remove any solvent residues of the system.
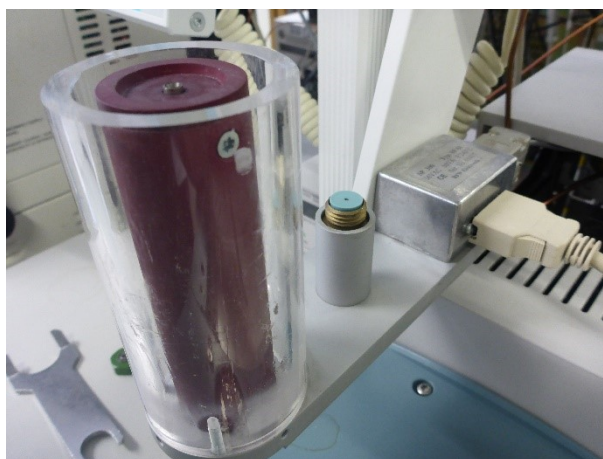
Figure 54 gas station septum.
Shown is the conditioning station on the left and the gas station with a green septum on the right. Source: *Matthias Reis 2018.*

Temperature of syringe (heater)

Differing temperatures in the SMM and the syringe might lead to conden-sation onto the syringe's surface if the needle temperature is set lower than that of the SMM. Therefore the syringe adapter heater was always set 10°C higher than the extraction temperature which was set in the single magnet mixer. Otherwise condensation leads to carryover as it is shown in figure 55.
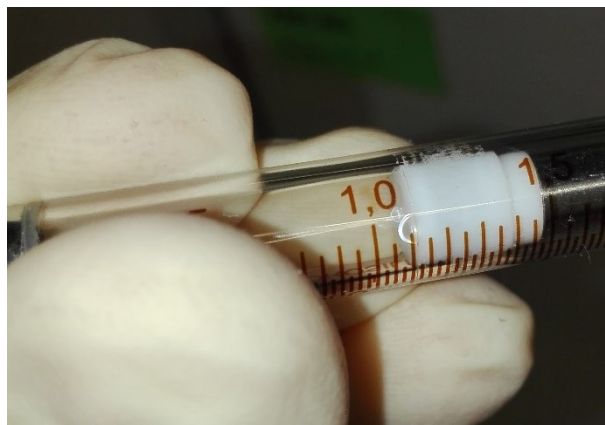
Figure 55 solvent residue in syringe.
Shown is the SPDE syringe containing a droplet of MilliQ®
residue. Source: *Matthias Reis* 2018.

The cycle composer software also allowed programming a temperature level which is set for the syringe (heater) during conditioning of the needle. A value of 80°C was chosen, which was held constant over the pre-determined conditioning time.

2.2.7.7. Summary of parameter optimization

	coating	Head-, Li-quid-space	Needle Pen. (mm)	strokes	Extr. temp. (°C)	Extr. flow rate (µl/s)	Desorp. temp. (°C)	Pre-desorp. time (s)	Desorp. volume (µl)	Desorp. flow speed (µl/s)	Init. temp time (min)	Purge Time (min)
heptachlor	225	HS	25	100	65	100	250	0	1500	10	2,6	2,8
aldrin	PDMS/AC	HS	25	100	75	100	280	30	1500	10	2,6	2,8
heptachlor cis epoxide	PDMS/AC	LS	25	100	55	100	280	30	1500	10	2,6	2,8
heptachlor trans epoxide	PDMS/AC	LS	25	100	75	100	280	30	1500	10	2,6	2,8
tolylfluanid	225	LS	35	100	65	150	280 (?)	0	1500 (?)	10 (?)	2,6	2,8
pethoxamid	225	LS	25	100	75	50	260	0	1500	30	2,6	2,8
dieldrin	PDMS/AC	LS	25	100	75	100	280	30	1500	10	2,6	2,8

Table 13 Summary of parameter optimization for SPDE. Source: *Matthias Reis* 2018.

According to results obtained from scan and single ion monitoring methods pethoxamid and tolylfluanid have the lowest response. As PDMS/AC seems to be the coating where most of the analytes are retained and desorbed well, especially both of these substances as well as heptachlor are better recovered when using the CT-225 needle. Therefore further investigations in recovery experiments could be taken by choosing this coating, as the response for the other compounds might be still high enough for a low level validation.

