From Source to Tap -
A Case Study of Organic Contaminants
in Raw and Drinking Water in the
Region of Uppsala, Sweden

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Abstract

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In this study, water samples from the surroundings of Uppsala were examined for residues of pesticides, pharmaceuticals and other contaminants characteristic for human fecal contamination. The aim was to perform a screening of water samples to investigate the level of pollution with the mentioned compound groups. Samples were taken from upstream and downstream river water, lake water, a drinking water treatment plant and treated drinking water. The contaminants were extracted from water samples with two different solid phase extraction (SPE) cartridges. Identification and quantification was achieved via separation with ultra-performance liquid chromatography (UPLC) followed by positive and negative electrospray ionization (ESI) coupled to a high-resolution time-of-flight (TOF) mass spectrometer. Out of 17 analyzed contaminants, 9 could be detected in environmental samples of which 5 were detected in finished drinking water. None of the targeted pesticides were found in environmental samples or drinking water. The average method recovery was 51% and 39% depending on the extraction method. The limit of quantification (LOQ) ranged from 0.07 ng L^{-1} up to 74 ng L^{-1}. Contaminant concentrations in environmental samples ranged from 0.55 ng L^{-1} to 40 ng L^{-1} and in drinking water from 0.22 ng L^{-1} to 8.0 ng L^{-1}. Compounds detected were atenolol, benzoylecgonine, bezafibrate, caffeine, carbamazepine, cotinine, diclofenac, metoprolol and nicotine. Compounds not detected were atrazine, cyanazine, isoproturon, ketoprofen, monensin, quinmerac and simazine. The results show that several of the target analytes were present in the environment and in drinking water at low but measurable concentrations, which shows that the drinking water treatment plant is not able to successfully remove the contaminants with conventional treatment techniques. A pilot plant with membrane technology is ineffective as well, except when coupled to granular activated carbon (GAC). Risk quotients were determined and revealed that no hazards for human health or aquatic organisms can be expected from the detected contaminant concentrations.

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Popular science summary

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In environmental research, many studies have been conducted detecting synthetic organic compounds from various origins in rivers, lakes, groundwater and even drinking water. Among these contaminants, pharmaceuticals and pesticides play a large role as well as indicators of human impact (substances that are excreted with urine and feces, for example the decomposition product of nicotine). These compounds have in common that they have a specific mode of action on defined organisms. Their presence in the environment is of concern since effects on plants, animals, microorganisms and humans cannot be excluded. In this study, water samples were analyzed for a spectrum of contaminants, consisting of selected pharmaceuticals, pesticides and compounds such as caffeine and nicotine. The target contaminants were extracted from the water samples with solid phase extraction cartridges, a method that allows removing the compounds from the water samples and concentrating them for further analysis. After being concentrated, the analytes were separated with the help of liquid chromatography and analyzed by using a time-of-flight mass spectrometer. The results of the analyses showed that out of the 17 targeted compounds, 9 could be found at different concentration levels in surface water samples. 5 of the compounds present in environmental samples were detected in treated drinking water. The concentrations found in environmental samples ranged from 0.55 ng L^{-1} to 40 ng L^{-1} and in drinking water from 0.22 ng L^{-1} to 8.0 ng L^{-1}, which shows that the drinking water treatment plant is not able to successfully remove the contaminants with conventional treatment techniques. New technologies are being tested for their removal efficiency concerning pharmaceuticals and pesticides. However, in the case of the studied treatment plant, a nanofiltration treatment step was not effectively removing the target contaminants. Only when coupled to a carbon treatment, contaminants were successfully removed from the water. In order to estimate the hazards of the detected concentrations and their impact on aquatic life or humans, risk quotients were calculated. None of the detected compounds is present at levels where an effect on organisms can be expected.

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List of abbreviations

ac	alternating current
APCI	atmospheric pressure chemical ionization
CI	chemical ionization
CID	collision induced dissociation
DBP	disinfection by-product
dc	direct current
DW	drinking water
DWTP	drinking water treatment plant
ECOSAR	ecological structure activity relationships
EI	electron ionization
ESI	electrospray ionization
FTICR	fourier transform ion cyclotron resonance
GAC	granular activated carbon
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
LC	liquid chromatography
LC-MS	liquid chromatography mass spectrometry
LLE	liquid-liquid extraction
LOD	limit of detection
LOEC	lowest observable effect concentration
LOQ	limit of quantification
LTQ	linear trap quadrupole
MALDI	matrix-assisted laser desorption/ionization
MEC	measured environmental concentration
MQ	Milli-Q water
NDMA	N-nitrosodimethylamine
NF	nanofiltration
NOEC	no observable effect concentration
NOM	natural organic matter
NPDWR	national primary drinking water regulation
PAC	powdered activated charcoal
PFAA	perfluorinated alkylic acid
PFC	perfluorinated compound
PNEC	predicted no-effect concentration
POP	persistent organic pollutant
PPCP	pharmaceuticals and personal care products
QIT	quadrupole ion trap
QqQ	triple quadrupole
QTOF	quadrupole time-of-flight
RQ	risk quotient
SF	sand filter
SOC	synthetic organic compounds
SPE	solid-phase extraction
std.dev	standard deviation
TOF	time-of-flight
UPLC	ultra-performance liquid chromatography
WWTP	wastewater treatment plant

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1 Introduction

Organic compounds occur naturally in water as for example from natural organic matter (NOM); however, xenobiotics found their way into natural water bodies. These synthetic organic compounds (SOC) can pose serious threats to humans and the environment. Knowledge is needed in order to estimate possible risks from organic contaminants in water resources. Furthermore, the SOC's interaction with sediments, with other organic compounds or during various treatment steps is of high relevance since complexing reactions, adsorption mechanisms and conversion processes have a strong impact on the treatment efficiency at various treatment steps in drinking water treatment plants and influence the choice of treatment solutions (Ivančev-Tumbas, 2014). The fact that contaminants can undergo physico-chemical and biological transformations in the environment with unsure outcome increase the complexity of the topic.

Major groups of xenobiotics in aquatic bodies include pesticides, pharmaceuticals and personal care products. These compounds have different chemical properties, yet they have in common that they are able to exert specific effects in organisms (Daughton and Ternes, 1999; Halling-Sørensen et al., 1998). In order to estimate possible hazards, it is therefore important to know at what concentrations which contaminants are present in aquatic bodies.

Improvements in molecular analysis have made the detection of many compounds even at ng L^{-1} level possible (Ivančev-Tumbas, 2014). Among the methods of molecular analysis, liquid chromatography (LC) coupled to high-resolution mass spectrometry (HRMS) is the most common. A widely used method to extract analytes from water samples prior to analysis is solid phase extraction (SPE) (Richardson and Ternes, 2014). Within the last decades, studies were conducted all over the world and revealed water contamination with pesticides, pharmaceuticals and personal care products at levels from few ng L^{-1} up to several µg L^{-1}.

2 Objectives and hypotheses

2.1 Objectives

This study aims to provide a screening of water from the Uppsala region for the occurrence and concentrations of a spectrum of organic contaminants.

Within this study, several river, lake and drinking water samples were taken and analyzed for a range of organic compounds. The largest fraction of these compounds consists of human and veterinary pharmaceuticals and pesticides. Furthermore, metabolites of caffeine, nicotine and cocaine were analyzed, since they can be used as indicators of fecal contamination or for the estimation of consumption patterns (Daneshvar et al., 2012; van der Aa et al., 2013). The study ranges "from source to tap" in order to consider the fate of contaminants in different water bodies.

2.2 Hypotheses

- River water (downstream the wastewater treatment plant) will show highest concentrations of contaminants, followed by lake water; drinking water will have the lowest contaminant concentrations.
- The drinking water treatment techniques sand filter, GAC, chloramination and nanofiltration are not able to remove all contaminants.
- No risks for humans result from the presence of the studied organic contaminants in drinking water.

3 Background

3.1 Pharmaceuticals

Pharmaceuticals are not completely transformed in the body but excreted in large amounts unchanged or slightly transformed (Heberer, 2002). Their persistency inhibits a removal during wastewater treatment or degradation in the environment (Daughton and Ternes, 1999; Ternes, 1998). Through the effluents of wastewater treatment plants, they are released into the aquatic environment (Heberer, 2002). In some cases, untreated waste water enters the aquatic system, e.g. by discharge without treatment or during storm events from flood overload (Daughton and Ternes, 1999). Pharmaceutically active compounds can furthermore enter groundwater bodies via leachate from landfills or from manufacturing sites (Heberer, 2002). Another possible contamination pathway is the use of veterinary drugs in livestock breeding, e.g. fish farms and poultry (Heberer, 2002; Richardson and Ternes, 2014). Pharmaceuticals in the aquatic environment were first detected in the beginning of the 1980s (Ternes, 1998; Watts et al., 1984). The attention towards exposure of pharmaceuticals in natural environments grew rapidly in the mid 90s (Halling-Sørensen et al., 1998). Throughout the last 10-20 years, pharmaceutically active compounds have been found in aquatic environments all over the world and are gaining more and more attention as an upcoming main topic in environmental chemistry (Daughton and Ternes, 1999; Halling-Sørensen et al., 1998; Heberer, 2002; Heberer and Stan, 1997). Pharmaceutically active compounds have also been detected in treated drinking water and tap water (Daughton and Ternes, 1999; Delgado et al., 2012; Heberer and Stan, 1997; Mons et al., 2013).

Pharmaceuticals are designed to have profound effects in the physiology of an organism, i.e. they have a determined mode of action. Their biological effect is their most important characteristic (Daughton and Ternes, 1999; Halling-Sørensen

et al., 1998). What is particularly problematic is that for the desired medical effect, pharmaceuticals have properties that are of high importance for their fate in the environment. For example their ability to pass lipid membranes, their persistency and their lipophilicity are needed for their efficacy in medical treatment but are causing problems in the environment (Halling-Sørensen et al., 1998). It is estimated that up to 3000 different substances are available for medical treatment, of which only a small part has been examined for their environmental occurrence and effects. Among these drugs are painkillers, antibiotics, antidiabetics, betablockers, contraceptors, lipid regulators, antidepressants and impotence drugs (Richardson and Ternes, 2014). Although many pharmaceuticals are responsible for acute or chronic effects in aquatic organisms, the concentrations that are typically observed in water samples are below the lowest observed effect concentrations (LOEC). Some are however occurring at alarmingly high levels, for example diclofenac, ciprofloxacin or propanolol (Richardson and Ternes, 2014).

3.2   Pesticides

Pesticides are used in agricultural production to protect plants from pests or plant diseases and to control weeds. The use of these chemicals led to a strong increase in yields and agricultural production (Bolognesi, 2003). The term 'pesticide' describes a whole range of products, including herbicides, insecticides, fungicides, growth regulators, biocides and many more. Pesticides are most commonly used as plant protection products (PPPs) in order to protect crops and to influence their life processes as well as to prevent the growth of undesired plants. They contain so-called 'active substances', which are chemicals, plant extracts, pheromones or micro-organisms that have a certain action against pests or undesired plant products (Pesticides - European Commission, 2015). Many of these chemicals show a high stability towards degradation and can persist in the environment over several years (Bolognesi, 2003). Although pesticides are designed to have an effect only on selected organisms, it cannot be excluded that they have toxic effects on others. Many pesticides are proven to have toxic effects on humans. Studies about the acute toxicity of pesticides have been conducted comparably often; however, the fate of these chemicals in the environment and delayed effects are less studied (Bolognesi, 2003). Not only local environments are affected but human health is threatened when pesticides enter surface water bodies, since the latter are a primary source of drinking water (Gilliom et al., 1999; Tani et al., 2012).

Pesticides enter surface water and wastewater as runoff from the sites where they are applied (Masiá et al., 2013). Among the most important pathways are spray

drift, surface runoff, leaching, improper operation of pesticide equipment as well as runoff from streets (Gerecke et al., 2002). Residues of pesticides can be found in food and drinking water. Exposure to humans is also possible via air (Bolognesi, 2003). The use of pesticides and biocides is not restricted to agricultural application; a broad range is used in urban areas as well, for example as preservatives in cosmetics or material protection agents such as in wood (Schoknecht et al., 2003; Wittmer et al., 2010). Pesticides that are used in urban areas, for example on lawns and streets or in building materials, are easily washed away during rain events and can therefore enter WWTPs (Gerecke et al., 2002). Materials that contain for example biocidal active ingredients might release them during their service life. Among these may be ingredients in carpets, plastic films, preservatives for masonry or coatings and wood preservatives that can be released into the air or into the water (Schoknecht et al., 2003).

Many studies focus more on transformation products of pesticides rather than examining the parent compound. The transformation products can be more toxic than the parent compounds and they can appear in much higher concentrations (Richardson and Ternes, 2014). This can be particularly problematic when parent compounds are below a certain guideline value and are therefore considered safe while their transformation products pose severe risks to the aquatic environment. Pesticides can be found in many streams and in groundwater almost everywhere and the concentration in water is strongly related to the amounts used in the respective area. Therefore, pesticide levels in water vary with the season, since during spring and summer more pesticides are applied (Gilliom et al., 1999). They also vary with the geographic area, showing the importance of agricultural and urban areas. Impacts on aquatic ecosystems and to human health are very likely. The occurrence of pesticides in mixtures increases the difficulty of their assessment and might lead to unpredictable toxic effects (Gilliom et al., 1999). Even if single pesticides are found in very low concentrations that might be considered harmless to aquatic life or human health, the effects resulting from combined action in a mixture can be significant (Chèvre et al., 2006). Areas with mixed land use and urban areas are considered to be just as important for the contribution with pesticides and herbicides as are agricultural areas (Wittmer et al., 2010). Many governments regulate pesticide concentrations in drinking water. In EU law for example, the maximum concentration of pesticides in drinking water is 0.5 µg L^{-1} (Masiá et al., 2013; Tani et al., 2012). Pesticide levels are regulated by the Council Directive 98/83/EC which allows a maximum total pesticide concentration of 0.5 µg L^{-1} and a maximum concentration for single pesticides of 0.1 µg L^{-1}. The total pesticide concentration is defined by the sum of all detected and quantified pesticides (during monitoring). However, it is not indicated in the directive which

compounds have to be included in the monitoring procedure. An exemption is made for the pesticides aldrin, dieldrin, heptachlor and heptachlor epoxide: for these compounds, individual concentrations of only 0.03 µg L^{-1} are acceptable (European Commission, 1998). Admission of new pesticides in the EU is regulated by the Council Directive 91/414/EEC, which defines the properties compounds are required to have in order to be approved for application (Council of the European Communities, 1991; EFSA, 2015). About 450 active substances are approved by the European commission and are available on the market (EU Pesticides database - European Commission, 2015). The National Primary Drinking Water Regulations (NPDWRs) of the USA list specific maximum concentrations for individual organic chemicals including some pesticides instead of having general values, as in EU law (US EPA, 2014).

3.3 Other contaminants

Not only pharmaceuticals and pesticides are in the focus of environmental research – personal care products, nanomaterials, synthetic musks, perfluorinated compounds (PFCs), disinfection-by-products (DBPs) and brominated flame retardants in water are of high importance as well (Richardson and Ternes, 2014). Some organic contaminants can have endocrine disrupting effects and have also been found in drinking water (Delgado et al., 2012). The term 'emerging contaminants' refers to compounds that are only recently detected in the environment in various quantities and that could have effects on human health or ecosystems. A database has been established and can be accessed online, containing over 1000 emerging pollutants in the environment (Emerging substances | NORMAN, 2015). Personal care products are often used for the tracking of human fecal contamination (Daneshhvar et al., 2012).

3.4 Liquid chromatography

The separation of components in complex mixtures is a crucial step for their identification and quantification. For many compounds, this is only possible with the means of chromatography, a set of methods that allows the separation of closely related compounds. Liquid chromatography (LC), in particular, is one of the most commonly used separation techniques in chemical analysis. This method is applicable in many fields of science and industries. The possibility of automation, the high sensitivity, the accuracy of quantification and its suitability for the separation of thermally fragile species are only some of the reasons for its high popularity (Skoog et al., 2007). Many studies base on the combination of LC and mass spectrometry (MS), allowing the detection of many polar micropollutants in the environment, such as pharmaceuticals and pesticides (Krauss et al., 2010). Throughout chromatographic separation, the sample that is to be examined is diluted in a mobile phase, which is then forced through a column containing a stationary phase (Skoog et al., 2007). In case of liquid-solid chromatography, the mobile phase consists of a liquid solvent whereas the stationary phase consists of packed silica or artificial polymers containing active sites. Among the active sites, octadecyl (C18) chains are the most common; however, phenyl groups, cyano groups, diols and octyl (C8) chains are also used (Harris, 2007). During the separation, the compounds in the samples are distributed to varying degrees between the stationary and the mobile phase. Depending on the properties of the compounds and the phases, some components are retained stronger by the stationary phases than others. These compounds move more slowly through the column than the compounds that are weakly retained by the stationary phase. The time that is needed by a specific compound to move through the column is referred to as retention time. Due to different retention times, compounds exit the column separately and can more easily be analyzed and quantified. The most common property after which compounds are separated is polarity (Skoog et al., 2007).