2.2.7.8. Validation of the method

Before validation the analytes were spiked in Milli-Q® water with a concentration of 0,003µg/l as this is the drinking water legislation's parameter value for most of the analytes. Another measurement was conducted at 0,005µg/l, the lowest calibration point. The permissible maximum concentration for pethoxamid and tolylfluanid is instead 0,1µg/l but they were also checked at this concentration levels. For both measurements the peak intensity compared to the noise was observed and a signal to noise ratio above 3 was determined. Seven of these mix-standards were produced for each validation level. Measurements of three validation levels were conducted. While the six point calibration (5 points for low level) for each level was obtained by a mix standard of all analytes, the Milli-Q® samples were spiked with corresponding concentrations according to the parameter values of the drinking water legislation. In low level validation the Milli-Q® samples were spiked with 0,01µg/l of aldrin, dieldrin, heptachlor, heptachlor cis epoxide, heptachlor trans epoxide and with 0,05µg/l of pethoxamid and tolylfluanid. In medium level validation the Milli-Q® samples were spiked with 0,03µg/l of aldrin, dieldrin, heptachlor, heptachlor cis epoxide, heptachlor trans epoxide and with 0,1µg/l of pethoxamid and tolylfluanid. In high level validation the Milli-Q® samples were spiked with 0,1µg/l of aldrin, dieldrin, heptachlor, heptachlor cis epoxide, heptachlor trans epoxide and with 1µg/l of pethoxamid and tolylfluanid. Between each calibration and sample run 1 stroke of a Milli-Q® blank was conducted and injected. After the results were evaluated, a mean value of the 7 replicates was taken, considering a relative standard deviation (RSD, %). While the mean recoveries were positive in a range from 75% to 125%, a maximum relative standard deviation of 25% was accepted.

Compounds	Target ions (m/z)[a]	Ret. times (min)	Min. quadr. range (µg/l)[b]	Corr. Coeff. R²	Method det.limit (µg/l)	Rec.&Prec. low conc. (%)[c]	Rec.&Prec. med. conc. 1 (%)[d]	Rec.&Prec. high conc. (%)[e]	Rec.&Prec. med. conc. 2(%)[f]
Heptachlor°	*272*, 274, 100	14,11	0,0121-0,0762	0,999	0,0121	121 (19)	155 (20)	76 (20)	87 (27)
Aldrin	*66*, 263, 265	15,49	0,0119-0,0985	0,999	0,0119	119 (11)	88 (10)	99 (11)	106 (23)
Hep-cis epo	*353*, 355, 81	17,37	0,0121-0,1166	0,999	0,0121	121 (12)	109 (24)	117 (13)	107 (7)
Hep-tra epo	*353*, 355, 81	17,59	0,0117-0,1185	0,999	0,0117	117 (16)	116 (20)	118 (13)	101 (9)
Tolylflu*	*137*, 238, 240	17,83	0,0842	0,999	0,0842	-	-	-	84 (15)
Pethox^	*260*, 131, 91	18,51	0,0443-0,0983	0,999	0,0443	89 (23)	108 (30)	94 (31)	98 (8)
Dieldrin	*79*, 263, 277	20,69	0,0097-0,0920	0,999	0,0097	97 (14)	97 (23)	92 (12)	88 (8)

Table 14 Validation data of SPDE-GC-MSBased on *Jochmann et al.* 2007. method.

* worked just for medium level validation.

° RSD at medium level didn't meet inclusion criteria (27% > 25%).

^ RSD at high level didn't meet inclusion criteria (31% > 25%).

[a] Peak used for quantification is in italic.

[b] Quadratic range, where the accuracy of the base peak and the ratio of the qualifiers are within the accepted range.

[c] Relative standard deviations (n=7, fortification level 0,01µg and 0,05µg/l for pethoxamid and tolylfluanid respectively).

[d] Relative standard deviations (n=7, fortification level 0,03µg and 0,1µg/l for pethoxamid and tolylfluanid respectively).

[e] Relative standard deviations (n=7, fortification level 0,1µg and 1µg/l for pethoxamid and tolylfluanid respectively).

[f] Relative standard deviations (n=7, fortification level 0,03µg and 0,1µg/l for pethoxamid and tolylfluanid respectively). New calibration, 0,5µg/l instead of 2µg/l

Despite the mass abundance of m/z 100 of heptachlor is highest, followed by 272 and 274, the quantification and qualification ions were chosen in a manner which is displayed in table 14. Since the response is higher when m/z 100 is used as quantifier, this wrong decision would be a limiting factor for quantification. The same failure appears for heptachlor trans epoxide (order highest – lowest abundance of m/z: 81, 353, 355) and heptachlor cis epoxide (order highest – lowest abundance of m/z: 353, 81, 355) with the latter one having an impact on the ratio of the two qualifiers compared to the quantifying ion. Especially for heptachlor trans epoxide a more than two times higher response could have been reached.

Compound	Lowest observed concentration (µg/l)	S/N Quantifier[a]	S/N Qualifier 1[a]	S/N Qualifier 2[a]	LOD (µg/l)
Heptachlor	0,0086	16,38	17,15	12,21	0,0086
Aldrin°	0,0006	9,86	7,97	7,76	0,0006
Heptachlor-cis epoxide	0,0094	38,86	23,83	3,23	0,0094
Heptachlor-trans epoxide	0,0081	9,73	12,26	6,67	0,0081
Tol-ylfluanid*	0,0221	6,91	3,76	6,79	0,0221
Pethoxamid	0,0323	25,13	7,81	6,98	0,0323
Dieldrin	0,0076	16,00	26,32	6,52	0,0076

Table 15 Limits of detection for SPDE-GC-MS validation. Source: *Matthias Reis* 2018.
°Obtained from a blank.
***Obtained just from medium level 2 validation (new calibration which differs from the first one).**
[a] Peaks with a signal to noise (S/N) ratio greater than 3 were accepted for validation.

The limits of detection were evaluated by observing all samples of all validation levels, including each calibration point (of the two) calibrations. As tolylfluanid was obtained from the second calibration for medium level 2, the corresponding LOD was set only for this concentration level. Heptachlor was also found at 0,0048 µg/l at the lowest calibration point for medium level 2 validation but since one of the two qualifiers by far exceeded the boundary for the quantifier/qualifier ratio (Q/q ratio) the results were not included. The same situation was found for pethoxamid in the first calibration: at 0,0069 µg/l the S/N ratios were 7,26; 3,88 and 2,50 respectively but the corresponding Q/q ratios were also far out of the allowed range.

Considering the wrong evaluation of quantifiers and qualifiers for heptachlor and heptachlor trans epoxide, which is responsible for a loss of response up to 200%, even lower

detection limits, with acceptable S/N ratios, might have been possible. Lower LODs might be possible for dieldrin, since the compound is still showing S/N ratios well above 3 at 0,0076 µg/l whereby no lower values could be obtained for the lowest calibration point at 0,005 µg/l because of the seemingly poor accuracy at this concentration. Interestingly aldrin is well analyzed even at very low concentrations. Within a blank the lowest concentration of 0,0006µg/l was found, still having adequate S/N ratios. Dieldrin as well as heptachlor epoxide are oxidation products of aldrin and heptachlor, respectively. During the extraction process by the SPDE needle, which takes about 30 minutes, there could be a possibility of aldrin and heptachlor to oxidize to its metabolites. While the recoveries for aldrin are in the desired range, heptachlor could not be validated at both medium validation experiments. Also at low and high level, the compound is scratching the edge of the boundaries (121% at 125% boundary and 76% at 75% boundary respectively). Moreover heptachlor is a highly volatile substance and this could lead to "leaks" throughout the extraction process. The vial is heated by the single magnet mixer to about 50°C and if the septum is not ensuring tightness some of the more volatile analytes could leak, past the needle and septum, into the air (*Nilsson et al.* 1998). Following two mechanisms could be assumed considering the poor recovery of tolylfluanid: Either the degradation point at 200°C or the aqueous hydrolysis DT50 of 1,9 days at 20°C and pH7. The compound is moreover very pH-sensitive (DT50 for aqueous hydrolysis at pH4 / 22°C 11,7 days and DT50 at pH9 / 20°C at 20°C 10 minutes). (Ultrapure Milli-Q®) water should has a constant pH-value of 7,0, therefore stability of tolylfluanid should be provided. Since the vial tray is mounted on top of the GC / oven it warms up. The temperature of the tray wasn't measured but it felt like 30 to 40°C. This could speed up the aqueous hydrolysis process. N,N-dimethyl-N'-p-tolysulphamide (DMST), the metabolite of tolylfluanid, built by aqueous hydrolysis wasn't observed either when analyzing for the specific mass transitions obtained from the EURL database. As pethoxamid also degrades at 200°C either a selective degradation in the inlet or a selective hydrolysis (for tolylfluanid) could be concerned for further investigations. The term "selective" is used because pethoxamid worked for the low and the second medium concentration level and almost worked for the first medium and high validation level. Furthermore peaks for pethoxamid were always observed, while for tolylfluanid, which just worked for the second medium concentration level, often no peaks at all were found. Interestingly the characteristic mass

transitions for tolylfluanid were also observed at the retention time of heptachlor trans epoxide. Using another chromatographic column, which broadens the time inbetween these two compounds could therefore also solve this issue.