In order to increase the efficiency of LC, the columns are packed with very small particles in the size range of 1.7-5 µm. This increases the active area of the stationary phase and therefore improves the resolution and run time in LC. The smaller the particles, the denser the packing in the column, and the higher the resulting backpressure. In high-performance liquid chromatography (HPLC), high pressures of up to 150 bar are occurring within the column (Harris, 2007). If one solvent or constant solvent mixture in the mobile phase is not sufficiently eluting all components in a specific time frame (isocratic elution), gradient elution can be used which involves the use of several solvents that differ in strength. In gradient elution, the concentration of a solvent B increases over time whereas the concentration of the initial solvent A is reduced likewise. The continuous gradient rapidly

elutes all components (Harris, 2007). In reversed-phase chromatography, the predominant type of chromatography, the stationary phase consists of a nonpolar substrate whereas the mobile phase consists of a highly polar solvent such as an aqueous solution with various concentrations of polar organic solvents. This leads to a faster elution of polar compounds and a slower elution of less polar compounds (see also Figure 1). Reversed-phase methods are capable of separating small polar non-ionic species (Skoog et al., 2007).
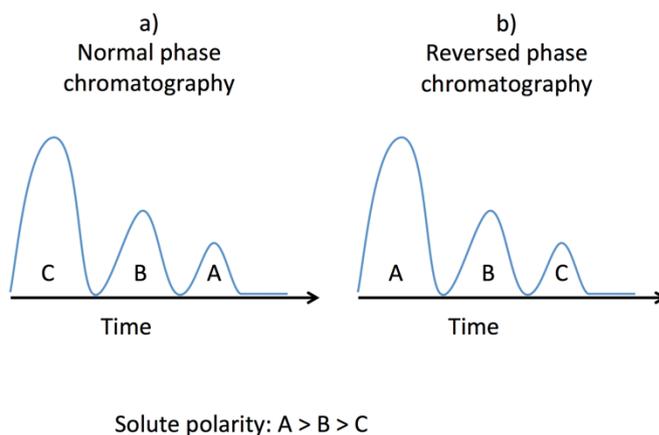
Figure 1: Polarity and elution in chromatography (after Skoog et al., 2007).

3.5   Mass spectrometry

Mass spectrometry (MS) is a widely used technique for the identification and quantification of individual compounds in a sample. It can be used to determine the structure of molecules, the elemental composition of a sample, the composition of complex mixtures and the occurrence of isotopes. High-resolution MS is a powerful tool for the determination of known and unknown compounds at low concentrations in mixtures and is of particular importance in environmental chemistry (Krauss et al., 2010; Skoog et al., 2007).

The resolution in mass spectrometry is the inverse of the resolving power. It describes the smallest mass difference at which separate peaks can be identified (Harris, 2007).

Depending on the task, different types of mass spectrometers are available, each of them having different benefits and limitations (Krauss et al., 2010). Their basic functional principles are outlined in chapter 3.5.1 to 3.5.5.

- **Triple quadrupole** (QqQ) and **quadrupole ion trap** (QIT) mass spectrometers are the first choice in target analysis due to their high sensitivity. However, their ability to detect unknowns is limited (Krauss et al., 2010).
- **Time-of-flight** (TOF) technology provides a lower sensitivity; however the resolution is much higher than for example in triple quadrupole mass spectrometers (Krauss et al., 2010).
- The main disadvantage of **Fourier transform ion cyclotron resonance** (FT-ICR) mass spectrometers is their high costs, which is why they are rarely used (Krauss et al., 2010).
- **Orbitrap** technology is getting more and more popular due to the high resolution, high mass accuracy and very high sensitivity (Krauss et al., 2010).

3.5.1   Quadrupole mass analyzers

The most important element of quadrupole mass analyzers is the quadrupole, consisting of four metallic rods that are arranged pairwise. These rods serve as electrodes and to each pair, positive or negative direct current (dc) is applied (Figure 2). The dc source is variable, meaning that the current can be increased over time (Skoog et al., 2007). Additionally to the direct current, a stronger alternating current (ac) is applied to the rods in a variable frequency. The ratio between ac and dc voltage is kept constant (slightly below 6), even when increasing the voltages during one run (Skoog et al., 2007).

Figure 2: Positive or negative direct current (dc) applied to the rods.

When an ion enters the quadrupole, the alternating current on the rods attracts and repels the ion and thereby influences its trajectory (Figure 3). If the ion is heavier or lighter than a target mass, it is diverted and neutralized at one of the rods. Only ions with a specific mass-to-charge ratio (m/z) are able to oscillate in resonance to the alternating current and to pass the quadrupole on a stable trajectory (Gates, 2014a; Harris, 2007; Skoog et al., 2007).

Figure 3: Schematic of a quadrupole. (Gates, 2014a) http://www.chm.bris.ac.uk/ms/images/quad-schematic.jpg

The mass-to-charge ratio (m/z) is defined by the ratio of the mass number of an ion to the number of charges on the ion. It is often referred to as 'mass' since most ions in MS are only charged singly. The quadrupole serves as a mass filter, since only ions within a narrow range of m/z values can pass the quadrupole successfully. The detector after the quadrupole recognizes at what current and frequency settings ions can pass and is thereby able to detect a whole mass spectrum in short time. Very common is the arrangement as a so-called triple quadrupole. In this instrument, a first quadrupole filters for mother ions, which are then dissociated in a collision cell. The ion fragments are then again separated in a subsequent quadrupole. The analysis of fragments allows a more reliable determination of compounds. Triple quadrupole instruments are an example of tandem mass spectrometry (Skoog et al., 2007).

3.5.2   Quadrupole ion-trap mass spectrometer

 Another type of mass spectrometer that can be combined with chromatographic instruments is the quadrupole ion-trap mass spectrometer. Ions are inserted into the ion trap, a chamber consisting of two end caps (electrodes) and a ring electrode. The ring electrode is supplied with a variable radio-frequency voltage. Ions inserted into the chamber can be stored for up to 15 minutes. The radio-frequency voltage stabilizes ions in the cavity on a circular trajectory, i.e. ions circulate in the ion trap. A change in the electric field results in destabilization of the circular trajectory of ions within a narrow m/z ratio. By increasing the voltage applied to the ring electrode, ions leave the trapping field depending on their mass to charge

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ratio. A detector captures the ejected ions and generates a signal (Harris, 2007; March, 2000; Skoog et al., 2007).

3.5.3 Time-of-flight (TOF) mass spectrometer

As the name describes, the mass of ions in a TOF instrument is calculated by their respective time of flight. Compounds are ionized and accelerated by an electric field before flying through a drift tube. Since the ions (theoretically) acquire the same kinetic energy, ions with a higher mass to charge ratio will arrive later at the detector than ions with a lower mass to charge ratio. The time an ion needs to "fly" from the ionizing source to the detector is in direct relation to its mass to charge ratio (Skoog et al., 2007). However, not all ions acquire the exact same kinetic energy, resulting in different times of flight for ions with the same mass. To tackle this problem, a reflectron element can be installed in the drift tube. This element reflects the ions, reverses their flight direction and improves resolving power (Harris, 2007). The reflectron consists of a decelerating field with a growing potential that is used to stop the ions. The time needed for an ion to enter the field and to leave it again does not depend on the initial velocity but solely on its mass. Like that, differences in kinetic energy can be extinguished and the resolution can be improved (Mamyrin, 2001).

3.5.4 Fourier transform ion cyclotron resonance (FTICR)

Among all broadband mass analysis techniques, FT-ICR provides the highest resolving power and mass accuracy. The precision in mass acquisition and the high resolution results from a different principle of measurement: instead of separating the ions before reaching the detector, masses are determined by measuring a frequency. The heart of the instrument consists of an ion trap analyzing cell in which the ions are accelerated by a radio-frequency signal. They are moving on a circular trajectory, a cyclotron. The ions are being excited by an electric pulse and acquire a frequency that is depending on their mass. This frequency can be measured very precisely and with the means of a frequency to mass conversion, the determination of the ions mass to charge ratio is possible (Heeren et al., 2004; Shi et al., 2000; Skoog et al., 2007).

3.5.5 Orbitrap mass spectrometers

In an orbitrap mass analyzer, static electric fields are used rather than dynamic electric fields which come to use in a quadrupole ion trap (Hu et al., 2005). The orbitrap consists of a central electrode (cathode) that is aligned coaxially through an outer electrode (anode). Both electrodes are supplied with a direct current (dc), creating a purely electrostatic field. Ions are entering the ion trap perpendicularly to the central electrode (red arrow in Figure 4) and start moving on an orbital trajectory around the inner electrode. Stabilized ions move axially along the inner electrode and

Figure 4: Cutaway view of the ion trap in an Orbitrap; image from (Hu et al., 2005).

rotationally around it. This process is called orbital trapping. The detection of ion masses works in a way similar to FTICR - an image current of the ions on their oscillating trajectory is recorded and goes through a conversion to produce a time-domain transient (Perry et al., 2008). Because the Orbitrap operates with a pulsed ion source, a coupled quadrupole for storage of ions is required in order to use continuous electrospray ionization (Hu et al., 2005).

All presented types of mass spectrometers have their advantages and disadvantages. However, hybrid instruments, in which a combination of quadrupole and TOF technology (QTOF) or a combination of linear ion trap and orbitrap technology (LTQ orbitrap) is used, provide excellent detection possibilities in various matrices (Kosjek et al., 2007; Krauss et al., 2010; Petrovic and Barceló, 2006). The LTQ Orbitrap in particular offers a high dynamic range and excellent sensitivity (Krauss and Hollender, 2008). In other cases, the use of different technologies in various runs is beneficial. For example, the use of a triple quadrupole for a first quantification and a separate run in a time-of-flight mass spectrometer for confirmation describes an established strategy (Krauss et al., 2010). The use of different mass spectrometers combined is referred to as 'tandem mass spectrometry'.

3.6   Ionization techniques in LC:

- Electrospray ionization (ESI); Electrospray ionization is the most common ionization technique for LC-MS. It is a comparably soft ionization method but lacks the ability to produce fragments. It is considered only successful for compounds with at least one N, P, O, S or metal atom whereas compounds containing only C, Si, H and halogens are not likely to be ionized by ESI. The analyte is intro-

duced and passed through an electrospray needle, which has a high potential difference with respect to the receiving cone (the counter electrode). In the space between needle tip and cone, the solvent evaporates, causing the small droplets from the needle spray to be ripped apart. The ionization results from the charge the molecules get due to the high potential difference between needle and cone (Gates, 2014b; Hug et al., 2014).

- Atmospheric-pressure chemical ionization (APCI); APCI shares some similarities with ESI. The analyte is ejected by a spray needle, which has, in contrast to ESI, no electric potential. Instead, ionization occurs with the help of a corona discharge needle. The electric corona is a plasma containing charged particles. After being ejected from the spray needle, the solvent evaporates. Meanwhile, the corona discharge needle is put under high voltage and therefore transforms surrounding H_2O to H_3O^+. The molecule from the analyte reacts with the H^+ from H_3O^+, leaving an ionized molecule $[M+H^+]$ and H_2O. APCI is in contrast to ESI not suitable for thermally labile compounds (Gates, 2014c; Harris, 2007).

- Matrix-Assisted Laser Desorption/Ionization (MALDI): For the ionization with MALDI, a matrix has to be created on a sample plate, isolating the analytes from each other. This is referred to as solid solution. Afterwards, a laser beam leads to the ejection of analyte and matrix molecules from the sample plate (matrix excitation). Just above the sample plate, proton transfer from the matrix molecules to the analytes ionizes analyte molecules. Furthermore, cations can attach to the analyte. $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$ ions are formed.

In gas chromatography, different ionization methods are used, such as electron ionization (EI) or chemical ionization (CI).

3.7   Analytical approaches in MS

In general, three different analytical approaches exist.

- In *quantitative target analysis*, compounds in the sample are measured and their retention time, mass and fragments are compared to a reference standard. A list of target ions is created before analysis and the analysis focuses on finding these defined ions in the sample. If new substances shall be detected, the analysis has to be run again with the respective reference standard (Krauss et al., 2010).

- If the reference standards are not available, quantification and confirmation of compounds has to be achieved with a different method. In *suspect screening analysis*, a list of suspected ions with known compound-specific information

is defined. Molecular formula and structure of the suspected ions can be used for the detection of compounds. Theoretically predicted isotope patterns, exact calculated m/z ratios, physico-chemical properties and related expected retention times of the suspect ions help in the detection of compounds. The possibility of false positive findings and false negatives are reasons for the rare appliance of suspect screening in environmental research (Krauss et al., 2010).

- In contrast to target analysis and suspect screening, *non-target screening* aims at detecting compounds of which no information is available or which might not be expected in the examined sample. The procedure usually involves the detection of peaks by exact mass filtering after chromatography, the deduction of a possible mass formula for the detected peak and the comparison of the findings with databases. In that way, various possible compounds can be estimated and ranked according to the possibility of appearance. For final confirmation, the findings can be compared to the measurement of a reference standard. Finding the most probable chemical structure to the molecular formula that is derived from the detected peak represents the main challenge in non-target screening (Krauss et al., 2010). An overview of a possible working procedure for suspect screening and non-target screening can be found in (Hug et al., 2014).

3.8 Solid phase extraction (SPE)

One of the most important sample preparation methods is solid phase extraction (SPE). The first SPE cartridges were introduced in 1978 (Hennion, 1999), and has developed tremendously ever since. SPE was introduced as an alternative of liquid-liquid extraction (LLE) and has replaced it successfully. LLE uses large amounts of solvents that are costly to dispose of and is more time consuming to use. The demand for more environmentally friendly laboratory methods increased the popularity of SPE (Hennion, 1999; Poole, 2003).

SPE is based on the partitioning of dissolved compounds between two phases, i.e. a liquid phase and a solid phase. The sample itself constitutes the liquid phase; the sorbent is the solid phase. When passing a liquid sample through a SPE device, the analytes bind to the sorbent. The bound analytes can afterwards be eluted with an appropriate solvent (Camel, 2003). One of the strongest advantages is the ability of SPE to extract many polar analytes from water samples. A broad range of sorbents is available on the market, from cross-linked copolymers over *n*-alkylsilica to graphitized carbons and more. Some sorbents have been developed especially for the analysis of polar analytes. When SPE is directly coupled to

chromatographic analysis (mostly LC), it is referred to as 'on-line' whereas an extraction separated from chromatography is called 'off-line' SPE. On-line SPE allows automatic operation and is less time consuming. In environmental analysis, the extraction of a broad range of compounds with various polarities via SPE and the subsequent analysis with LC-MS is a very popular approach, e.g. for the analysis of organic compounds in water samples (Hennion, 1999).

SPE devices exist as cartridge and disks; however, with the basic principle being the same, the main difference is the format. Whereas disks are only available from industrial manufacturers, cartridges can be prepared in the lab. Disks are much more expensive than cartridges but have the advantage of being less prone to clogging and pressure drops within the SPE device than cartridges. Therefore, disks allow higher sample flows and shorter sample processing times (Poole, 2003). They both are limited with regards to handling samples with suspended solids and prior filtration is necessary to avoid clogging. Some manufacturers integrate filters into their SPE cartridges (Hennion, 1999).

4 Materials and methods

4.1 Target analytes

The target analytes included in this study are presented in Table 1. The targeted pesticides include herbicides (atrazine, cyanazine, isoproturon, quinmerac, simazine) and an anthelmintic against parasitic worms (albendazole-sulfone). The targeted pharmaceuticals include beta blockers (atenolol, metoprolol), blood lipid regulators (bezafibrate), psychoactive drugs (carbamazepine), pain killers (diclofenac, ketoprofen) and veterinary drugs (ketoprofen, monensin). Compounds referred to as personal care products are commonly used as fecal source indicators. Compounds of this group that were included in this study were caffeine, nicotine, a metabolite of nicotine (cotinine) and a metabolite of cocaine (benzoylecgonine). This range of compounds was already used in previous studies. It is based on usage amounts and was established to identify sources of fecal contamination. If contamination with fecal matter was found via the identification of E. coli bacteria, the selected range of compounds should help identify from which source the contamination came from (e.g a household or a poultry farm). The compounds analyzed in this study are part of this range and represent a compromise – only compounds that were found to work well with the developed method were used.

Table 1: Properties of the analyzed contaminants; K_{ow} is the octanol-water partitioning coefficient, S_w is water solubility.

Name	Type	Mass [g mol^{-1}]([a])	Molecular formula([a])	log K_{OW} ([c])	S_w
Albendazol-sulfone	Pesticide	297.3302	$C_{12}H_{15}N_3O_4S$	1.10	1449 mg L^{-1} ([b])
Atenolol	Pharmaceutical	266.33608	$C_{14}H_{22}N_2O_3$	-0.03	13300 mg L^{-1}([a])
Atrazine	Pesticide	215.68326	$C_8H_{14}ClN_5$	2.82	34.7 mg L^{-1}([a])
Benzoylecgon-ine	Metabolite of cocaine	289.32636	$C_{16}H_{19}NO_4$	-1.32	3820 mg L^{-1}([c])

Bezafibrate	Pharmaceutical	361.8194	$C_{19}H_{20}ClNO_4$	4.25	1.55 mg L^{-1} ([c])
Caffeine	Beverage ingredient	194.1906	$C_8H_{10}N_4O_2$	0.16	21600 mg L^{-1} ([a])
Carbamazepine	Pharmaceutical	236.26858	$C_{15}H_{12}N_2O$	2.25	18 mg L^{-1} ([a])
Cotinine	Metabolite of nicotine	176.21508	$C_{10}H_{12}N_2O$	0.34	$1*10^6$ mg L^{-1} ([a])
Cynazine	Pesticide	240.6927	$C_9H_{13}ClN_6$	2.51	170 mg L^{-1} ([a])
Diclofenac	Pharmaceutical	318.130469	$C_{14}H_{10}Cl_2NNaO_2$	4.02	4.47 mg L^{-1} ([c])
Isoproturon	Pesticide	206.28412	$C_{12}H_{18}N_2O$	2.84	72 mg L^{-1} ([d])
Ketoprofen	Pharmaceutical	254.28056	$C_{16}H_{14}O_3$	3.00	51 mg L^{-1} ([a])
Metoprolol	Pharmaceutical	267.3639	$C_{15}H_{25}NO_3$	1.69	$1*10^6$ mg L^{-1} ([a])
Monensin	Pharmaceutical	670.87088	$C_{36}H_{62}O_{11}$	5.43	
Nicotine	Tobacco ingredient	162.23156	$C_{10}H_{14}N_2$	1.00	$1*10^6$ mg L^{-1} ([a])
Quinmerac	Pesticide	221.63972	$C_{11}H_8ClNO_2$	2.87	
Simazine	Pesticide	201.65668	$C_7H_{12}ClN_5$	2.40	6.2 mg L^{-1} ([a])

[a] PubChem, [b] Epi Suite ™, [c] DrugBank, [d] WHO, 2003

(Table 1 continued)

4.2 Sampling site selection

Sampling sites were selected according to the natural flow in the aquatic system.
Sites along river Fyris upstream and downstream of Uppsala were included.
Furthermore, a lake sample directly after the city (Lake Ekoln) and one at a
drinking water treatment plant (Lake Görväln) were taken. The distaance between
the lakes is approximately 50 km. In the treatment plant, samples were taken at
different treatment steps: from the intake, collecting unfiltered water from a depth
of 22m; after the sand filter treatment (SF), after the granular activated carbon
(GAC) and from finished drinking water (DW). The water gets disinfected with
UV light and chloramine (NH_2Cl) before being introduced into the water supply
system. Furthermore, samples from a pilot plant with nanofiltration technology
(NF) and GAC were taken. Tap water samples from the treatment plant (DWTP
DW), school (DW School) and supermarket (DW Supermarket) were taken in
order to account for possible contamination from within the water supply system.
The school and supermarket receive the treated drinking water from the described
DWTP. A map with sampling sites is attached in Appendix A. Figure 5 gives an
overview of the sampling sites, Figure 6 shows the drinking water treatment plant
(DWTP) and its treatment steps.

The carbon in the pilot plant is Norit GAC 1240W, the columns were 2.5 m
high and filled with 1 m carbon at a width of 9 cm. The flow rate in the pilot plant

GAC was 1 L min^{-1} with a contact time of 6 min. Contact time is the same as in full-scale GAC. The nanofiltration module is a Pentair HFc module for capillary nanofiltration.
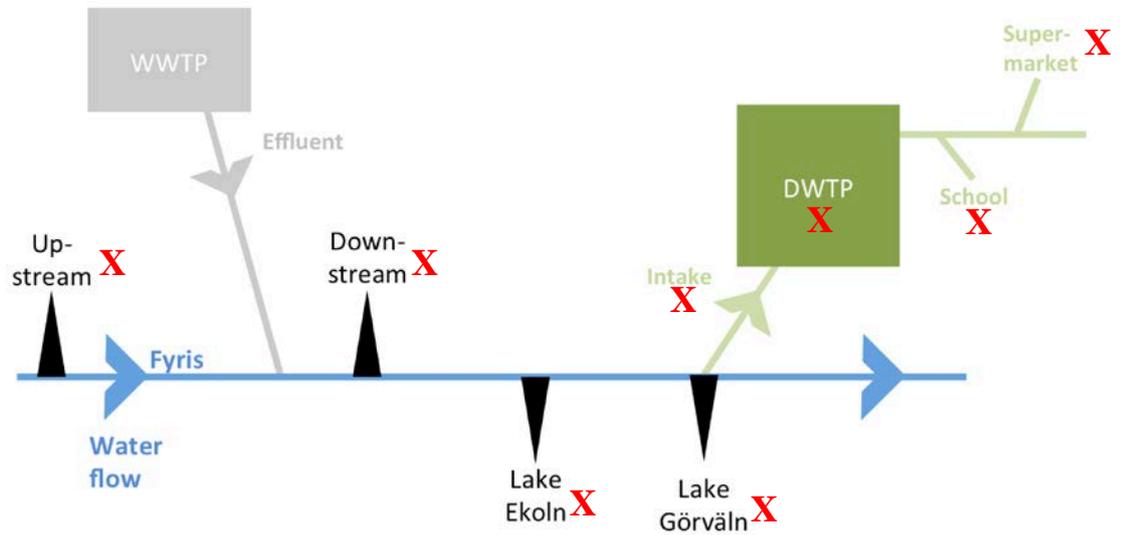
Figure 5: Schematic of sampling points (not to scale); samples were taken from river Fyris before (upstream) and after (downstream) passing Uppsala, in Lake Ekoln, Lake Görväln, inside the drinking water treatment plant (DWTP), in a school and in a supermarket. Sampling sites are indicated with a red X.
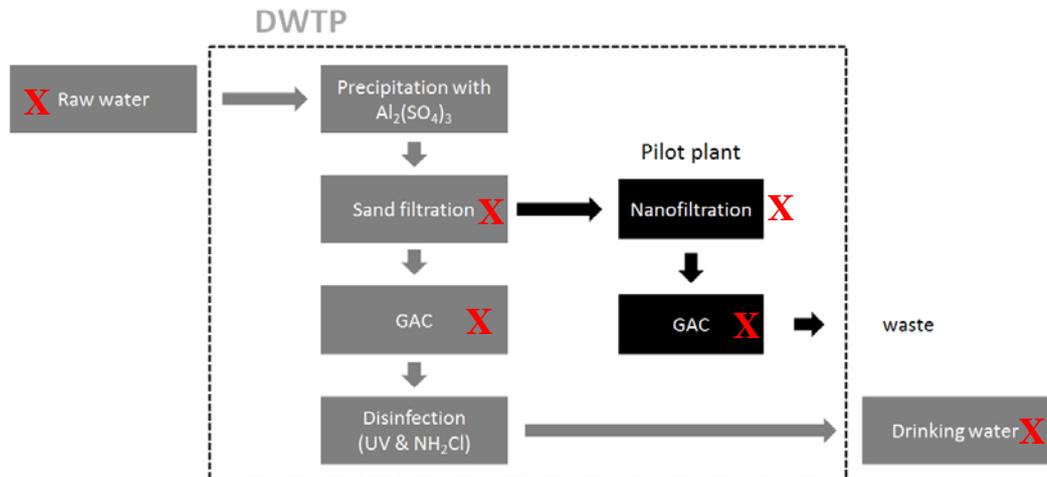
Figure 6: Schematic view of the treatment steps in the DWTP including the pilot plant; raw water undergoes treatment by precipitation with aluminium sulfate, sand filtration, GAC and disinfection with UV light and chloramine; water in the pilot plant is taken after the sand filtration step of the full-scale plant and undergoes nanofiltration and GAC treatment Sampling sites are indicated with a red X. (illustration after Ahrens et al., 2014)

4.3   Sampling

Samples were taken as grab samples, providing information about the current situation at the specific sampling site. A triplicate sample was taken at the site Fyris Downstream, while a duplicate was taken from the drinking water at the plant (DWTP DW). All other samples were taken onefold.

The samples from Lake Görväln and within the DWTP were taken on 9th March 2015. The samples Fyris Upstream, Fyris Downstream and Lake Ekoln were taken on 31st March 2015. On April 17th, tap water samples from school and supermarket were taken as well as one sample from the pilot plant coupled to GAC, since the carbon had been exchanged one week prior to the sampling (Pilot NF + new GAC). After sampling, the samples were stored at 5 °C.

The GAC in the pilot plant was exchanged on 18th of November 2014 and 13th of April 2015, i.e. 5 months before the first sampling date and 1 week before the third sampling date. Since the removal efficiency of GAC decreases with its age, samples were taken to compare the effect of old and new GAC.

The river samples were taken with a stainless steel bucket and an attached rope. The bucket was lowered into the river from a bridge in order to collect samples from the middle of the stream. The collected water was then transferred into a stainless steel container (Sharpsville containers; Figure 7). Several filled buckets were needed to fill one of the steel containers. The sample from Lake Ekoln was taken with a stainless steel bucket by walking with waders into the lake from the shoreline. The lake sample from Görväln was taken by using an inlet from the drinking water treatment plant (DWTP) that collects water from 5 m depth. This was a compromise, since no boat was available to collect water from the lake surface and the shoreline would not allow walking in with waders. The sample from the 5 m inlet is representative for the surface water, while the intake of the treatment plant collects raw water for treatment from a depth of 22 m, using water from deeper layers of the lake. The samples in the DWTP Görväln were collected directly with stainless steel containers after the various treatment steps. Tap water samples were taken in the lunchroom of a local supermarket as well as in the kitchen of a primary school. Control samples were taken in the lab from common taps as well as from a Millipore station.

Figure 7: Sampling of river water downstream and transfer of sampling water into stainless steel containers.

Samples were taken with stainless steel cans that have a volume of ~12 L. (Sharpsville containers). Only 5 L of the collected water was used for analysis within this study. The containers are usually used by the department to collect samples for persistent organic pollutants (POPs) and are internally called POP cans. For simplicity, they will therefore be referred to as POP cans from now on. Inside the cans, a pipe leads to the bottom of the can, allowing pumping out water from the can without opening the top lid (Figure 8).
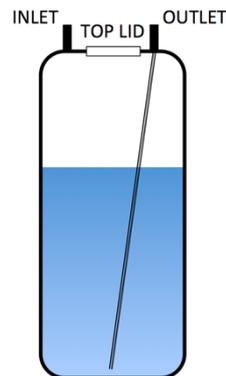
Figure 8: Schematic view of a POP can.

4.4   Solid phase extraction (SPE)

Water samples were extracted in the POPs laboratory at the Department of Aquatic Sciences and Assessment, Section for Organic Environmental Chemistry and Ecotoxicology at SLU, Uppsala. Solid phase extraction (Figure 9) was carried out with Waters Oasis HLB (1 g) cartridges and Agilent Bond-Elut ENV (1 g) cartridges using a Waters extraction manifold SPE station. The two cartridges were used for two reasons: first, the performance of the cartridges should be compared; second, the spectrum of compounds that can be extracted with each cartridge differs. Before extraction, the cartridges were preconditioned with methanol (HPLC grade) by filling the cartridges twice completely and afterwards flushing them with Milli-Q water. Water samples were filtered with glass fiber filters (Whatman GF/F, 0.7 µm, burned at 400 °C before use) into 5 L glass bottles (VWR chemicals) and spiked with 250 µL of a mass-labeled internal standard mix prior to extraction (concentration level $c = 200$ ng mL^{-1}, in methanol). The internal standards were taken out of the freezer, acclimatized to room temperature and vortexed before being added to the samples in the 5 L glass bottles. The internal standard was added to enable future analyses in larger screening studies but were not used in this study. A list of compounds included in the internal standard mix can be found in Appendix E. SPE usually took place over night. For each extraction, a batch of 6 bottles were connected via tubes to the SPE station and a vacuum

Figure 9: Solid phase extraction (SPE).

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suction pump sucked the sampling water through the cartridge. Speed was adjusted to approximately one drop per second (the loading time was approximately 10-15 h). After extraction, the cartridges were washed with 10 mL of Milli-Q water and 5 mL of 5% methanol solution before being eluted three times with 10 mL of HPLC-grade methanol (Merck KgA) into separate glass tubes. The eluate was stored in a freezer at -18 °C.

4.5 Evaporation

The eluate was concentrated in a N-Evap 112 Nitrogen Evaporator (Organomation Associates Inc.) and pooled afterwards. Glass tubes were rinsed twice with HPLC-grade Methanol (Merck KgA) when pooling the eluate. The pooled eluate was concentrated further to a level of 500 µL before transferring it into a vial (Agilent Technologies). Test tubes were rinsed twice with 500 µL HPLC-grade ethanol (VWR chemicals). The vials were again concentrated to 500 µL and filled up to 1 mL with Millipore water. See Figure 10 for a schematic overview of the procedure.
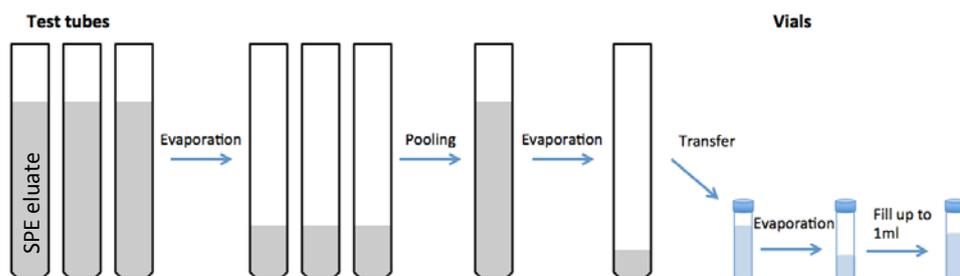
Figure 10: Schematic view of eluate concentration.

An overview of chemicals and devices used can be found in Appendix D.

4.6 Instrumental analysis: Ultra-performance liquid chromatography and high-resolution mass spectrometry

Chromatographic separation of target compounds was performed with a Waters Acquity UPLC H-Class system. Columns used were a Waters HSS-T3-C18 column (silica skeleton) for positive ion mode and a Waters BEH-C18 column (polymer skeleton) for negative ion mode. Run time was 21 minutes.

Table 2: Parameters and settings of the LC method.

Parameter	Positive mode	Negative mode
Column type	HSS-T3-C18	BEH-C18
Dimension	2.1 mm x 100 mm	
Particle size	1.8 µm	1.7 µm
Injection volume	5 µL	
Flow rate	0.5 mL/min	
Column temperature	40 °C	

Mobile phase A consisted of Milli-Q water with 5 mM ammonium formate and 0.01% formic acid. Mobile phase B consisted of acetontrile and 0.01% formic acid. A and B were used for positive ion mode. The mobile phase composition gradient for positive mode can be found in Table 3. Solvent concentrations were increased and reduced constantly over time according to Table 3. For example solvent A had an initial concentration of 95% and decreases linearly from minute 0.5 to minute 16 while solvent B had an initial concentration of 5% and increases steadily to 95% in the same time.

Table 3: Gradient program LC (positive mode).

Time (min)	Solvent A (%)	Solvent B (%)
0.00	95.0	5.0
0.50	95.0	5.0
16.00	5.0	95.0
19.00	1.0	99.0
19.10	95.0	5.0
21.00	95.0	5.0

Mobile phase C consisted of Milli-Q water with 5 mM ammonium acetate and 0.01% ammonia. Mobile phase D consisted of acetonitrile and 0.01% ammonia. C and D were used for analysis in negative ion mode. The mobile phase composition gradient for positive mode can be found in Table 4. See above for an explanation of the gradient concentration change.

Table 4: Gradient program LC (negative mode).

Time (min)	Solvent C (%)	Solvent D (%)
0.00	95.0	5.0
0.50	95.0	5.0
16.00	5.0	95.0
19.00	1.0	99.0
19.10	95.0	5.0
21.00	95.0	5.0

Screening of the water extracts for the target compounds was performed by a time-of-flight mass spectrometer (Waters Xevo G2-S QTOF) with positive (ESI+) and negative (ESI-) electrospray ionization. Target compounds were analyzed in resolution mode. The resolution mode is an instrument setting that allows a higher mass accuracy (which is needed to differentiate between peaks) while having a lower sensitivity during analysis. The data was collected with a MS^E method, which allows the recording of two acquisition modes (high and low collision energy) at the same time.

Table 5: Parameters and settings for the time-of-flight mass spectrometer.

Parameter	Setting
Capillary voltage (V) positive mode	350
Capillary voltage (V) negative mode	400
CID collision energy low (V)	4
CID collision energy high (V)	10 - 45
Resolving power (R) at m/z = 556.2766	~ 30.000
Mass error during calibration (ppm)	< 2
Cone voltage (V)	30
Desolvation gas flow rate (L h^{-1})	700
Source temperature (°C)	120
Desolvation temperature (°C)	450
Collision cell pressure (bar)	~ $2.5\ e^{-5}$
Cone gas flow (L h^{-1})	25
Mass range (m/z)	100-1200
Scan time (s)	0.25
Interval (s)	10
Acquisition time (min)	0.1 - 19.9

Waters UNIFI Scientific Information System (Version 1.7) was used for instrument control, analysis settings, calibration and quantification as well as for qualitative analysis. Compound confirmation was done with the help of spiked Milli-Q and tap water samples and the detected peaks of the respective compound. For data extraction, a mass tolerance of 6 ppm (target match tolerance) and a retention time window of 1 minute were set up. During positive mode, hydrogen, sodium and ammonium adducts were searched for. Ionization resulted in $[M+H]^+$ $[M+Na]^+$ and $[M+NH4]^+$ ions in positive mode and $[M-H]^-$ ions in negative mode.

4.7 Quality control

4.7.1 Preparation of calibration standards

Calibration solutions were prepared by dilution of standard solutions with Milli-Q water for final concentrations of 0, 10, 25, 50, 100 and 150 ng mL-1 (ppb) of the respective target compound. A list of the added compounds in the calibration solutions can be found in Appendix B.

4.7.2 Lock spray settings

During the MS runs, a lock mass solution with Leucine Enkephalin (Waters) was injected continuously to compensate for a shift in mass determination during measurement. In positive mode, lock masses (m/z) were 278.1135 and 556.2766 and a cone voltage of 30.0 V and a capillary voltage of 300 V was applied. In negative mode, the respective masses were 236.1041 and 554.262, while cone voltage and capillary voltage where similar.

4.7.3 Extraction efficiency and method recovery

Extraction efficiency and method recovery were calculated with the help of contaminant concentrations in blank and spiked samples of Milli-Q and drinking water. The spiked samples were spiked with a mixture of native compounds (same as in calibration solutions, see Appendix B) and internal standard mixes. Internal standards contain mass-labeled compounds while native compounds are identical to the ones found in the environment. The extraction efficiency accounts for the method's ability to extract the target compound from a sample, covering adsorption and elution in the SPE cartridge as well as ionizability and detectability in LC-MS. It was calculated with the help of Milli-Q samples that were treated identically to the environmental samples. Spiking with internal standard and native mixes was done before extraction with SPE cartridges. Three replicates were prepared of each blank and spiked sample. The concentration in blank Milli-Q water is considered to be background and therefore has to be subtracted. The calculation can be seen in Equation 1,

$$\text{Extraction efficiency } (\%) = \frac{C(MQ_{spiked}) - C(MQ_{blank})}{c_{theoretical}} * 100$$

Equation 1: Calculation of extraction efficiency.