No carryover with adequate S/N and Q/q ratios was observed within the blank which was injected inbetween samples and calibration points, except for aldrin. Since the relative carryover for aldrin (0,0005-0,001µg/l) compared to the lowest validation level (0,01µg/l) is considered as high further investigations could be taken into conditioning or heating the syringe to completely remove aldrin from the system.

On the next side the results are illustrated graphically.
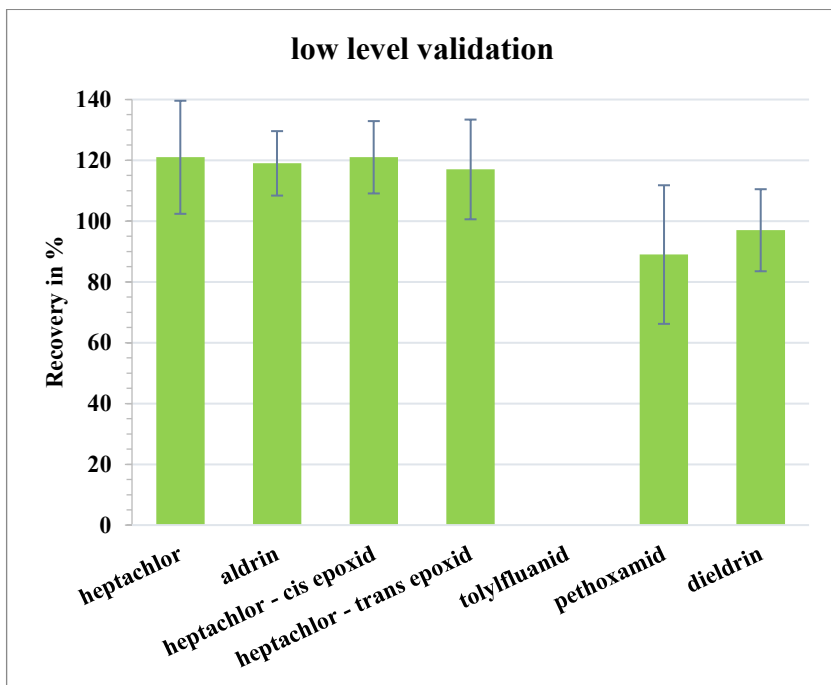
Figure 57 low level validation.
Green bars represent compliance with the boundary conditions and error bars show the relative standard deviation in %. Source: *Matthias Reis* **2018.**

Figure 56 medium level 1 validation.
Green bars represent compliance with the boundary conditions, while red bars show compounds which didn't meet the criteria; error bars show the relative standard deviation in %. Source: *Matthias Reis* **2018.**

Figure 58 medium level 2 validation.
Green bars represent compliance with the boundary conditions, while red bars show compounds which didn't meet the criteria; error bars show the relative standard deviation in %. Source: *Matthias Reis* **2018.**
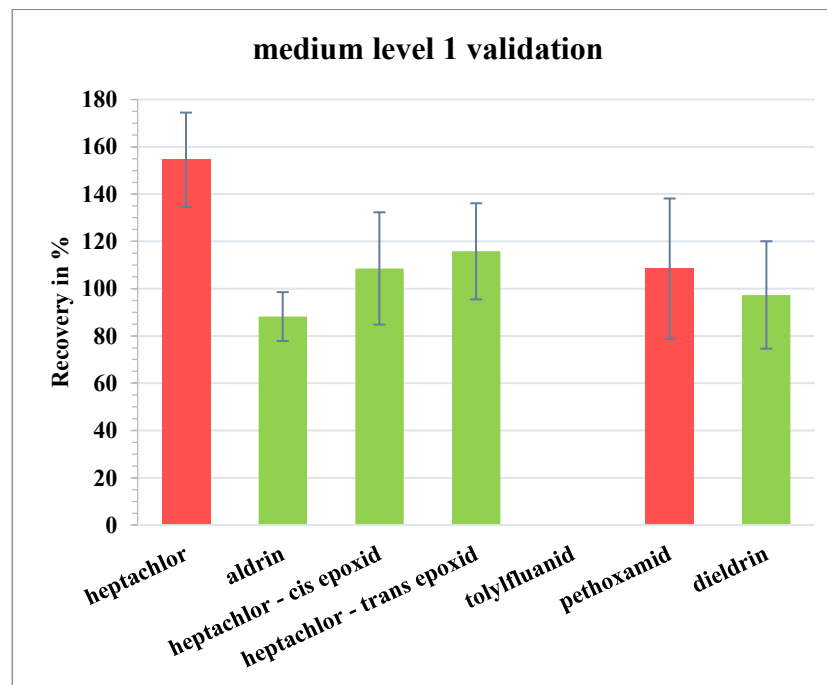