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where *C(MQ)* is the average (*n=3*) measured concentration of the respective compound in spiked or blank Milli-Q water and $c_{theoretical}$ is the concentration of spiked compounds that was added to the sample.

The method recovery accounts not only for the extraction efficiency, but also includes matrix effects, as for example ion suppression by other compounds than the target compound. It can be regarded as a means of how well the method is generally able to detect the targeted compounds. Like the Milli-Q water blanks, drinking water blanks and spiked drinking water samples were treated exactly as the environmental samples. The blank drinking water sample is again subtracted to account for background noise. The calculation for the recovery can be found in Equation 2,

$$\text{Method recovery (\%)} = \frac{C(DW_{spiked}) - C(DW_{blank})}{c_{theoretical}} * 100$$

Equation 2: Calculation of method recovery.

where *C(DW)* is the average (*n=3*) measured concentration of the respective compound in spiked or blank drinking water and $c_{theoretical}$ is the concentration of spiked compounds that was added to the sample. A summary of extraction efficiencies and recoveries, calculated per compound for the two different SPE cartridges can be found in Table 6. Extraction efficiencies and recoveries are also shown graphically in Appendix C.

The concentrations measured during instrumental analysis were adjusted with the recovery results. That means, if for a specific compound a recovery of for example 30% was acquired, the detected concentration in the environmental sample was recalculated to correct for the low recovery and to calculate the actual amount in the sample.

4.7.4 Limit of quantification (LOQ) and limit of detection (LOD)
The limits of quantification (LOQs) of the described method were calculated with the help of response values from spiked drinking water samples. The exact calculation can be found in Equation 3. LOQ values are presented in chapter 5.1, Table 6.

$$\text{LOQ} = \left[c_{spiking} \middle/ \frac{r(DW_{spiked})}{200} \right] + 2 \left[std.\,dev\big(C(DW_{spiked})\big) \middle/ \frac{r(DW_{spiked})}{200} \right]$$

Equation 3: Calculation of the limit of quantification (LOQ). $c_{spiking}$ is the concentration of the target compound that was added to the sample; r is the average ($n=3$) response that was recorded for the spiked drinking water samples. 200 is the threshold value for response below which the quantification program does not integrate peaks; $std.dev$ is the standard deviation of the measured concentrations in the spiked drinking water samples.

The standard deviations were calculated and added to the limit of quantification in order to gain reliability of the calculation. By adding two standard deviations, it can be assured that in spiked samples the limit of detection is true for approximately 97.5% of the samples (see Figure 11).
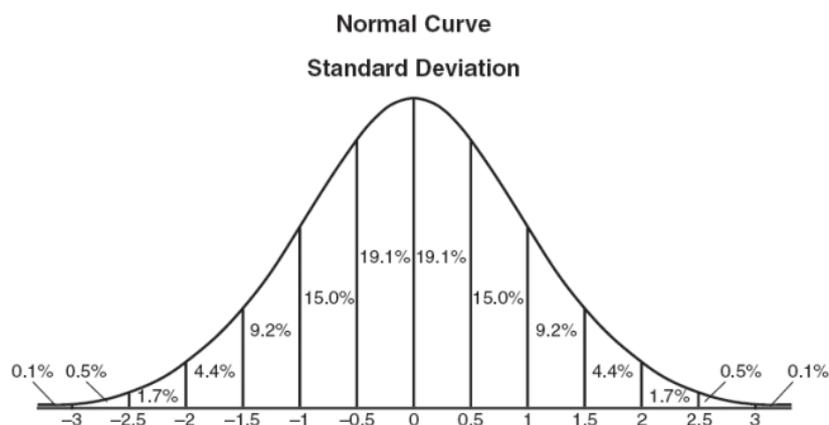
Figure 11: Normal curve and standard deviations.
(http://www.regentsprep.org/regents/math/algtrig/ats2/normal67.gif)

The limit of detection (LOD) in this study is the same as the limit of quantification (LOQ), since the instrument type used (QTOF) has a very high resolution and quantification precision. While other studies use signal to noise ratios as a means to differentiate between the detection and quantification limit, the noise in the described method is so low that no reasonable conclusion could be drawn from such approach.

5 Results

5.1 Method validation

As mentioned before, two different SPE cartridges were used for extraction: Waters Oasis HLB and Agilent Bond-Elut ENV. The average extraction efficiency was 97±7% for HLB cartridges and 63±8% for ENV cartridges, while the average method recovery was 51±6% for HLB cartridges and 41±4% for ENV cartridges. A paired, two-tailed Student t-test was conducted for extraction efficiencies and recoveries of HLB and ENV cartridges and revealed that both extraction efficiency ($p = 0.0067$) and recovery ($p = 0.0022$) were significantly different between the two groups, assuming a level of significance of $\alpha = 0.05$. Therefore it can be concluded that HLB cartridges performed significantly better than ENV cartridges, concerning extraction efficiency (53% better extraction efficiency with HLB) and recovery (25% better recovery with HLB cartridges). The results of both HLB and ENV extractions are presented below; however, since the results conceived from the HLB extraction are considered more reliable, the data evaluation and conclusions will be based on only HLB results. Contaminant levels in blank samples were <LOQ and are not presented.

The following compounds have not been detected, although the extraction efficiency and recovery would have allowed detection: atrazine, cyanazine, isoproturon, ketoprofen, monensin, quinmerac, simazine.

The LOQ of diclofenac was calculated with results from both HLB and ENV cartridges, since the automatic peak integration in the Waters UNIFI software did not integrate diclofenac peaks in all spiked samples, even though they were clearly visible. In the current version of the software, manual peak integration is not possible. This procedure was an exemption; the calculation of the LOQ with response

values from both HLB and ENV extractions was only made for diclofenac. For all other compounds, response values of only one extraction method were used.

Table 6: Summary of extraction efficiencies (± standard deviation), recoveries and detection limits for HLB and ENV cartridges.

	HLB			ENV		
	Extraction efficiency	Method recovery	LOQ [ng L^{-1}]	Extraction efficiency	Method recovery	LOQ [ng L^{-1}]
Albendazol-sulfone	100 ± 17 %	75 ± 9.3 %	0.91	84 ± 12 %	73 ± 5.2 %	0.74
Atenolol	31 ± 0.77 %	18 ± 1.4 %	0.27	29 ± 1.5 %	21 ± 0.3 %	0.67
Atrazine	96 ± 5.0 %	36 ± 2.4 %	0.63	76 ± 5.3 %	31 ± 2.0 %	0.71
Benzoylecgonine	110 ± 6.9 %	71 ± 7.2 %	0.13	77 ± 11 %	64 ± 6.2 %	0.14
Bezafibrate	120 ± 6.8 %	91 ± 7.4 %	0.67	100 ± 8.0 %	86 ± 4. 0%	0.58
Caffeine	100 ± 4.5 %	70 ± 8.8 %	3.0	67 ± 7.0 %	50 ± 9.6 %	4.5
Carbamazepine	110 ± 4.6 %	47 ± 3.6 %	0.16	74 ± 6.3 %	39 ± 2.9 %	0.19
otinine	100 ± 11 %	86 ± 12 %	0.38	46 ± 12 %	39 ± 5.0 %	0.57
Cyanazine	96 ± 4.9 %	54 ± 4.7 %	1.0	81 ± 8.6 %	47 ± 5.1 %	1.2
Diclofenac	84 ± 7.9 %	6.6 ± 3.8 %	74	63 ± 6.3 %	13 ± 7.4 %	74
Isoproturon	85 ± 4.1 %	31 ± 2.4 %	0.23	73 ± 7.2 %	26 ± 1.2 %	0.25
Ketoprofen	120 ± 5.7 %	65 ± 6.3 %	4.0	84 ± 6.7 %	52 ± 1.9 %	3.6
Metoprolol	120 ± 4.8 %	21 ± 2.1 %	0.22	14 ± 19 %	22 ± 2.4 %	0.22
Monensin	81 ± 5.2 %	67 ± 6.7 %	0.07	69 ± 5.5 %	60 ± 6.5 %	0.08
Nicotine	14 ± 0.76 %	68 ± 12 %	1.1	31 ± 14 %	33 ± 5.6 %	1.6
Quinmerac	160 ± 28 %	14 ± 2.0 %	5.6	20 ± 1.8 %	2 ± 0.6 %	28
Simazine	120 ± 4.5 %	40 ± 2.6 %	1.2	90 ± 13 %	33 ± 2.6 %	1.4
Average:	97 ± 7 %	51 ± 6 %		63 ± 8 %	41 ± 4 %	
t-test: *p*-value recovery				0.0067		
t-test: *p*-value extraction efficiency				0.0022		

5.2 Concentrations and composition profiles

The concentrations that were found at each sampling point using either HLB or ENV are shown in Figure 12. The error bars show standard deviations for the sampling points where more than one sample was taken: at the site downstream of Uppsala ("Fyris Downstream"), a triplicate was taken, while the treated drinking water in the treatment plant ("DWTP DW") was taken as a duplicate. See chapter 4.2 for an overview and description of the sample names. The highest total contaminant concentration was found at the site Downstream, while the lowest concentrations were detected in the water that was filtered by the pilot plant coupled to a granular active carbon filter (Nano new GAC).
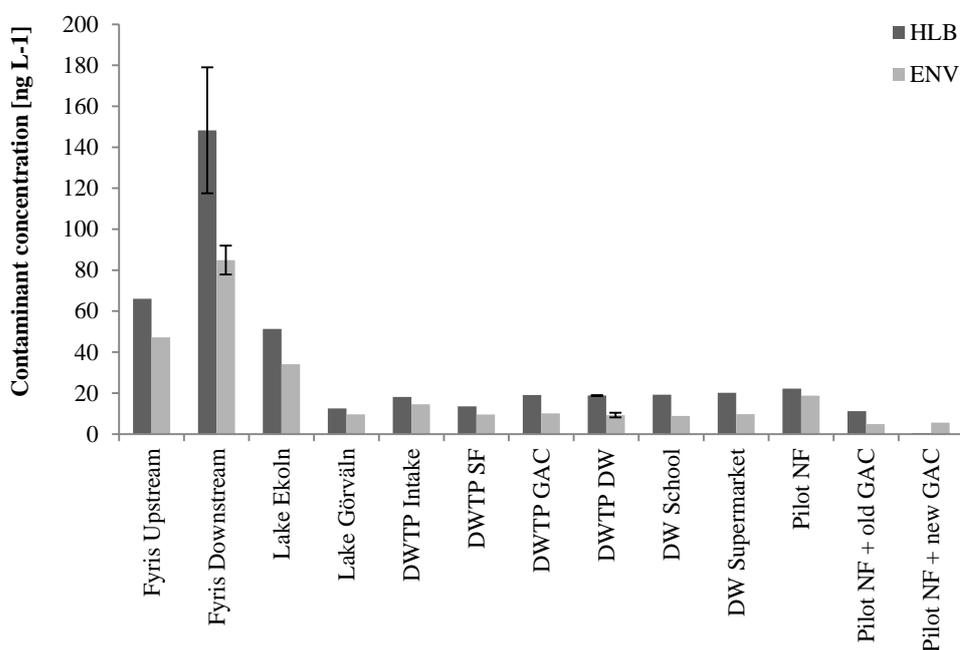
Figure 12: Contaminant concentrations [ng L^{-1}] at all sampling points, results from extraction with HLB and ENV cartridges. Samples were analysed onefold except of Fyris Downstream ($n = 3$) and DWTP DW ($n = 2$).

All calculated environmental concentrations of both extraction methods are shown in Table 7 and Table 8. All findings are presented graphically below, except for the concentrations of albendazol-sulfone (0.35 ng L^{-1} - 1.3 ng L^{-1}). Albendazol-sulfone was only detected in the samples "Pilot NF + old GAC" and "Pilot NF + new GAC", i.e. only in the samples where the nanofiltration technique was coupled to a GAC treatment (Table 7). This is further discussed in chapter 6.2.

Table 7: HLB concentrations [ng L^{-1}] of all contaminants; values in brackets are < LOQ.

	LOQ [ng L^{-1}]	Fyris Upstream	Fyris Down-stream	Lake Ekoln	Lake Görväln	DWTP Intake	DWTP Sand Filter	DWTP GAC	DWTP Drinking Water	DW School	DW Supermarket	Pilot NF	Pilot NF + old GAC	Pilot NF + new GAC
Albendazole-sulfone	<0.91	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.3	(0.35)[*]
Atenolol	<0.27	26	20	5.2	0.71	<LOQ	0.66	0.58	(0.22)[*]	0.70	0.86	<LOQ	<LOQ	<LOQ
Atrazine	<0.63	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Benzoylecgonine	<0.13	<LOQ	0.64	0.55	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.06
Bezafibrate	<0.67	<LOQ	1.99	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Caffeine	<3.0	32	38	19	<LOQ	6.8	<LOQ	6.5	8.0	7.2	6.9	7.7	4.3	<LOQ
Carbamazepine	<0.16	<LOQ	4.65	3.7	3.0	3.0	3.2	2.6	3.2	3.2	3.3	2.8	<LOQ	<LOQ
Cotinine	<0.38	2.9	6.0	5.1	4.4	4.1	4.6	4.7	4.6	4.1	4.9	5.0	1.1	<LOQ
Cyanazine	<1.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Diclofenac	<74	<LOQ	(26)[*]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Isoproturon	<0.23	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ketoprofen	<4.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Metoprolol	<0.22	1.4	40	15	3.2	3.0	3.7	3.2	1.7	3.5	3.7	1.1	<LOQ	<LOQ
Monensin	<0.07	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Nicotine	<1.1	4.2	11	3.3	1.2	1.3	1.4	1.4	1.1	(0.48)[*]	(0.64)[*]	5.6	4.4	<LOQ
Quinmerac	<5.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Simazine	<1.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

[*]values in brackets are below LOQ; peaks were clearly visible, but the values cannot be quantified reliably.

Table 8: ENV concentrations [ng L^{-1}] of all contaminants; values in bracket are <LOQ.

	LOQ [ng L^{-1}]	Fyris Upstream	Fyris Down-stream	Lake Ekoln	Lake Görväln	DWTP Intake	DWTP Sand Filter	DWTP GAC	DWTP Drinking Water	DW School	DW Super market	Pilot NF	Pilot NF + old GAC	Pilot NF + new GAC
Albendazole-sulfone	<0.74	(0.35)*	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.91	0.85
Atenolol	<0.67	24	16	4.1	(0.43)*	(0.48)*	(0.43)*	(0.44)*	<LOQ	(0.51)*	(0.63)*	<LOQ	<LOQ	<LOQ
Atrazine	<0.71	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Benzoylecgonine	<0.14	<LOQ	0.68	0.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Bezafibrate	<0.58	<LOQ	1.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Caffeine	<4.5	16	16	9.3	<LOQ	(4.3)*	<LOQ	<LOQ	(1.9)*	<LOQ	<LOQ	(3.7)*	(2.0)*	<LOQ
Carbamazepine	<0.19	<LOQ	3.6	3.2	2.5	2.4	2.7	2.6	2.6	2.7	3.1	3.0	<LOQ	<LOQ
Cotinine	<0.57	1.9	3.9	3.0	2.5	2.8	2.6	3.2	3.3	3.1	3.4	3.8	0.94	<LOQ
Cyanazine	<1.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Diclofenac	<74	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Isoproturon	<0.25	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Ketoprofen	<3.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.7
Metoprolol	<0.22	0.85	28	10	2.3	2.2	2.2	2.2	0.95	2.5	2.7	6.5	<LOQ	<LOQ
Monensin	<0.08	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Nicotine	<1.6	5.0	15	3.9	1.9	2.4	1.6	1.7	(0.48)*	<LOQ	<LOQ	1.7	(1.1)*	<LOQ
Quinmerac	<28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Simazine	<1.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

*values in brackets are below LOQ; peaks were clearly visible, but the values cannot be quantified reliably.

Nicotine was present in almost every sample (Figure 13). The same is valid for cotinine, a metabolite of nicotine (Figure 14). The recoveries (HLB) were 68±12% for nicotine and 86±12% for cotinine. Both compounds showed a strong increase in concentration between the sampling points upstream and downstream. The concentration of both compounds decreased again in samples from lake Ekoln and lake Görväln but stayed comparably stable afterwards. Samples from the nanofiltration pilot plant (Pilot NF) showed an increase in concentration when compared to the intake water. In the samples from the nano pilot plant coupled to fresh granular activated carbon (Pilot NF + new GAC), the concentrations decreased to <LOQ.
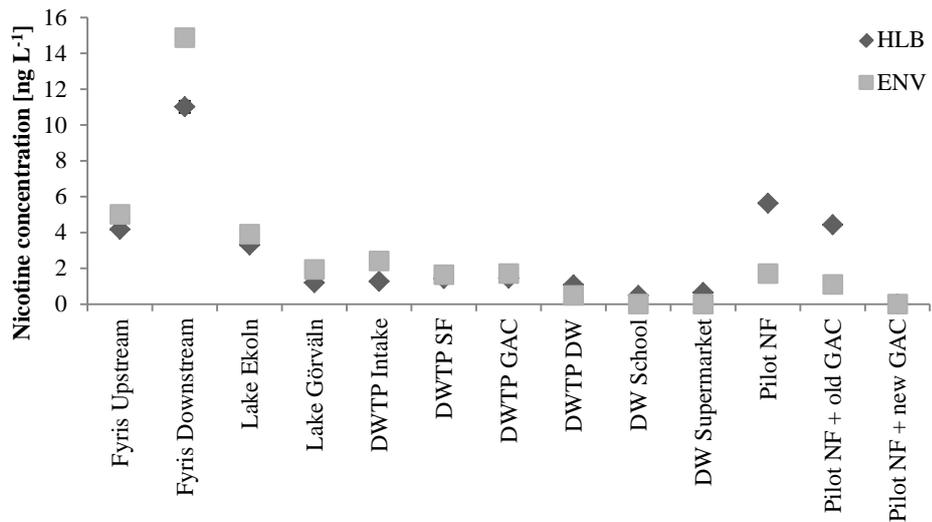
Figure 13: Nicotine concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.
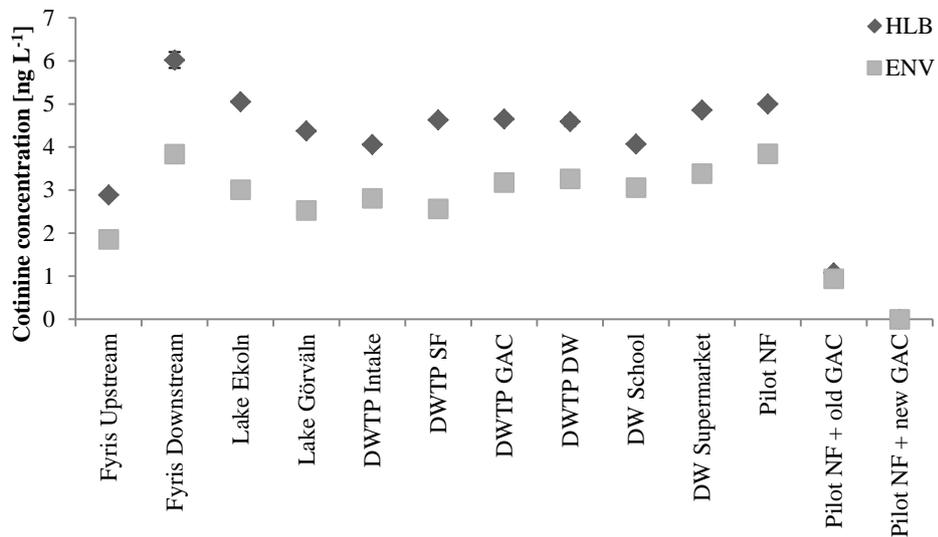
Figure 14: Cotinine concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.

Both atenolol (Figure 15) and metoprolol (Figure 16) are active ingredients in pharmaceuticals that are prescribed to prevent heart attacks. They belong to the group of beta blockers. The recovery (HLB) for atenolol was 18±1.4%, for metoprolol 21±2.1%. Atenolol concentrations showed a high standard deviation between the triplicate measurements (downstream) and is the only compound with a higher concentration upstream than downstream. Both compounds decreased in concentration between the sampling points downstream and Lake Görväln and remained constantly low throughout the sampling points in Lake Görväln, the treatment plant as well as in tap water samples.
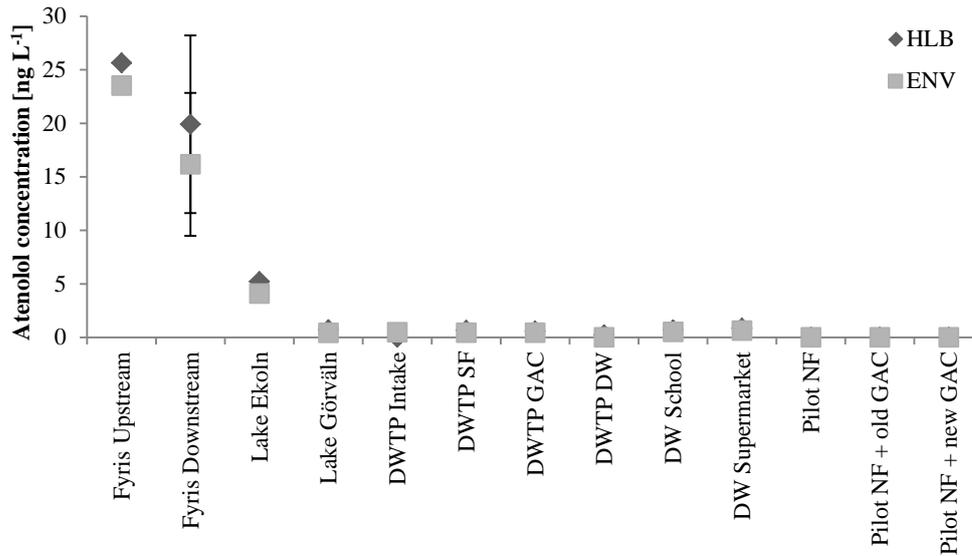
Figure 15: Atenolol concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.
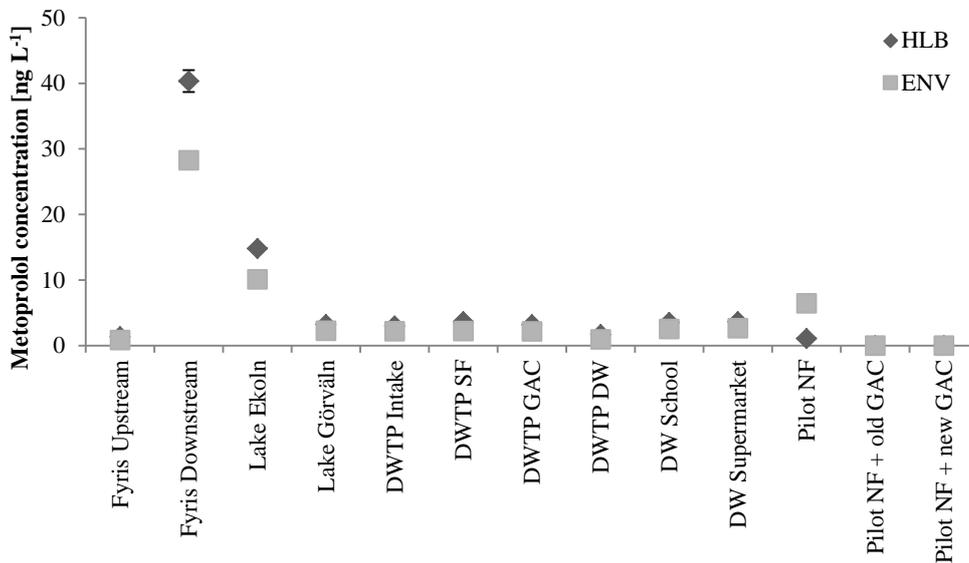
Figure 16: Metoprolol concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.

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The two pharmaceuticals carbamazepine (Figure 17) and bezafibrate (Figure 18) were found in concentrations below 5 ng L^{-1}. The recoveries (HLB) were 47±3.6% for carbamazepine and 91±7.4% for bezafibrate. Carbamazepine is used in the treatment of epilepsy, shizophrenia, bipolar disorder and neuropathic pain. Bezafibrate works as a blood lipid regulator. While bezafibrate was only detected at one sampling site (Fyris Downstream), carbamazepine appeared in almost every sample. The latter showed a similar concentration pattern as e.g. cotinine: a strong increase in concentration at the downstream sampling point was followed by a decrease in concentration in the lake samples while staying comparably constant throughout the treatment steps, disappearing only in the pilot plant coupled to GAC.
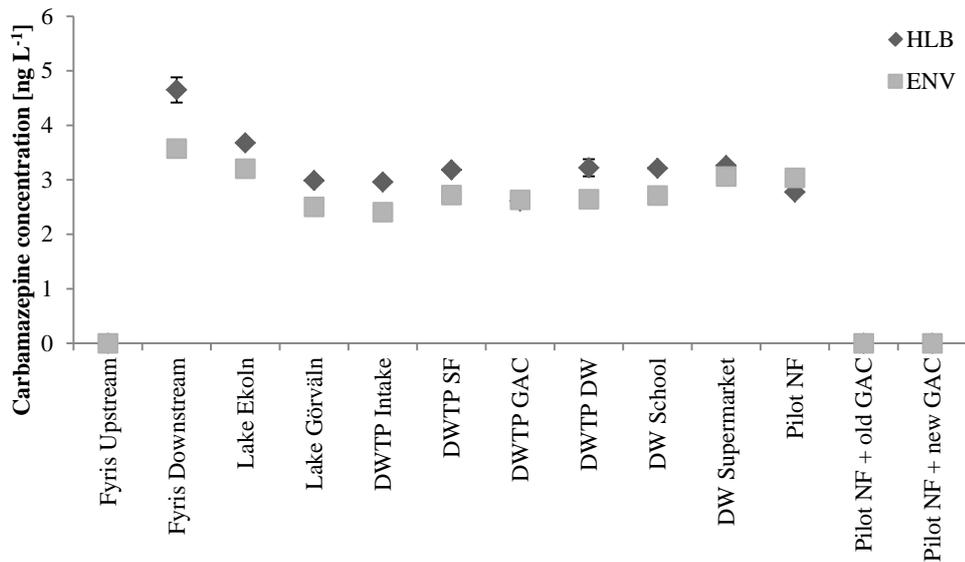
Figure 17: Carbamazepine concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.
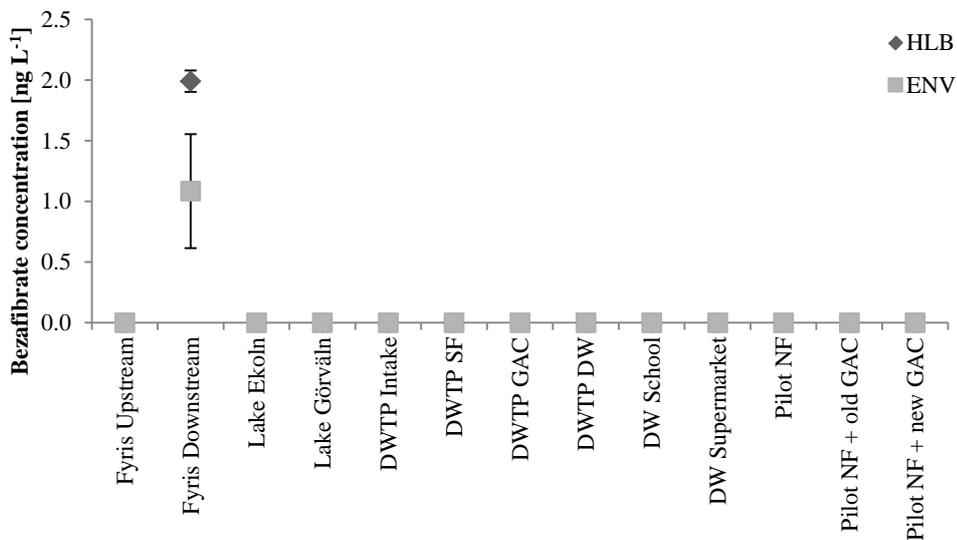
Figure 18: Bezafibrate concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.

Neither benzoylecgonine nor caffeine belong to the group of pharmaceuticals or pesticides, but still are often included with the PPCP (pharmaceuticals and personal care products). Benzoylecgonine (Figure 19) had a recovery (HLB) of 71±7.2% while caffeine (Figure 20) had a recovery of 70±8.8%. Benzoylecgonine is a metabolite of the illegal drug cocaine and could only be detected in low concentrations (<1 ng L^{-1}). It was only detected in the downstream river water and the first lake sample (Ekoln). Caffeine however was among the compounds of which the highest concentrations were detected. Caffeine concentrations reached 38 ng L^{-1} in the downstream river water. The concentration distribution appears to be similar as described for cotinine and carbamazepine: increasing in concentration downstream of Uppsala, but decreasing again with the lake samples while remaining constant throughout different treatment steps in the treatment plant.
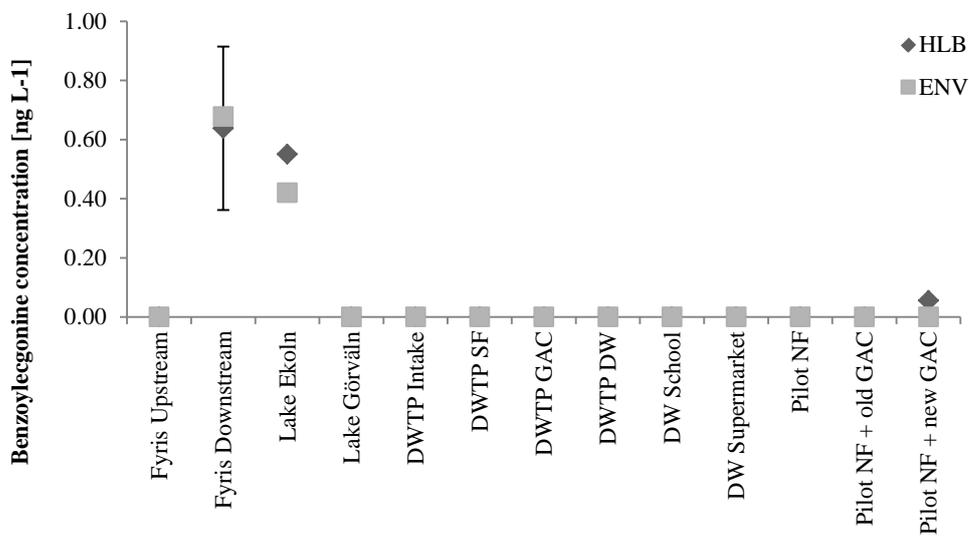
Figure 20: Benzoylecgonine concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.
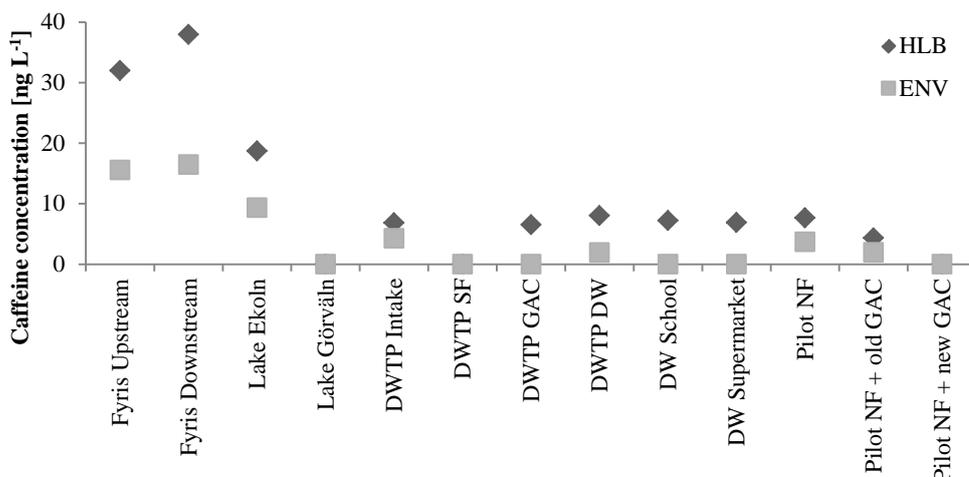
Figure 19: Caffeine concentration [ng L^{-1}] at all sampling points; values <LOQ were set to zero.

Figure 21 shows a comparison of measured concentrations of different compounds in treated drinking water at the treatment plant and the downstream river water. Out of 9 compounds that were detected in downstream river water, 6 could still be detected in drinking water in concentration up to 8.0 ng L^{-1} (caffeine). A strong difference in concentrations can be seen, when compared to downstream river water.
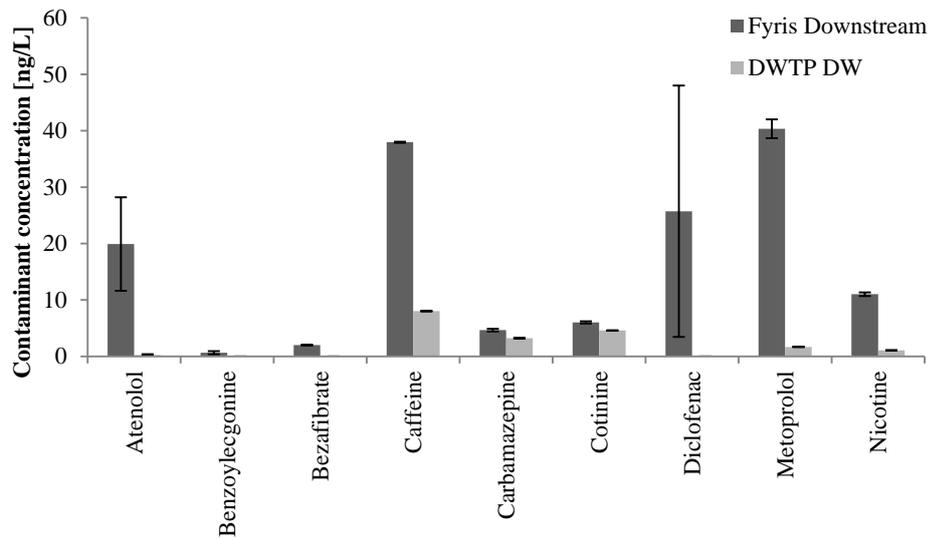
Figure 21: Direct comparison between contaminant concentrations downstream and in treated drinking water at the treatment plant. Error bars represent standard deviations of the replicates.

A composition profile for the water at all sampling points can be found in Figure 22. This graph shows the relative composition of each sample, expressed as percentage of all contaminants measured. It has to be emphasized that no absolute values can be derived from such graphic. Caffeine, metoprolol, nicotine, cotinine and carbamazepine were present in most samples and were not removed effectively by the treatment plant. Although their total concentration decreased, the proportions stayed constant. Atenolol had a higher share in the composition upstream of Uppsala and became less present throughout later sampling points (compared to other compounds). The sample "Pilot NF + new GAC" was the only sample that showed a completely different composition than the other samples.