Figure 59 high level validation.
Green bars represent compliance with the boundary conditions, while red bars show compounds which didn't meet the criteria; error bars show the relative standard deviation in %. Source: *Matthias Reis* **2018.**
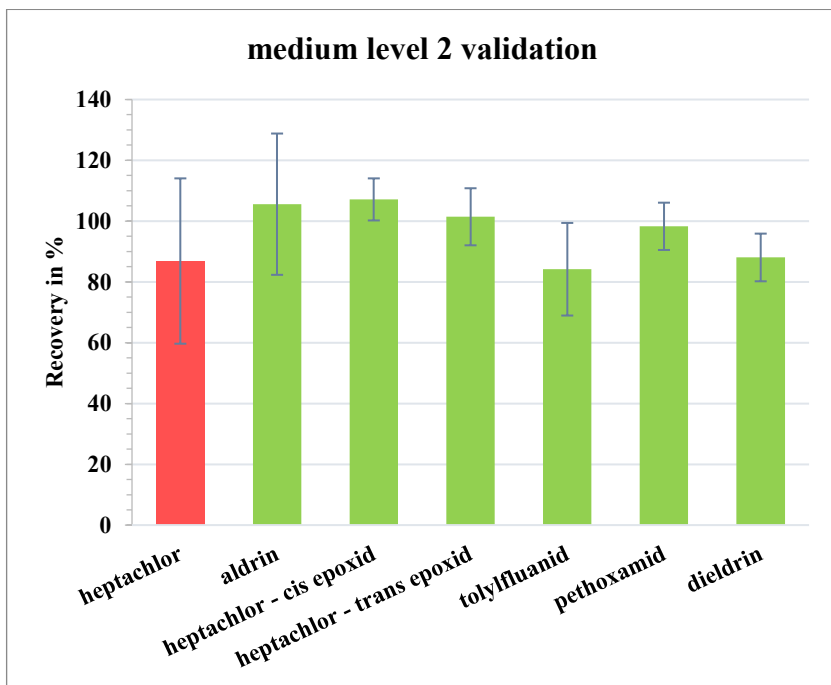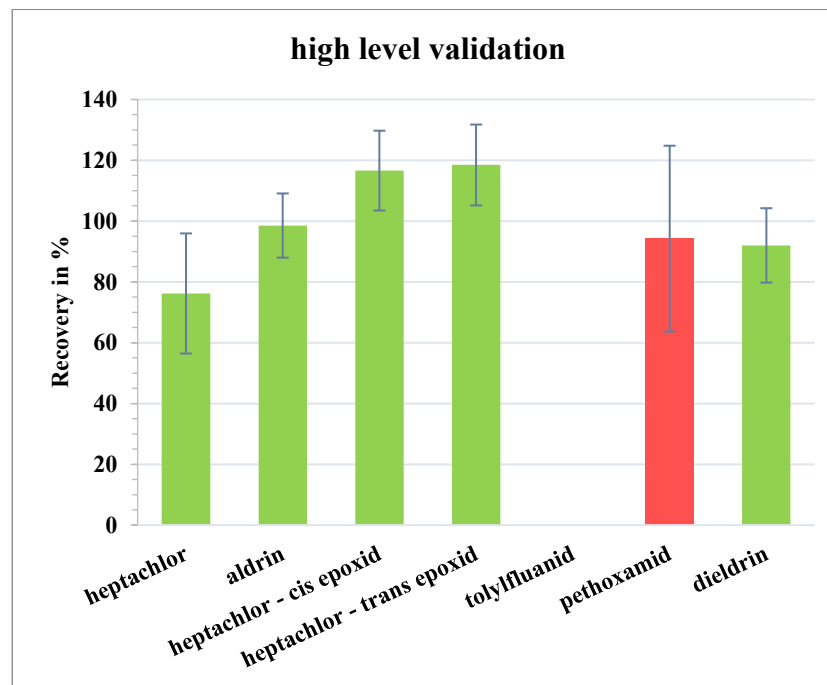
3. Conclusion / Summary