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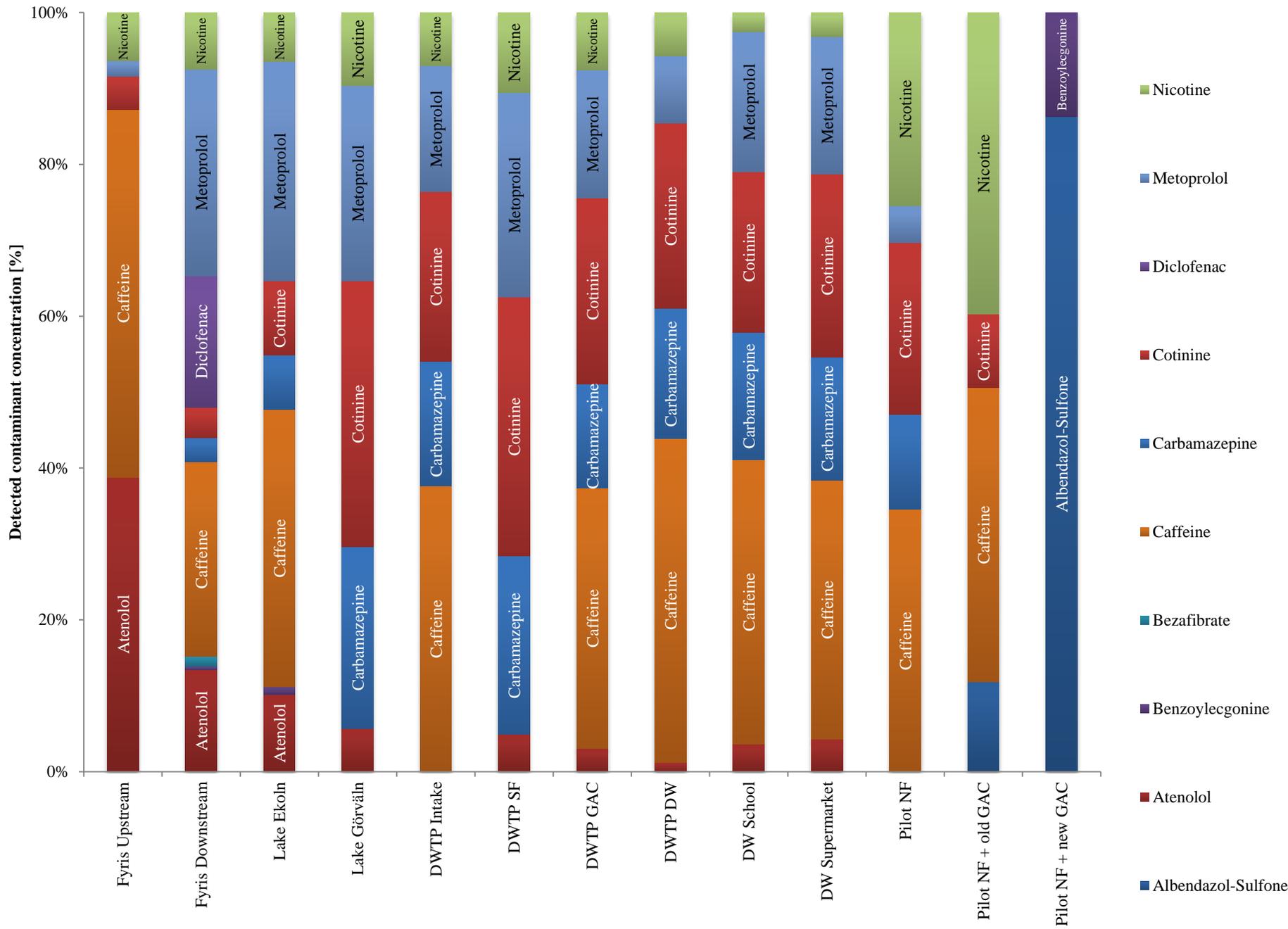
Figure 22: Composition profiles of all sampling sites. Atrazine, cyanazine, isoproturon, ketoprofen, monensin, quinmerac and simazine were not detected.

5.3   Efficiency of the treatment steps in the drinking water treatment plant and the pilot plant

The drinking water treatment plant was not able to remove all compounds that were detected in environmental samples. Even in treated drinking water, some compounds were still detected. Figure 23 and Figure 24 show the concentrations after different treatment steps, normalized to intake water, which are depending on the removal efficiency of the full-scale drinking water treatment plant and the pilot plant with membrane technology. The graphs show what fraction of the measured concentration in the intake water is present after several filter steps. A value higher than 100% would suggest a formation inside the treatment plant. This is unlikely, and alternative explanations are discussed later. Neither the full-scale treatment plant nor the nanofiltration treatment (without GAC) in the pilot plant was able to remove the compounds successfully. Only when coupling nanofiltration treatment to new granular activated carbon, compounds were completely removed from the water.
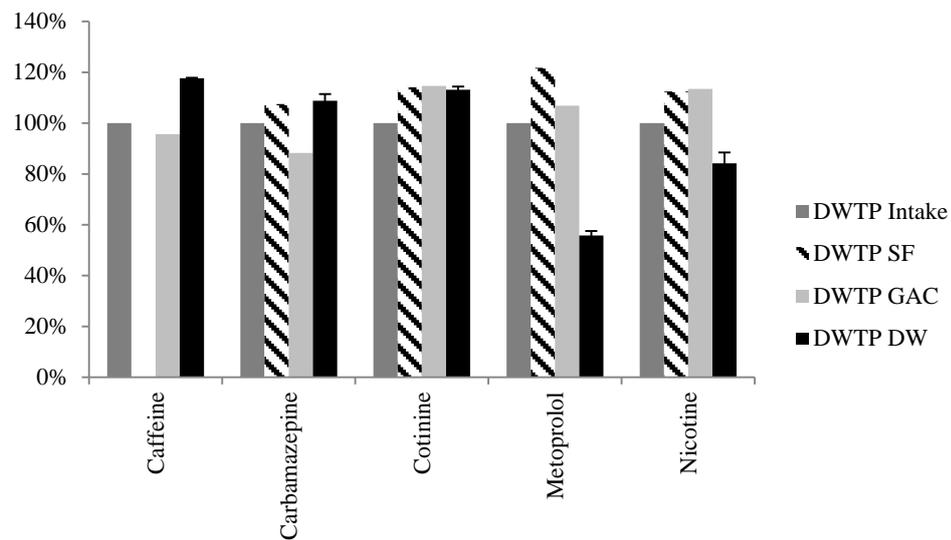
Figure 23: Concentrations normalized to intake water in the full scale treatment plant.
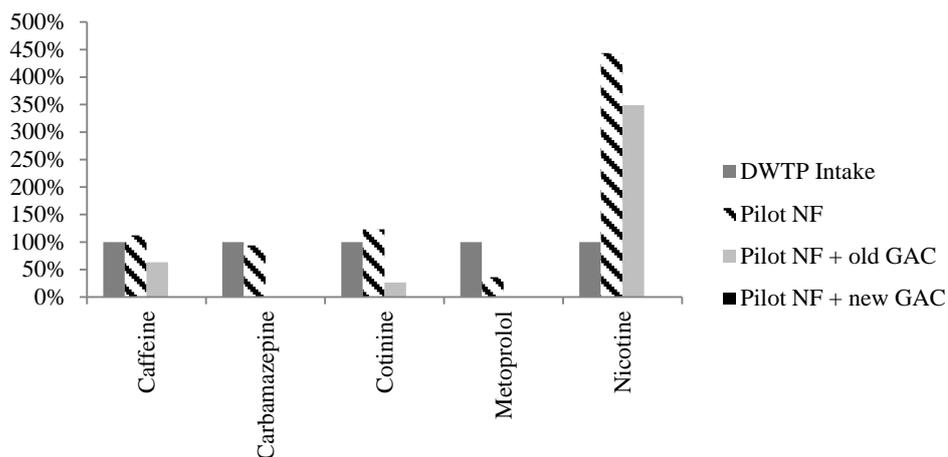
Figure 24: Concentration normalized to intake water in the pilot plant.

5.4 Risk quotient MEC/PNEC and comparison between common doses and daily human intake

Drinking water is our main food stuff with a consumption of 2.0-2.5 L day^{-1} for adults (including water used for cooking, hot beverages etc.; Westrell et al., 2006). In order to estimate whether the detected concentrations pose a risk to humans or the environment, risk quotients (RQ) were calculated for the detected compounds. The risk quotient is a ratio between the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC), see also Equation 4. The PNEC values were derived from literature data. If RQ >1, a potential risk for aquatic organisms can be assumed, if RQ <1, the compound is assumed to be harmless at the measured concentration.

$$RQ = \frac{MEC}{PNEC}$$

Equation 4: Calculation of risk quotients (RQ) with the help of measured environmental concentrations (MEC) and predicted no-effect concentrations (PNEC).

A summary of MEC, PNEC and RQ can be found in Table 9. PNEC values were derived from literature data. In previous studies, PNEC values were calculated from ecological structure activity relationships (ECOSAR) models (van der Aa et al., 2013), calculated from no observable effect concentrations (NOEC) tested with aquatic organisms (Komori et al., 2013), estimated from toxicity assays (Deo and Halden, 2013) or derived from ecotoxicity tests with crustaceans (Cleuvers, 2005;

Savino and Tanabe, 1989; Valcárcel et al., 2011). Values in the present study were calculated for drinking water (DW) from the DWTP and downstream river water. Drinking water is consumed daily; therefore the risk estimation for drinking water is the most relevant to assess. The calculation of downstream water risk quotients gives estimates for potential environmental hazards.

Table 9: Predicted no-effect concentrations (PNEC), measured environmental concentrations (MEC) and calculated risk quotients (RQ) for detected compounds. PNEC values were derived from literature data and origin from modelling simulations and toxicity tests.

	PNEC [ng L^{-1}]	MEC (DWTP DW)	RQ (DWTP DW)	MEC (Fyris Downstream)	RQ (Fyris Downstream)
Atenolol	310000[a]	<LOQ	0	20	$6.4*10^{-5}$
Benzoylecgonine	4900[b]	< LOQ	0	0.64	$1.3*10^{-4}$
Bezafibrate	10000[c]	< LOQ	0	2.0	$2.0*10^{-4}$
Caffeine	5200[c]	8.0	$1.5*10^{-3}$	38	$7.3*10^{-3}$
Carbamazepine	250[c]	3.2	$1.3*10^{-2}$	4.7	$1.9*10^{-2}$
Cotinine	5200[d]	4.6	$8.8*10^{-4}$	6.0	$1.2*10^{-3}$
Diclofenac	10000[c]	< LOQ	0	26	$2.6*10^{-3}$
Metoprolol	7900[a]	1.7	$2.1*10^{-4}$	40	$5.1*10^{-3}$
Nicotine	2400[e]	1.1	$4.5*10^{-4}$	11	$4.6*10^{-3}$
∑RQ			0.016		0.040

[a] Cleuvers, 2005, [b] van der Aa et al., 2013, [c] Komori et al., 2013, [d] Deo and Halden, 2013, [e] Valcárcel et al., 2011

Risk quotients varied between $7.1*10^{-7}$ and $1.3*10^{-2}$ for drinking water and between $6.4*10^{-5}$ and $1.9*10^{-2}$ for downstream river water, i.e. they were much lower than 1. Even the sum of all risk quotients, ∑RQ, stayed well below 1 (0.016 for drinking water, 0.040 for downstream river water). That means, for the detected compounds no hazardous effect is assumed. However, it should be noted that these calculations neither include all compounds that might be present in the water, nor do they include effects of mixture toxicity. Furthermore, the PNEC values were derived for aquatic organisms and toxicity to humans can be very different. Therefore, the uncertainties of the presented risk quotients are high when applied to humans. Even if a safety factor of 100 would be applied, the RQ would still be below 1 for all compounds except carbamazepine.

An average consumption of two liters tap water per person and day is a conservative assumption for risk assessment, covering the upper percentiles of the population. This value is valid for different countries and people of different sex, age or health condition (Westrell et al., 2006). The total amount of contaminant that is

taken up within one year, assuming a consumption of two liters tap water per day, can be found in Table 10. Even if contaminant concentrations as high as in the downstream river water were assumed, the yearly uptake would be far lower than common doses.

Table 10: Calculated yearly uptake of contaminants assuming a consumption of 2 L drinking water per day; n.d.= not detected

	Drinking water	Downstream river water	Common doses
	Yearly uptake [mg]	Yearly uptake [mg]	
Atenolol	n.d.	0.015	25 – 100 mg tablets ([a])
Benzoylecgonine	n.d.	0.0005	~ 100 mg cocaine (nasal) ([b])
Bezafibrate	n.d.	0.0015	400 mg tablets ([a])
Caffeine	0.0059	0.028	100 mg/cup of coffee ([c])
Carbamazepine	0.0024	0.0034	100 – 400 mg tablets ([a])
Cotinine	0.0034	0.0044	1 mg nicotine per cigarette ([d])
Diclofenac	0.0000	0.019	50 – 100 mg tablets ([a])
Metoprolol	0.0012	0.030	25 – 200 mg tablets ([a])

[a]Drugbank, [b]Huestis et al., 2007, [c]MedlinePlus Medical Encyclopedia, 2015, [d]Benowitz and Jacob, 1984

6 Discussion

6.1 Method performance

In general, method performance was good, even though the recovery showed great variations between different compounds. See chapter 4.7.3 for an explanation of recovery. Considering the broad range of compounds that were included in the method, this is not surprising. While most compounds were recovered well, others achieved low recovery rates. Albendazol-sulfone, benzoylecgonine, bezafibrate, caffeine and cotinine were recovered at rates above 70%, which can be considered high. Values between 40% and 70% can still be considered satisfactory and were achieved for carbamazepine, cyanazine, ketoprofen, monensin, nicotine and simazine. Values below 40% were found for atenolol, atrazine, diclofenac, isoproturon, metoprolol and quinmerac. At this low level of recovery, quantification of the compounds lacks precision, but nonetheless allows detection and rough concentration estimations (Vieno et al., 2006).

When comparing recoveries to literature data, mostly values above 75% were achieved. For example atenolol: 81-106% (Castiglioni et al., 2005; Vieno et al., 2006); atrazine: 80-90% (Hildebrandt et al., 2008; Masiá et al., 2013); bezafibrate: 76% (Castiglioni et al., 2005); caffeine: 78% (Santos et al., 2005), carbamazepine 89-98% (Castiglioni et al., 2005; Metcalfe et al., 2003a; Santos et al., 2005); diclofenac: 78% (Santos et al., 2005); ketoprofen: 80% (Santos et al., 2005); metoprolol: 87-104% (Vieno et al., 2006); simazine: 80-90% (Hildebrandt et al., 2008; Masiá et al., 2013). However, these studies focused on specific compounds of the same classes whereas the presented work aimed at a broad screening of various different compounds. Technically, it is possible to detect the target compounds with better recoveries for each individual compound, but for the simultaneous detection of the described compounds, it is not possible to perform an optimization of the method and the recovery values were therefore considered acceptable. If the method was optimized for single compounds, for example by adding clean-up

steps or adjusting the pH during chromatography, other compounds could not be detected at the same run and multiple samples per site had to be analyzed.

The limit of quantification (LOQ) for most compounds was below 2 ng L^{-1}. Only few compounds had higher LOQ values: caffeine, diclofenac, ketoprofen and quinmerac. In some cases, the measured concentration in the sample was lower than the calculated LOQ. They were presented in brackets in Table 7 but not included in the data evaluation. Comparing the calculated LOQ values to LOQs from literature data reveals that the presented method was able to detect compounds at comparable levels. Vieno et al.(2006) demonstrated LOQs in drinking water of 6.5 ng L^{-1} for atenolol, 2.2 ng L^{-1} for metoprolol and 0.2 ng L^{-1} for carbamazepine. Masiá et al. (2013) showed LOQ values of 6.0 ng L^{-1} for simazine, 1.0 ng L^{-1} for isoproturon and 6.0 ng L^{-1} for atrazine. Another study calculated LOQ values of 1.07 ng L^{-1} for atenolol, 0.1 ng L^{-1} for bezafibrate, 1.3 ng L^{-1} for carbamazepine (Castiglioni et al., 2005). Generally speaking, the presented LOQ values are low, with few exemptions. Caffeine for example had a LOQ of 3.07 ng L^{-1}, but since it was detected in most samples at much higher concentrations, the higher LOQ is not a problem. The calculation of the LOQ of diclofenac (LOQ = 74 ng L^{-1}) was problematic, since it was not always detected even in spiked samples, and the recovery of diclofenac was very low. This is surprising, since it has been shown to be well extractable with HLB cartridges (Öllers et al., 2001). Quinmerac and monensin had LOQ values of 5.6 ng L^{-1} and 0.07 ng L^{-1} and have not been detected in any sample, yet a presence at very low concentrations cannot be excluded.

For some compounds, the peak identification with the Waters UNIFI software in the current version (v1.7) caused problems since it does not allow manual integration. Therefore, peaks were clearly visible but impossible to quantify. Examples are carbamazepine at the sampling site upstream or diclofenac in spiked samples and at the site downstream.

Concerning the differences between the two SPE cartridges, it was shown that HLB cartridges performed significantly better than ENV cartridges with respect to the detected compounds. The method recovery of target analytes was on average 25% better with HLB cartridges than with ENV. Therefore, the use of just HLB cartridges but with several repetitions per sample should be considered for future studies. This would increase reliability of the collected data and would allow statistical analysis including variances.

6.2 Comparison of detected concentrations with literature data

The concentrations detected in the current study are low when compared to data from previous studies (see Table 11). Some compounds were within the ranges of the data presented in literature (atenolol, carbamazepine, cotinine, metoprolol), Others were well below, between 64% to 99% lower than the values presented in the literature (benzoylecgonine, bezafibrate, caffeine, diclofenac, nicotine). It can be concluded that the contamination of surface waters in the region of Uppsala is low for the presented compounds. However, it is important to stress that:

- other contaminants are likely to be present,
- degradation of compounds might lead to the creation of more hazardous secondary molecules,
- mixture toxicity can lead to unpredictable, toxic effects, and
- effects on biota in water can be severe due to continuous exposure and cumulative effects as well as multigenerational exposure (Halling-Sørensen et al., 1998).

Table 11: Comparison of detected concentrations with literature data. (n.d. = not detected).

Compound	Fyris Downstream [ng L^{-1}]	DWTP DW [ng L^{-1}]	Literature data (surface water) [ng L^{-1}]	Reference
Albendazol-Sulfone	n.d.	n.d.	not available	
Atenolol	20	n.d.	11.8 - 25	Vieno et al., 2006
Atrazine	n.d.	n.d.	4.6 - 18.6	Masiá et al., 2013
Benzoylecgonine	0.64	n.d.	1 - 16	van der Aa et al., 2013
			26.8	Baker and Kasprzyk-Hordern, 2011
Bezafibrate	2.0	n.d.	350	Ternes, 1998
Caffeine	38	8.0	105	Kim et al., 2007
Carbamazepine	47	3.2	1.4 - 66	Vieno et al., 2006
Cotinine	6.0	4.6	4 - 14	Metcalfe et al., 2003b
Cyanazine	n.d.	n.d.	50-810	Rebich et al., 2004
Diclofenac	26	n.d.	150	Ternes, 1998
Isoproturon	n.d.	n.d.	280 (max. conc)	Kreuger et al., 2010
Ketoprofen	n.d.	n.d.	120	Ternes, 1998
Metoprolol	40	1.7	3.8 - 116	Vieno et al., 2006
Monensin	n.d.	n.d.	n.d. - 37	Song et al., 2007
Nicotine	11	1.1	32.3	Baker and Kasprzyk-Hordern, 2011
Quinmerac	n.d.	n.d.	90 (max. conc.)	Kreuger et al., 2010
Simazine	n.d.	n.d.	n.d. - 48	Masiá et al., 2013

The concentrations of the detected compounds follow the pattern that was expected. Upstream of Uppsala, 5 compounds were detected in low concentrations, a result of effluents from small-scale municipal and private wastewater treatment

systems. Although the area surrounding the sampling site is characterized by agriculture, no pesticides were found. This is either due to no or low use of the target pesticides at the sampling time, a degradation of the pesticides into breakdown products, or the successful prevention of pesticide leakage. Furthermore, some pesticides like cyanazine and atrazine are banned from the market and not in use anymore today. They were included because they could still be found in other areas. The sampling took place in spring; therefore some pesticides might be applied later in the year, at different growing stages and seasons. Downstream of Uppsala, shortly after the effluent of the municipal wastewater treatment plant enters the stream, a total of 9 contaminants was detected. All of the compounds detected in upstream river water were still present. Downstream, concentrations were highest for almost all of the detected compounds (only exception: atenolol). Pharmaceuticals and personal care products consumed in the city of Uppsala were not efficiently removed by the wastewater treatment plant and entered the river with the effluent (Daneshvar et al., 2009). Concentrations were expected to decrease again at the next sampling point at Lake Ekoln. Here, dilution can be regarded as the most important factor. The river water enters the lake and gets diluted by about 40% in average in the lake water (Nõges et al., 2007). The compounds are probably not removed or degraded yet, but rather watered down. In the case of benzoylecgonine, the concentration fell below the LOQ. For other compounds, a decrease in concentration could be observed. On average, the concentrations were reduced by 51% between the site downstream and Lake Ekoln. At the sampling point in Lake Görväln, concentrations decreased even more. Compared to Lake Ekoln, concentrations in Lake Görväln are reduced by 66% on average. Further dilution is one reason for this decrease. However, since the sampling points are quite far from each other (~50 km), degradation mechanisms gain importance. Phototransformation and biotransformation may lead to the breakdown of the original compound into smaller fragments, sorption to particles or sediments can cause the retention of contaminants (Daneshvar et al., 2009). Furthermore, it is important to mention that the composition profile changed between the sampling location at Ekoln and Görväln. The different concentration and composition profile is most likely due to the fact that only a smaller fraction of the water at Görväln (about 25%) comes actually from Lake Ekoln. The other 75% come from other parts of Lake Mälaren, the lake to which both Görväln and Ekoln belong to (Wallin et al., 2000). It is important to mention that the water of Lake Ekoln and at Lake Görväln differ with regards to their chemistry. The water of Lake Ekoln has a higher density and therefore sinks and accumulates in deeper layers when entering Lake (Görväln Köhler, S. 2015). Therefore, the water that is taken up from the DWTP at a depth of 22m is likely to be mainly water from Lake Ekoln (Köhler, S. 2015).

Within the drinking water treatment plant at Lake Görväln, concentrations remained relatively constant. For some compounds, they even seemed to increase (caffeine, cotinine, nicotine). A magnification of pharmaceuticals and personal care products within the treatment plant is highly unlikely, the higher concentrations are probably detected due to reduced ion suppression and matrix effects (Cullum et al., 2004) since the water is cleaner. In water samples with many matrix compounds, these compete with the target analytes to get a charge during ionization. The targeted compounds get suppressed by other compounds that are present in the sample. As a result, fewer are ionized and therefore not detected in the mass spectrometer. When the water is cleaner, more of the suppressing matrix compounds have been filtered out and suppression is lower. This makes the detection of target analytes easier and detected concentrations seem to be higher. This is also a possible explanation why albendazole-sulfone was only detected (<LOQ) in the samples from the pilot plant combined with GAC treatment, i.e. in the water that is expected to be the cleanest of all samples. The pesticide was not found in the samples "Intake", "DWTP SF" or "Pilot NF" although the water was going through these treatment steps before (see also chapter 4.2, Figure 6). It is highly unlikely that the pesticide entered the pilot plant between the nanofiltration step and the GAC. However, since the recovery of albendazol-sulfone was high (75%±9.3) and the LOQ was low (0.91 ng L^{-1}) it is surprising that it was not found in other samples. Either the detection of this compound was possible in the cleanest samples due to reduced suppression and matrix effects, or errors in measurement caused a false finding. It is important to mention, though, that albendazole-sulfone was found in a concentration below the limit of quantification. That means, the quantification is not reliable. The occurrence of albendazole-sulfone was only discussed here since the peak was clearly visible in the chromatogram. For future studies it would be recommendable to test recoveries with more matrix samples. As described, drinking water from the tap was used in this study to include matrix effects, since drinking water already contains matrix compounds. However, in order to account for the various samples, other matrices should be included as well, for example to derive direct recovery values for river water.

For the treated drinking water, compounds that were present at the DWTP showed the same concentration in the tap water sample. This shows that compounds do not undergo degradation in the water supply system, nor does contamination occur after the treatment.

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6.3 Treatment efficiency and treatment alternatives

Neither the full-scale treatment plant, nor the nanofiltration step in the pilot plant was able to successfully remove the target contaminants. This becomes clear when comparing the compounds that were still present in the intake water. All of the 5 compounds that were detected in the intake water are still present in samples from the full-scale treatment plant and the nanofiltration step (without GAC). Only when coupling the nanofiltration technology to a GAC treatment, 2 compounds could be removed with "old" GAC (in use for 5 months), while all of them were <LOQ after being treated with "new" GAC (in use for 1 week). This shows that the pilot plant with nanomembrane technology alone had almost no impact on contaminant concentrations. Only when coupled to granular activated carbon (GAC), concentrations decreased; therefore the removal of the target compounds was therefore most likely only performed by the carbon treatment step. In the full-scale treatment chain is also a carbon treatment included; however, it is exchanged less often and its performance might have been reduced at the time of sampling.

The average contaminant removal in the full-scale treatment plant was 4%; the only compound reduced in concentration was metoprolol with a reduction of about 40% (full-scale). The other compounds remained more or less unchanged (Figure 23, Figure 24). Conventional drinking water treatment plants with a combination of flocculation, sedimentation and sand filtration mechanisms have already before been shown to be ineffective in the treatment of organic micropollutants (Sarkar et al., 2007).

Nicotine appeared to increase strongly in the pilot plant. Since magnification of nicotine within the plant is unlikely, reduced matrix effects probably lead to a better detection of the target compound. The good removal efficiency of activated carbon for many pesticides, pharmaceuticals and other synthetic organic compounds (SOC) has been attested by several other studies. However, GAC is no guarantee for the efficient removal of all SOCs (Ivančev-Tumbas, 2014). While some membrane filtration studies were effective in contaminant removal, there have been studies that confirm the low removal rates of the nanofiltration (without GAC) that were presented (Ivančev-Tumbas, 2014). It can be concluded that the membrane technology that is currently tested in the pilot plant is not an appropriate option for a full-scale solution, since it has not successfully removed the contaminants in question.

Other studies showed that pharmaceuticals are poorly removed from water during the drinking water treatment process by the means of sand filtration or flocculation with iron(III)-chloride. However, they were successfully removed with granular

activated carbon (GAC) or ozonation (POSEIDON, 2004; Ternes et al., 2002). One study revealed that four tested pharmaceuticals in water matrices could be removed by treatment with ozone (O_3) or ozonation combined with hydrogen peroxide (H_2O_2). The removal of pharmaceuticals by ozonation was also shown by Rosal et al. (2010), where some personal care products remained in the water, but pharmaceuticals were removed successfully. It was also shown that UV radiation in combination with titanium dioxide (TiO_2) or ozone (O_3) and TiO_2 is able to remove some pharmaceuticals from water (Benitez et al., 2011). However, it has to be remembered that during disinfection steps in drinking water treatment with oxidation reagents like chlorine, ozone, chloramine, etc., so-called disinfection by-products (DBPs) can be formed that might have severe genotoxic or carcinogenic effects (Richardson and Ternes, 2014). One example presented in Richardson and Ternes, 2014, was the formation of toxic N-nitrosodimethylamine (NDMA) after the reaction of a fungicide with ozone. The treatment of PPCPs with chlorine disinfection is not as efficient and leads to unknown transformation products (POSEIDON, 2004).

Although no pesticides were found at quantifiable levels in this study, possible options to reduce pesticide loads in environmental water sources are outlined shortly here. Gerecke et al. (2002) indicated that proper handling of farming equipment can reduce pesticide input into the environment dramatically. Teaching and information programs will train farmers for proper pesticide management. Furthermore, regulations for pesticides in urban environments should be reviewed due to the adhering long-term effects. Sarkar et al. (2007) were able to remove isoproturon successfully with an approach based on powdered activated charcoal (PAC) sorption and consecutive nanofiltration. Ozonation is most likely able to remove pesticides as well, since the ozone molecule is able to react with a broad range of organic compounds (Rosal et al., 2010). Preoxidation with chlorine combined with coagulation was a reliable method examined by Ormad et al. (2008) for pesticide removal from drinking water. Preoxidation with ozone (without combined coagulation) was found to be successful for most pesticides as well, while a treatment with coagulation alone was least efficient. Lastly, preoxidation with ozone and subsequent treatment with activated carbon removed almost all pesticides from the water (Ormad et al., 2008).

Another approach than removing the contaminants in the drinking water treatment plant would be to prevent them from entering the environment in the first place, i.e., increasing the removal efficiency of the wastewater treatment plant (Benitez et al., 2011). Ozonation is common and repeatedly recommended in drinking water treatment but can as well be applied in wastewater treatment, since it is able to

remove micropollutants (Rosal et al., 2010). For most PPCPs, only advanced technologies like ozonation or activated carbon lead to successful treatment. Sludge-retention time is important for the biological degradation of PPCPs; increasing the duration of this time can be a measure to reduce PPCPs loads (POSEIDON, 2004). In a study of Schaar et al. (2010), contrast media (pharmaceuticals used in X-ray chromatography) could not successfully be removed from wastewater; however, most of the examined compounds were removed by ozonation.

6.4   Risk quotient, human health hazards and mixture toxicity

The study aimed at providing information about contaminant concentrations from source to tap. It is therefore of primary interest, which contaminants can be found in treated drinking water or tap water and also to investigate whether these pose a risk to human health. The calculation of risk quotients is a common measure (Cleuvers, 2005; Gabet-Giraud et al., 2014; van der Aa et al., 2013) for risk evaluation of contaminants. The concentrations found in the drinking water samples were low and do not pose a risk to human health even when consumed on a daily basis. The risk quotients as well as the sum of risk quotients were far below 1, indicating that there is no direct hazard from the individual compounds. The same is valid for the concentrations in the most polluted sample (Downstream). However, these calculations do not include mixture toxicity effects, nor do they include the various other compounds that might be present in the water samples. The application of risk quotients for the assessment of risks for humans is common but questionable since the PNEC values were derived from tests with aquatic organisms. Large insecurities can occur from the differences in exposure between humans and aquatic organisms. However, the risk quotients calculated are so low that no risks for humans are expected.

The estimation of risk quotients with PNEC values has been criticized. On the one hand, the parameters established depend strongly on the quality and quantity of collected data for toxic effects of single compounds. Differences between field and lab conditions can hardly be accounted for. Huge variations in PNEC values for individual contaminants can be found in the literature. Furthermore, contaminants rarely occur as single contaminants in the environment. Rather, a mixture is present and the PNEC value cannot account for mixture effects (Chèvre et al., 2006). Chèvre et al. (2006) suggest a model based on species sensitivity distributions (SSDs) to calculate risk quotients for pesticides, allowing for the inclusion of mixture effects. However, this approach is only valid for substances with similar modes of action and could not be applied to calculate effects that result from for example (theoretical) interactions between pharmaceuticals and pesticides.

The addition of concentrations can overestimate mixture toxicity but can be seen as a kind of realistic worst-case scenario, It was shown to be appropriate for hazard assessment of defined chemical mixtures, but even more for compounds of which availability of information on toxicity or molecular mechanisms was restricted (Backhaus et al., 2000). It should also be noted that even worse cases can exist because of the limitation of including target analytes only.

7 Conclusion and outlook

The current study aimed at analyzing water samples for investigating the occurrence of a range of pesticides, pharmaceuticals and personal care products. Compared to other studies, the range of compounds analyzed was quite narrow - therefore, only limited information about the contamination situation in surface water and drinking water from Uppsala can be drawn. However, the calibration solutions prepared for the analysis contained a number of other compounds. Therefore, a deeper analysis of the samples (including more compounds) is possible and will provide more information.

The main findings of this study show that surface water in Uppsala is contaminated with low levels of pharmaceuticals and personal care products. Contaminants were found to remain present throughout the sampling sites. For most compounds, concentrations were lower before the river Fyris passed Uppsala (upstream). The main source of the targeted compounds was found to be the WWTP effluent. This was known prior to the study (Daneshvar, 2012); however, the tracking of contaminants of different groups from source to tap was not performed before in this area. After the effluent of the communal WWTP entered the stream (downstream), concentrations raised to levels of up to 38 ng L^{-1} (caffeine). In the downstream river water, concentrations were highest for almost all compounds, the only exception being atenolol. Concentrations decreased in the two lake samples (Lake Ekoln and Lake Görväln) due to dilution effects. Within the DWTP, concentrations remained stable and contaminants were not removed efficiently with the conventional treatment techniques of flocculation, sand filtration, GAC and disinfection with UV light and chloramine. Out of a total of 17 contaminants analyzed, 6 could be found in drinking water (atenolol, caffeine, carbamazepine, cotinine, metoprolol, nicotine). The full-scale DWTP was neither able to remove these compounds effectively, nor to reduce their concentrations. The pilot-scale DWTP currently tested was ineffective as well, unless coupled to an activated carbon treatment step. Without carbon treatment, 5 of the target analytes could be detected in treated water from

the pilot plant. When coupled to new GAC, all targeted compounds were removed successfully.

The occurrence of contaminants in drinking water was a known issue, and it seems that the search for an appropriate and cost-effective treatment method for drinking water is not completed, since the tested nanofiltration technique performed unsatisfactorily. The use of GAC is effective but expensive, therefore alternatives are needed. It has to be emphasized, that with the current knowledge about these contaminations, hazards are neither expected for humans nor aquatic organisms. This was shown by calculating risk quotients for individual compounds based on PNEC values. The summarized RQ values ($\sum$RQ) of the detected contaminants were 0.016 for drinking water and 0.040 for the most polluted environmental sample, indicating that no hazard results from the examined water samples.

For the compounds selected, the used analytical methods worked satisfactorily. One suggestion presented was the use of only one extraction cartridge while taking more repetitions per sampling point for the inclusion of variations in the dataset. As the method was developed for a broad compound range screening, future projects will shed light on possible contaminations with other compounds than those targeted for in the current study. It also has to be mentioned that the samples in this study were taken as grab samples, i.e. they only represent the contamination situation of a short time window. Hence it would also be interesting to see the results of future studies, for example with samples from different seasons than in the current study.