Despite SPE is widely used as sample preparation method throughout the literature, the findings of this work showed that some analytes can not be recovered at trace levels. The evaporation process might affect the stability of some analytes resulting in poor recoveries. Furthermore SPE is a time consuming process leading to higher labour costs and slow sample throughput. Compared to SPE the SPDE technique turns out to be an efficient, time consuming (since the CTC-PAL is fully automated an online) sample preparation processs according to a sparse amount of relevant studies and the actual work. Not only are the analytical results (LODs and LOQs, sharp peaks) comparable to other, widely used methods like SPE or SPME but also from the economical perspective using SPDE leads to less expenditures (through reduction of working hours, less solvent), since analysis and moreover also sample preparation is fully automated by the CTC-PAL-GC-MS-system. When it comes to multi-methods where many analytes of a sample have to be analyzed at the same time, it is necessary to investigate the physicochemical properties of each compound accurately because they will influence the parameters as well as the coating of the needle, which has to be used for the extraction and desorption process as shown in this work. While for analytes like aldrin, dieldrin, and heptachlor cis-/trans-epoxide a PDMS/AC coating ensures good retention, high responses and recovery rates within the limit values at each - low, medium and high concentration -validation level, the observed peak area of pethoxamid is small compared to the other compounds. Despite that, pethoxamid also could be recovered at a low and medium concentration levels and nearly (relative standard deviation was 5% above the limit) at a high concentration level. Heptachlor and tolylfluanid show peak responses, comparable in area, which are the second lowest after pethoxamid. Furthermore both of the first mentioned analytes share a low stability in water due to aqueous hydrolysis. Especially for tolylfluanid this phenomenon could have taken place at most of the conducted experiments as the molecule sometimes couldn't be recovered at all. All three of the latter substances, heptachlor, pethoxamid and tolylfluanid are better retained with the CT-225 coating, therefore further investigations should be considered. While the 7 pesticides under investigation are one part among 50 substances which have to be analyzed according to the drinking water

legislation further studies could tackle a multi-method of all 50 pesticides, carried out by SPDE sample preparation.

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Appendix

SPDE – recommended operating temperatures

CHROMTECH

| Phase	Max.-Temp.	Recommended Operating Temp.	Conditioning
SPDE PDMS:	280°C	200-280°C	30min@250°C
SPDE CT-5:	280°C	200-280°C	30min@250°C
SPDE PDMS/AC:	280°C	200-280°C	30min@250°C
SPDE 1701:	250°C	180-230°C	30min@220°C
SPDE 225:	230°C	160-210°C	30min@200°C
SPDE WAX:	260°C	200-250°C	30min@220°C

How to: cycle programming

Instrument → CTC PAL Edit Cycles…

Choosing the cycle which want to be edited or clicking on "New Cycle" for creating a new cycle. Therefore a the A-GC Inj cycle will be used as template.

New cycle variables can be inserted or existing variables can be edited. Default values and upper limits can be set. Attention: when setting too high or low values some parameters (vial needle penetration) can lead to damage of the needle.
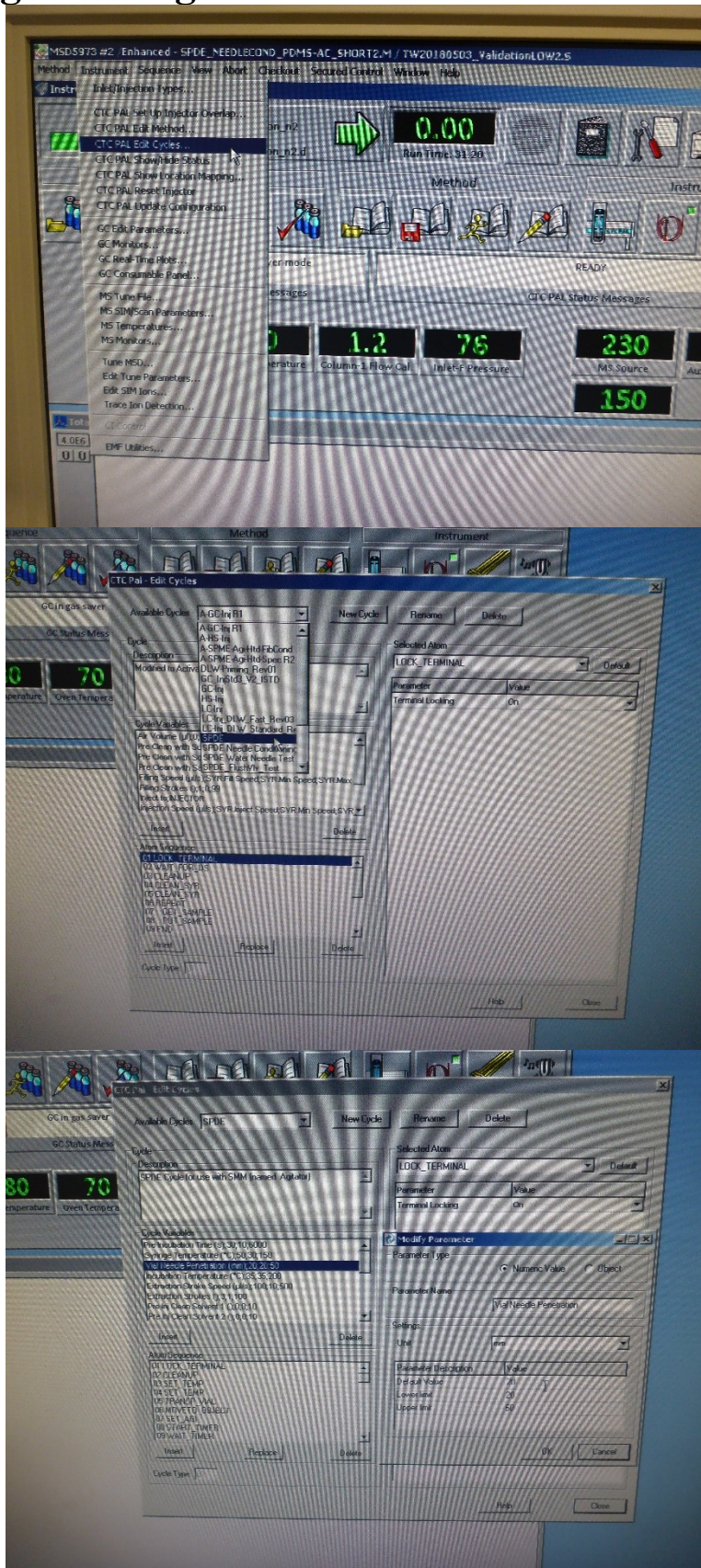
Insertion of a new atom sequence (i.e.: a sequence which is then executed by the pal during the method run) is conducted by clicking on the desired spot and pressing the "Insert" button. The chosen spot / atom sequence will then be copied to a duplicate. In this example the cleaning step of the syringe was copied.
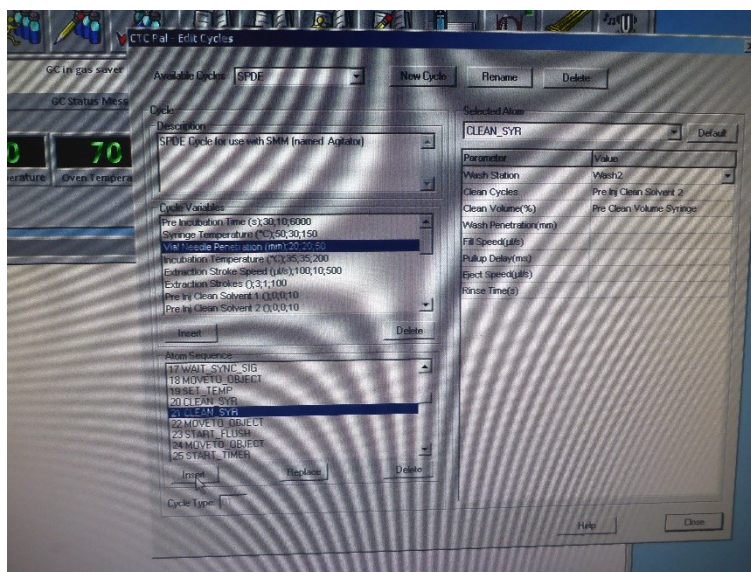

The duplicated atom can be changed by opening the pull down menu of the "Selected Atom". A list, showing the available atoms is displayed, where the atom of interested can be selected.