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10 Appendix

10.1 Appendix A

Coordinates of sampling points and map of the sampling sites

Table 12: Coordinates of sampling points.

| Sampling point	Coordinates (Decimal Degrees; WGS84)
Fyris Upstream	60.011027 17.717783
Fyris Downstream	59.831660 17.661302
Lake Ekoln	59.757547 17.638128
Lake Görväln / DWTP	59.417038, 17.753173
School	59.417694 17.791111
Supermarket	59.726361 17.789361
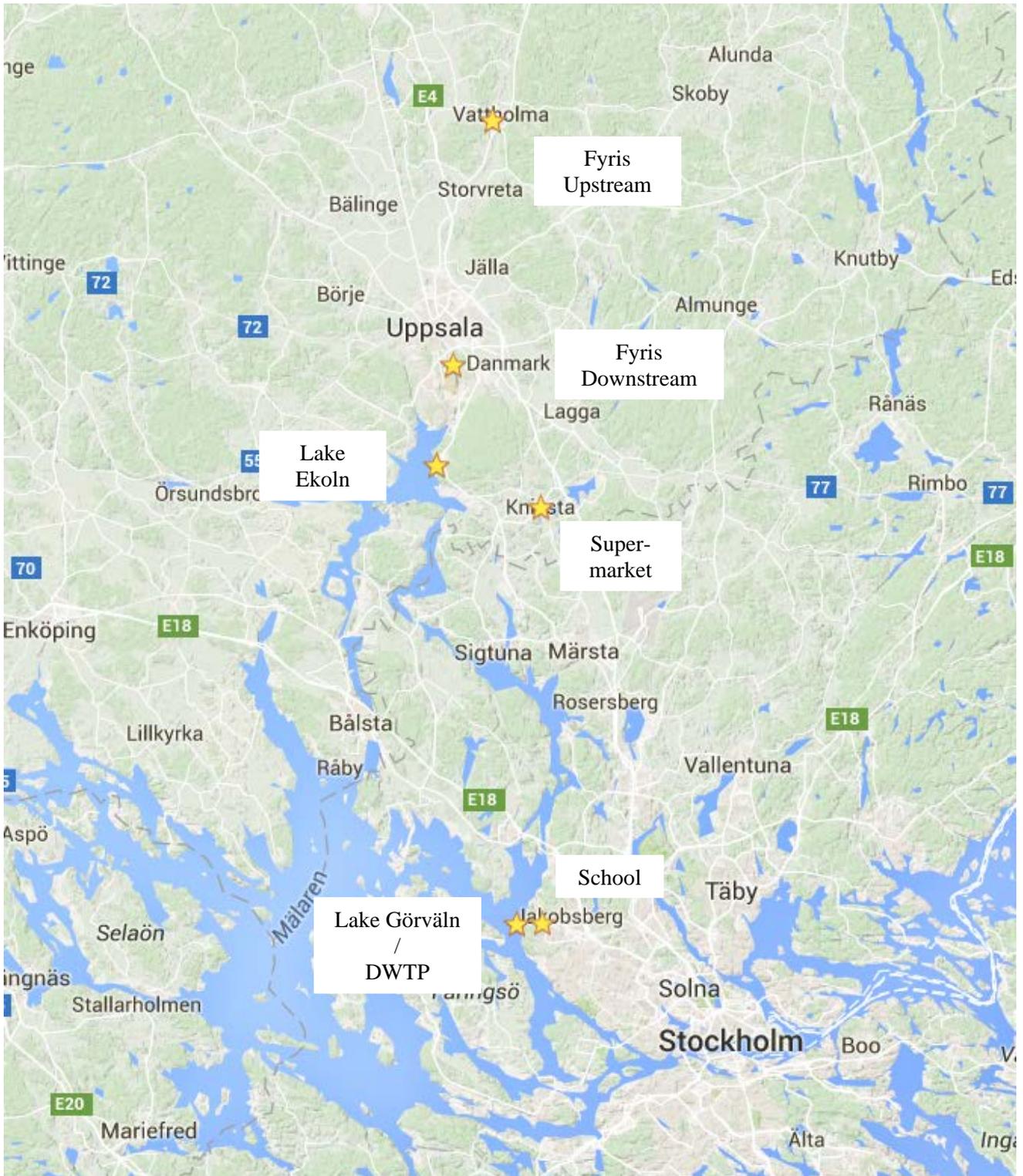
Figure 25: Map with sampling points. Source: Google Maps.

10.2   Appendix B

Table 13: List of compounds in the calibration solution; compounds examined in this study are highlighted.

Pesticides:

2,4-D	Diklorvos	Hexytiasox	Pendimetalin	Triflusulfuronmetyl
Acetamiprid	Dikofol	Imazalil	Penkonazol	Trinexapak-etyl
Aklonifen	Dimetoat Diuron	Imidakloprid	Permetrin	Trinexapak-syra
Alaklor	Endosulfan-alfa	Iprodion	Pikloram	Tritikonazol
Aldrin	Endosulfan-beta	**Isoproturon**	Pikoxystrobin	Vinklozolin
Alfa-cypermetrin	Endosulfansulfat	Jodsulfuronmetyl	Pirimikarb	
Amidosulfuron	Epoxikonazol	Karbendazim	Prokloraz	
Amisulbrom	Esfenvalerat	Karbofuran	Propamokarb	
AMPA	Etofumesat	Karfentrazonetyl	Propikonazol	
Atrazin	Fenitrotion	Karfentrazonsyra	Propoxykarbazon	
Atrazindesetyl	Fenmedifam	Klomazon	Propyzamid	
Atrazindesisopropyl	Fenpropidin	Klopyralid	Prosulfokarb	
Azoxystrobin	Fenpropimorf	Klordan-alfa	Protiokonazol-destio	
Beta-cyflutrin	Flamprop	Klordan-gamma	Pyraklostrobin	
BAM	Florasulam	Klorfenvinfos	Pyroxsulam	
Benazolin	Fluazinam	Kloridazon	Quinoxyfen	
Bentazon	Fludioxonil	Klorpyrifos	Rimsulfuron	
Bifenox	Flupyrsulfuronmetyl	Klotianidin	Siltiofam	
Bifenox-syra	Fluroxipyr	Kvinmerak	**Simazin**	
Bitertanol	Flurprimidol	Lambda-cyhalotrin	Spiroxamin	
Boskalid	Flurtamon	Linuron	Sulfosulfuron	
Cyanazin	Flusilazol	Mandipropamid	Taufluvalinat	
Cyazofamid	Flutriafol	MCPA	Terbutryn	
Cybutryn	Foramsulfuron	Mekoprop	Terbutylazin	
Cyflufenamid	Fuberidazol	Mesosulfuronmetyl	Terbutylazindesetyl	
Cyflutrin	Glyfosat	Metabenztiazuron	Tiakloprid	
Cykloxidim	HCH-alfa	Metalaxyl	Tiametoxam	
Cyprodinil	HCH-beta	Metamitron	Tifensulfuronmetyl	
Cypermetrin	HCH-delta	Metazaklor	Tiofanatmetyl	
Deltametrin	HCH-gamma	Metiokarb	Tolklofosmetyl	
Difenokonazol	Heptaklor	Metolaklor	Tolylfluanid	
Diflufenikan	Heptaklorepoxid	Metrafenon	Tribenuronmetyl	
Diklobenil	Hexaklorbensen	Metribuzin	Trifloxystrobin	
Diklorprop	Hexazinon	Metsulfuronmetyl	Trifluralin	

Pharmaceuticals:

Acetaminophen	**Diclofenac**	Naproxen
Amitryptiline	Diltiazem	Oxazepam
Amlodipine	Enalapril maleate	Propylparaben
Atorvastatin	Ethylparaben	Ramipril
Bezafibrate	Fluconazole	Saccharin
Bicalutamide	Furosemide	Sertraline
Budesonid	Hydrochlorothiazide	Simvastatin
Caffeine	Ibuprofen	Sucralose
Candesartan	Irbesartan	Tamoxifen
Cetirizine	Ketoconazole	Terbutaline
Citalopram	**Ketoprofen**	Valsartan
Climbazole	Lamotrigine	Venlafaxine
Clopidogrel hydrogensulfat	Lidocaine	Zolpidem tartrate
Desvenlafaxine	Losartan	
Diazepam	Methylparaben	

Fecal source tracing:

Albendazole sulfone	**Diclofenac**	**Monensin**
Albendazole sulfoxide	Fenbendazole-sulfon	Narasin
Atenolol	Fenylbutazon	Naproxen
Atrazine	Flubendazole	**Nicotine**
Bentazone	Flubendazole, 2-amine	Paraxanthine
Benzoylecgonine	Fluorescent brightener 71 (DAS-1)	**Quinmerac**
Bezafibrate	Gemfibrozil	**Simazine**
Blankophor (DAS-2)	Ibuprofen	Toltrazuril
Caffeine	**Isoproturon**	Toltrazuril-sulfone
Carbamazepine	Ivermektin	Triclosan
Cotinine	**Ketoprofen**	Vedaprofen
Creatinine	Meloxicam	Nandrolone (17b)
Cyanazine	**Metoprolol**	

PFAA:

PFBA (perfluorobutanoic acid)

PFBS (perfluorobutane sulfonic acid)

PFDA (perfluorodecanoic acid)

PFDoDA (perfluorododecanoic acid)

PFHpA (perfluoroheptanoic acid)

PFHxA (perfluorohexanoic acid)

PFHxS (perfluorohexane sulfonic acid)

PFNA (perfluorononanoic acid)

PFOA (perfluorooctanoic acid)

PFOS (perfluorooctane sulfonic acid)

PFPeA (perfluoropentanoic acid)

PFUnDA (perfluoroundecanoic acid)

10.3 Appendix C

Extraction efficiencies and recoveries - graphical presentation
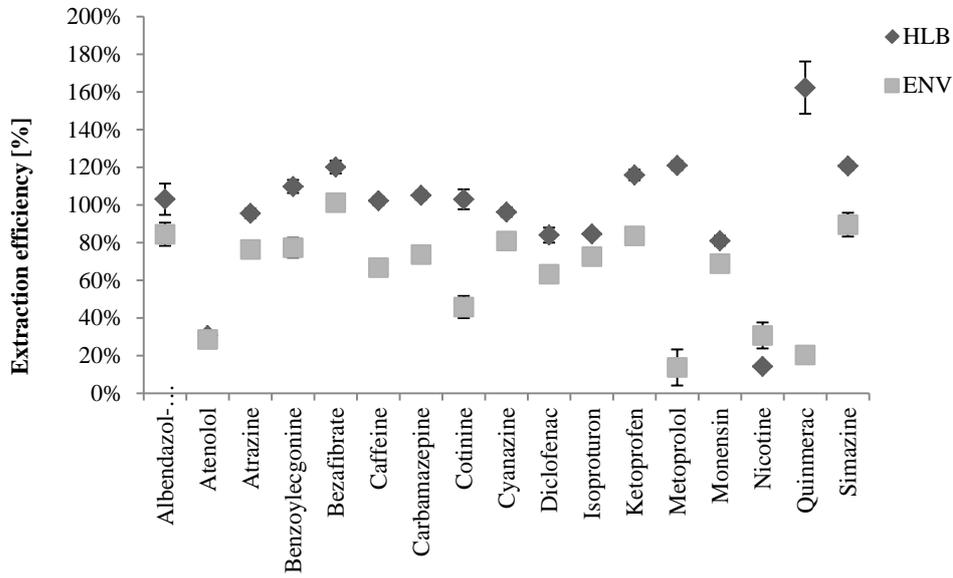
**Figure 26:** Extraction efficiency of HLB and ENV cartridges.
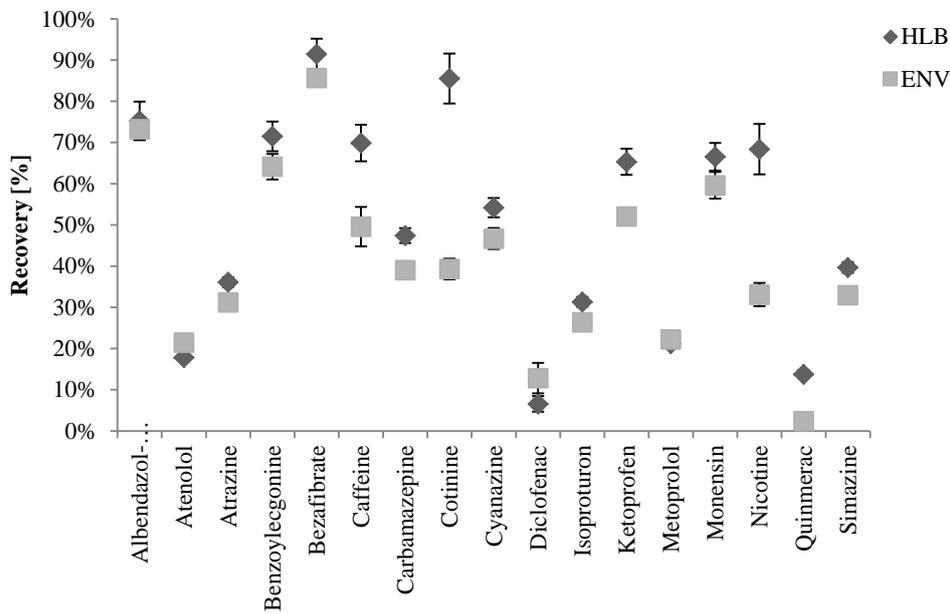
Figure 27: Recoveries of HLB and ENV cartridges.

10.4   Appendix D

List of chemicals and devices used in the lab:

Pop-Cans	Sharpsville Container; NSF Component; max capacity 3 gal. P/N 29748PS; Year 2013
Pump	Masterflex L/S; Cole Parmer; model 7528-10; 6-600 RPM
Pumping module	Masterflex easy-load 3; model 77800-62
Tubings	Masterflex Norprene 06404-15; Mfg by Saint-Gobain
Filter station	Millipore Corporation; Bedford, Masssachusetts, U.S.A 01730; Filter Diameter 142 mm
Glassfiber Filters	Whatman, GE Healthcare Life Sciences; Glass Microfibre Filters; GF/F; Diameter 142mm; 25 circles; Cat no. 1825-142; GE Healthcare UK Unlimited, Amersham Place Little Chalfont; Buckinghamsihre HP7 9NA, UK
Glass bottles	VWR Borosilicate 3.3; 5000 mL; 215-2330
SPE station	Waters, Milford, Massachusetts U.S.A;
SPE HLB	Waters; Oasis HLB 200cc; 1g; LP Extraction Cartridge; Part-no. 186000117; sorbent batch no. 101A
SPE ENV	Bond-Elut ENV; 1000mg; 6 mL; 30/PK. Agilent Technologies; Part No 12255012
SPE reservoir	BondElut; Reservoir - 2 Frits; 20 ml capacity; 100/PK. Part no. 12131017; Agilent Technologies
Methanol 99%	LiChrosol hypergrade for LC-MS; Methanol,; Merck KGaA, 64271 Darmstadt, Germany; Purity > 99.9%
Ethanol 99%	AnalaR NORMAPUR; Ethanol absolute; VWR Chemicals; VWR International, 201 rue Carnot, 94126 Fontenay sous Bois, France; Product 20821.310; Batch 13E170515

Ultrapure water	EMD Millipore Corporation Milli-Q water
Pipettes	VWR Ultra High Performance (100-1000 µl)
	VWR Ergonomic High Performance (1-10 ml)
Pipette tips	VWR
N-Evaporator	Organomation Associates Inc. 266 River Road West Berlin, MA 01503 U.S.A; model N-Evap 112 Nitrogen Evaporator
Vials	Agilent Technologies, Vial, screw, 2ml; part no. 5182-0716
Vial caps	Agilent Technologies, Cap, 9mm, blue screw, part no. 5182-0717
Falcon tubes	Corning Incorporated, Corning, NY, 14831; 50ml Centrifuge Tube (sterile) part no. 430290
Centrifuge	Eppendorf Centrifuge 5810
pH Indicator paper	Macherey-Nagel GmbH & Co. KG; Neumann-Neander-Str. 6-8, 52355 Düren, Germany; Special Indicator paper; 90211; 90213; 90207
pH indicator sticks	VWR Prolabo; Paper dosatest; code 35 311.604; VWR International, 201 rue Carnot, 94126 Fontenay sous Bois, France
Sonicator	Branson 5510
LC-MS	Xevo G2–S QTOF

10.5   Appendix E

List of compounds added in internal standard mix:

Fecal source tracing:
D3-benzoylecgonine
D7-atenolol
D10-fenylbutazon
D3-17b-nortestosteron
13C6-diclofenac
D3-meloxicam
D3-paracetamol
D3-ABZ-SO
D3-ABZ-SO2
D6-Cholesterol
D3-toltrazuril sulfon
D6-Bentazon

PFAA:
13C8 FOSA
d3-N-MeFOSAA
d5-N-EtFOSAA
d3-N-MeFOSA
d5-N-EtFOSA
d7-N-MeFOSE
d9-N-EtFOSE
13C4 PFBA
13C2 PFHxA
13C4 PFOA
13C5 PFNA
13C2 PFDA
13C2 PFUnDA
13C2 PFDoDA
18O2 PFHxS
13C4 PFOS

Declaration*

I,

Name, First name	KLÖCKNER, PHILIPP
Born on	23. 11. 1989
Matriculation number	594451

hereby declare on my honor that the attached declaration,

- ☐ Homework/Presentation
- ☐ Bachelor Thesis
- ☒ Master Thesis
- ☐ Diplom Thesis,

has been independently prepared, solely with the support of the listed literature references, and that no information has been presented that has not been officially acknowledged.

Supervisor	Karin Wiberg, SLU Uppsala
Lecturer	Prof. Dr. Thilo Streck, Universität Hohenheim
Thesis topic	From source to tap – A study of organic contaminants in raw and drinking water in the region of Uppsala, Sweden
Semester	SS 2015

I declare, here within, that I have transferred the final digital text document (in the format doc, docx, odt, pdf, or rtf) to my mentoring supervisor and that the content and wording is entirely my own work. I am aware that the digital version of my document can and/or will be checked for plagiarism with the help of an analyses software program.

UPPSALA   21. 09. 2015   P. Klöckner

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