"Move to object" for example means that the pal is moving to a defined object like the single magnet mixer, gas station, injector and so on.
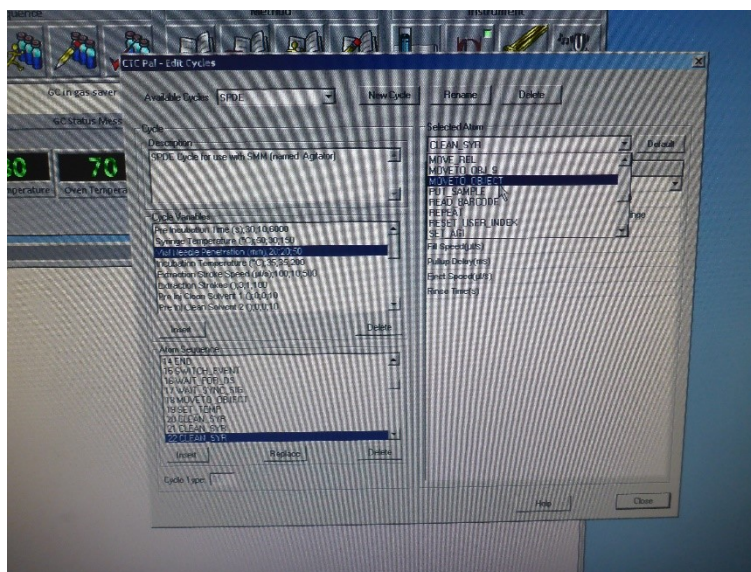
When the desired atom was selected a parameter list with attributed values will be displayed (on the right side). After the desired values were selected, pressing on the bottom "Replace" saves the changes which have been made.In this example "Home" was choosing as object name (where the syringe moves to). At home there is no needle penetration (contrary to the object SMM)
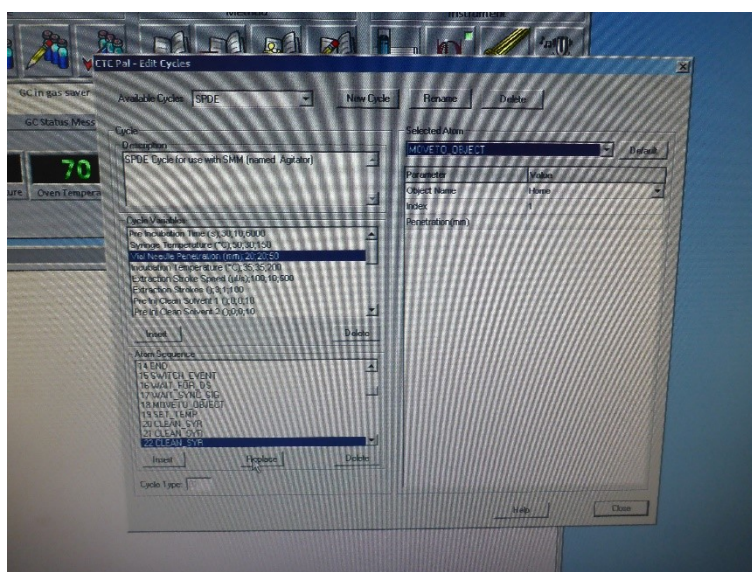
How to: Testing the flushing line

Since nitrogen supply line is splitted after the control valve, supplying the gas station as well as the syringe (adapter) the latter one could be broken because of high frequently movement of the pal head. A working flushing line is very important since it prevents the needle (coating) from degradation at the conditioning process. Furthermore the nitrogen stream transports analyte residues away from the stationary phase.

By programming a short cycle where the pal just executes a "flushing" atom sequence, the plunger of the syringe moves up, opening the syringe's side port and starts flushing by opening the flush valve at the back of the syringe adapter. When filling a 20ml with water it is possible to just push up the guide until the needle dives into the water. If nitrogen is coming through it will be recognized by observing bubbles.
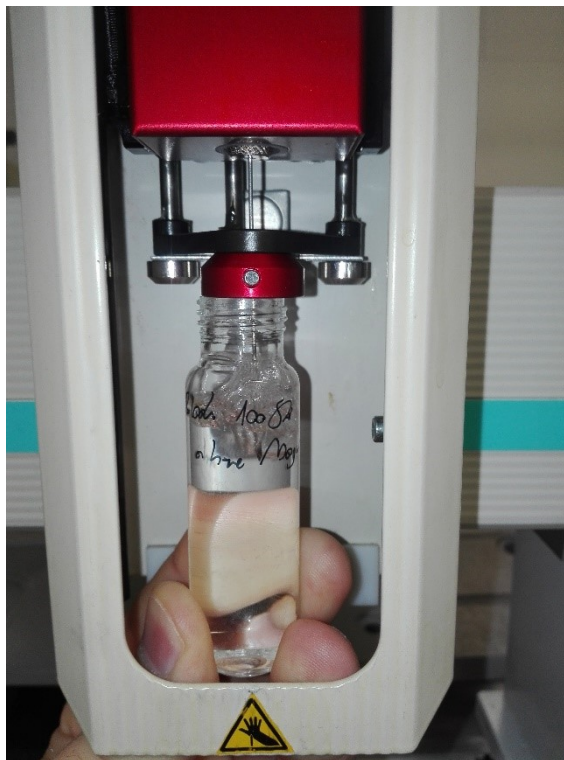
Attention for malfunction: needle guide & co.

When the pal and SPDE needle are operating many sequences it could happen that the needle guide will slightly change its position. If this phenomenon occurs then the needle will be wrongly guided and the pal will stop operating because of an integrated protection function, which observes appearing forces, pushing against the pal head when it wants to move down. Another reason for malfunction might be loosened plugs. The connection plug from the pal operating surface to the conditioning station tends to loosen itself sometimes because of vibrations of the pal.